



AAHP - 2026

**VI Biennial Poultry Health Conference and
National Symposium on**

“STRENGTHENING POULTRY HEALTH: TRANSFORMING FARMERS ECONOMY”



20-21, January 2026
Nagpur Veterinary College, MAFSU, Nagpur

COMPENDIUM

Jointly Organized by

**Department of Veterinary Pathology,
Nagpur Veterinary College, MAFSU, Nagpur**

and

Association of Avian Health Professionals

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VI Biennial Poultry Health Conference and National Symposium on

“STRENGTHENING POULTRY HEALTH: TRANSFORMING FARMERS ECONOMY”

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Maharashtra Animal and Fishery Sciences University, Nagpur

The Genesis

The Maharashtra state is bestowed with unique, rich and diverse animal and fisheries resources that contribute significantly to the gross product of the state. For sustainable management, conservation and augmentation of these animal and fisheries resources, the Maharashtra Animal & Fishery Sciences University was established under the State Act No. 1998 (MAH. XVII of 1998) on 3rd December, 2000 with its Headquarter at Nagpur; the famous City of Oranges located centrally in the country and connected well with different parts by road, rail and air. The university has been carved out of the four agricultural universities in the state by transferring five Veterinary Colleges, one Post Graduate Institute of Veterinary and Animal Sciences and one Dairy Technology College. Considering the need for developing additional human resource in the fields of Fishery Science and Dairy Technology, two Fishery Science Colleges and an additional Dairy Technology College were established by the university after five years of its formation. His Excellency Hon. P.C. Alexander, Chancellor of University & Governor of Maharashtra inaugurated the university establishment on 3rd December, 2000.

Thus beginning with the five Veterinary Colleges, one Post Graduate Institute of Veterinary & Animal Sciences and one Dairy Technology College at different geographical locations of the state in December 2000, University made a commendable progress in developing Fishery Faculty and strengthening Dairy Technology Faculty by establishing two new Fishery College at Nagpur & Udgir and one Dairy Technology College at Udgir. Besides ten colleges in three faculties, Director of Instructions, Director of Research and Director of Extension Education, the University has a well-developed Students Welfare Section, University Library, Bio-informatics Center and training and placement centers. With the new concepts of research and extension responsibility, the University plans to initiate and coordinate research projects with the other Universities, institutions of ICAR.

Establishing Excellence in education and research

University established highly specialized and well developed infrastructure to support advanced teaching and research in veterinary medical sciences.

- India's first Veterinary Nuclear Medicine Center (VNMC) at Mumbai
- Western Region Referral Laboratory for Meat and Meat Products Quality Standards at Mumbai
- Swine Fever Surveillance Center at Nagpur
- Centre for One Health at Nagpur
- Wildlife Research and Training Centre at Nagpur
- Poultry Research and Training Centre at Nagpur
- Embryo Transfer Technology Laboratory at Akola
- Mineral & Hormonal Assay Laboratory at Akola

Nagpur Veterinary College, Nagpur

With a commitment to the highest standards, the unit seamlessly integrates Teaching, Research, and Extension services to fulfill its multifaceted mission, Nagpur veterinary College was established on 1st July 1958. Late Hon,ble Shri Y. B. Chavan, the then Chief Minister of the Govt. Of Bombay inaugurated this college in the convocation Hall of Nagpur University. The college was named as Nagpur Veterinary College, on the similar line as Bombay Veterinary College. Initially the Departments of Anatomy, Physiology and Animal Husbandry were established and then other departments in phases were established. In 1968 this college was affiliated to Nagpur University and the administrative control was with the state department of Animal Husbandry.

Establishing Excellence in education and research

The college hosts highly specialized and well developed infrastructure

- Swine Fever Surveillance Center at Nagpur
- Centre for One Health at Nagpur
- RVC Unit
- Poultry Research and Training Centre
- Livestock Farms
- Veterinary Hospital

Department of Pathology

Department of Pathology is imparting educational courses in U.G., P.G. and Ph.D. degree program and also provides diagnostic post mortem services and advanced laboratory diagnostic facilities to poultry and livestock farmers and field veterinarians. Post graduate research is concentrated in the field of toxico-pathology, molecular diagnostics for endemic and re-emerging diseases and also on nanoparticles.

Diagnostic services offered by the department:

- Diagnostic post-mortem for domestic and wild animals, as well as poultry
- Histopathology
- Clinical pathology
- Molecular diagnostics for viral, bacterial, and mycoplasmal agents
- Isolation of viruses using embryonated eggs or cell culture
- Mycotoxin analysis of feed samples (Qualitative)

ACKNOWLEDGEMENTS

The organizers would like to thank the following companies and organizations for their generous support for organizing VI Biennial Poultry Health Conference and National Symposium AAHP – 2026.

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Dr. Niteen V. Patil

Ph.D., FADS, FNAVS, FANSI, FANA (Animal Nutrition)

Vice Chancellor

डॉ. नितीन व. पाटील

पि.एच.डी., एफ.ए.डी.एस., एफ.एन.ए.डि.एस. एफ.ए.एस.आय., एफ.ए.एन.ए. (पशुपोषण शास्त्र)

कुलगुरु



MESSAGE

I am pleased to note that Nagpur Veterinary College in collaboration with Association of Avian Health professionals is hosting **VI Biennial Poultry Health Conference (AAHP-2026) and National Symposium at Nagpur during January 20-21, 2026**. The theme of the conference is “Strengthening Poultry Health: Transforming Farmers Economy” is apt looking into the importance of maintaining poultry health in ensuring profitability to the poultry enterprise. Poultry sector is one of the fastest growing sectors, boosting economy of India’s agriculture sector. Poultry industry is very dynamic sector facing challenges due to emerging health problems which needs to be addressed on priority.

The conference will address the existing and future needs of the rapidly growing poultry industry. I hope this conference will provide an opportunity to the scientist working in this field, practitioners, industry personnel and poultry farmers to interact, discuss, deliberate and devise strategies for promoting poultry industry.

I wish the conference a grand success.

(Niteen Patil)



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डॉ.शि. वि.उपाध्ये, एम.व्ही.एससी., पीएच.डी., एफआयएससीएस, एफएनएव्हीएस
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Dr. S. V. Upadhye, M.V.Sc., Ph.D., FISVS, FNAVS
Director (Instructions) & Dean (Faculty of Veterinary & Animal Sciences)



Message

Greetings from Maharashtra Animal and Fishery Sciences University, Nagpur. We are privileged for being bestowed with the honor to organize the prestigious National event “VI Biennial Poultry Health Conference (AAHP-2026) and National Symposium” during January 20-21, 2026 on “Strengthening Poultry Health: Transforming Farmers Economy”. We take immense pleasure to welcome all the delegates and members nationwide to be our guests for the event. It will be an update on the advances in poultry health. Scientific deliberations will be one of the most important facets of the conference. The best-known national luminaries in various fields will enlighten us with their dedicated work and vast experience. The scientific program has been planned to hold the interest of both the beginners and the experts. We promise you an academic event that will enhance not just the academics but also bring to you recent advances in Poultry health.

We welcome the members, post-graduates and other colleagues to a conference. Nagpur is famous for its diversity of cultures and religion, Varhadi foods and historical heritage. You are also cordially invited to join us for enjoying the timeless beauty and warm hospitality of the wonderful City of Oranges, the tiger capital, Nagpur. We would also make an effort to extend all possible comforts to all of you during the event.

(S. V. Upadhye)



संशोधन संचालनालय
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डॉ. नितीन व. कुरकुरे
संचालक संशोधन

Dr. Nitin V. Kurkure, Ph.D., DICVP, FNAVS, FIAVP
Director of Research



MESSAGE

I am glad to know that Nagpur Veterinary College, Nagpur, is hosting the **VI Biennial Poultry Health Conference (AAHP-2026) and National Symposium at Nagpur during January 20-21, 2026. The theme of the conference “Strengthening Poultry Health: Transforming Farmers Economy”** highlights our commitment to advancing the poultry health and transforming farmers economy.

The theme of the symposium is very apt for the current scenario of our poultry industry which has been facing many problems in the form of newly emerging diseases. The scientific deliberations will be a key feature, with esteemed national experts sharing their insights and experiences.

Join us for an enriching experience filled with new concepts, in depth discussions. We look forward to welcoming you to the city of oranges.

Warm regards,

N.V. Kurkure



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संचालक, विस्तार शिक्षण

Dr. S. W. Bonde, MVSC, Ph.D., FSAPI, FFSVBB

Director Extension Education



MESSAGE

It's a matter of great pleasure to know that the Department of Pathology, Nagpur Veterinary College, Nagpur is organizing VI Biennial conference of Association of Avian Health Professionals (AAHP-2026) on “Strengthening Poultry Health: Transforming Farmers Economy” during January 20-21, 2026.

I am confident that the conference will encompass a broad range of topics in poultry health from foundation concepts to the latest advancements. The carefully curated scientific program is designed to engage both beginners and experts, featuring esteemed national luminaries who will be sharing their insights and experiences. The attendees can look forward to thought-provoking discussions on key issues and valuable opportunities to connect with leading experts in the field. I congratulate Associate Dean, Nagpur Veterinary College Nagpur, organizing secretary and their team for their untiring efforts in organizing this conference. I wish the organizers of AAHP-2026 a grand success and complement the editorial team for bringing out this compendium.

(Dr. S. W. Bonde)

Director (Extension Education)
MAFSU, Nagpur



Dr J M Kataria
President



**Association of Avian
Health Professionals**
(Regd No. 545 of 2010
(AP Societies Registration
Act No. 35 of 2001)
Rajendranagar, Hyderabad

MESSAGE

It is indeed a great honor and immense pleasure that the 6th Biennial Poultry Health Conference of the Association of Avian Health Professionals (AAHP) and National Symposium (AAHP-2026) with theme “Strengthening Poultry Health: Transforming Farmers Economy” is being organized during 20-21st January, 2026 by the Department of Veterinary Pathology, Nagpur Veterinary College, MAFSU, Nagpur. This event is a significant opportunity for poultry health professionals to come together, share their knowledge and advance the field of poultry medicine. I extend a warm welcome to the delegates from research organizations, veterinary universities, poultry industry, field veterinarians and post-graduate students from various institutions and collages.

Rapid expansion of poultry industry increases risk of infectious diseases. Emerging and evolving pathogens remain a serious threat to the poultry industry causing considerable economic impact. Public health concerns and consumer pressure to reduce the use of antimicrobials in poultry production is increasing. Control of foodborne pathogens, which is of critical importance to public health, remains a major challenge for the poultry industry. Novel tools and interventions are absolutely essential to control and reduce poultry health and disease challenges.

The theme of the conference “Strengthening Poultry Health: Transforming Farmers Economy” is very relevant and the topics covered in this conference are crucial for the health and well being of poultry. I am looking forward to fruitful deliberations, interactions and scalable outcomes in the forthcoming conference. I am confident that the discussions and collaborations that take place here will lead to meaningful advancements in the field of poultry medicine.

I congratulate the organizing committee for sincere efforts in scrupulously designing the technical sessions of the conference keeping in view the latest trends and future scope of innovative diagnostic and vaccine technologies as well as other disease control and health management tools in poultry.

I wish the AAHP conference-2026 a grand success


(J M Kataria)



Dr M R Reddy
General Secretary



**Association of Avian
Health Professionals**
(Regd No. 545 of 2010
(AP Societies Registration
Act No. 35 of 2001)
Rajendranagar, Hyderabad

MESSAGE

I am very glad that the 6th Biennial Poultry Health Conference of the Association of Avian Health Professionals (AAHP) and National Symposium (AAHP-2026) with theme “Strengthening Poultry Health: Transforming Farmers Economy” is being organized during 20-21st January, 2026 by the Department of Veterinary Pathology, Nagpur Veterinary College, MAFSU, Nagpur. I on behalf of the AAHP family thank the Nagpur Veterinary College, MAFSU, Nagpur for hosting this conference.

Poultry disease challenges have evolved in line with the evolution of large scale poultry production. Consequently, the strategies for disease prevention, diagnosis, and control have also evolved and must continue to evolve. Since the goal of a poultry operation is to convert feed into food as economically as possible, it is critical to manage both the risk and consequence of disease challenge. Poultry veterinarians and other poultry health professionals will have to play a bigger role in the future and they should equip and have stronger focus on poultry health, welfare and food safety. Advances in poultry health care and management bring significant economic benefits, increased production, reduced mortality and improved quality of meat and eggs. Under these circumstances, a great responsibility lies on the scientists and entrepreneurs to strive hard to overcome the difficulties.

The theme of this conference is very apt for the current scenario of our Poultry Industry which has been facing many problems in the form of newly emerging diseases, antimicrobial resistance, welfare and food safety.

I hope that through this two-day conference you will discover complex poultry health challenges and will find better solutions. Together, we will make the AAHP stronger for a healthy and sustainable poultry industry.

I sincerely appreciate the efforts made by the organizers. I wish you all a stimulating and memorable conference

M R Reddy



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Dr. J. P. Korde, M.V.Sc., Ph.D.
Associate Dean



Message

We are honored to host the VI Biennial conference of Association of Avian Health Professionals (AAHP-2026) from January 20-21, 2026, under the theme “Strengthening Poultry Health: Transforming Farmers Economy” This is a significant milestone for our institute, and we are excited to welcome delegates and members from across the nation.

The conference will cover a wide range of topics in poultry health, from foundational concepts to the latest advancements. We have curated a scientific program designed to engage both beginners and experts, featuring esteemed national luminaries who will share their insights and experiences. We are expecting thought-provoking discussions, debates on key issues, and opportunities to connect with leading experts in the field.

Join us for a conference filled with knowledge and networking. We are committed to ensuring your comfort and enriching your experience during this exciting event.

We look forward to welcoming you to Nagpur for AAHP-2026!

Warm regards,

(J. P. Korde)
Associate Dean



Department of Veterinary Pathology
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Dr. P. M. Sonkusle
Organizing secretary

MESSAGE

Dear Friends and Colleagues,

It is a great pleasure and an honor to Department of Veterinary Pathology, Nagpur Veterinary College to welcome the delegates from different parts of India for the VI Biennial Poultry Health Conference (AAHP-2026) and National Symposium on “Strengthening Poultry Health: Transforming Farmers Economy” during January 20-21, 2026 at the Silver Jubilee Auditorium, Nagpur Veterinary College, Maharashtra Animal & Fishery Sciences University, Nagpur, India.

Poultry diseases remain one of the major threats to poultry production in India. There is a need to reduce disease burden in poultry production by improving our understanding of host, pathogen and environment interaction in poultry. Poultry veterinarians and other poultry health professionals will have to play a bigger role in the future and they should equip and have stronger focus on poultry health, welfare and food safety.

The AAHP 2026 will provide a wonderful forum for scientific and industrial fraternity to refresh and share the knowledge base and explore the possibilities for healthy production and wealthy nation through effective poultry management. The Conference will serve to offer plenty of opportunities to meet and interact with the leading scientists and researchers, and colleagues from industries. I am sure that, the two day deliberations of this symposium would help the Indian Poultry Industry to overcome the threats and face new challenges confidently for strengthening poultry health and transforming farmers economy.

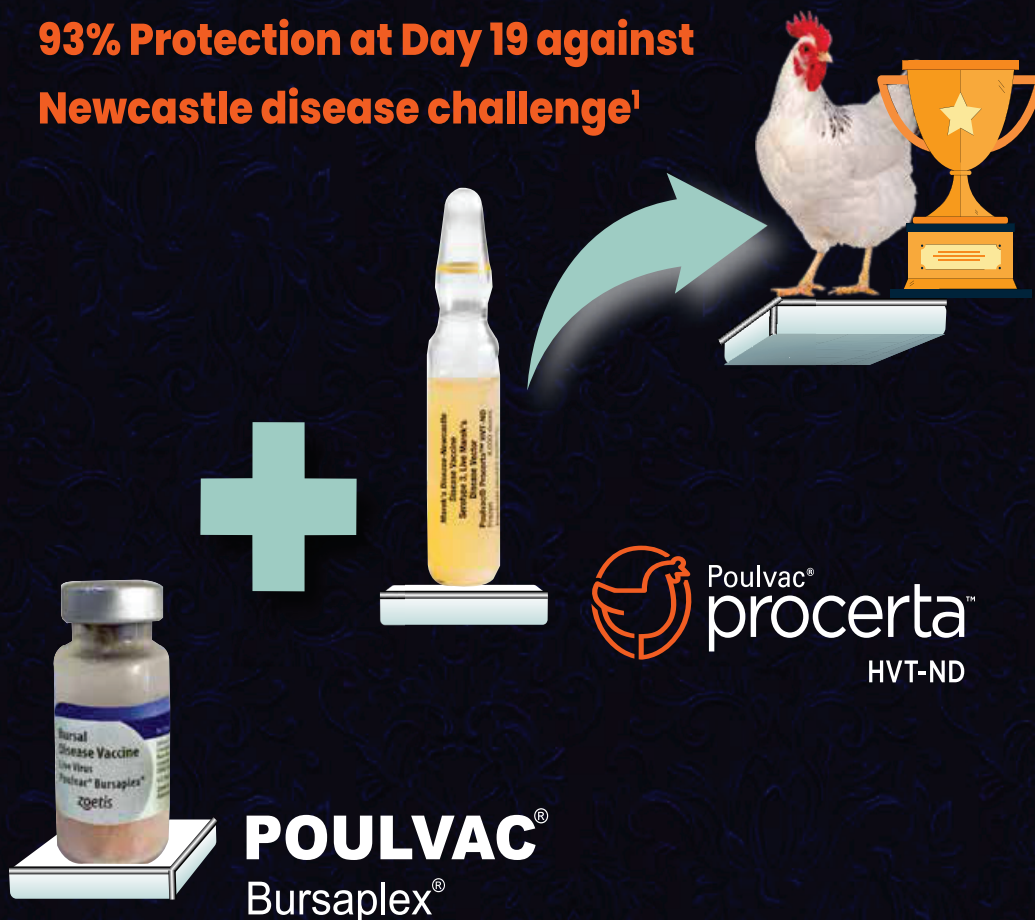
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With best wishes,

P.M. Sonkusale

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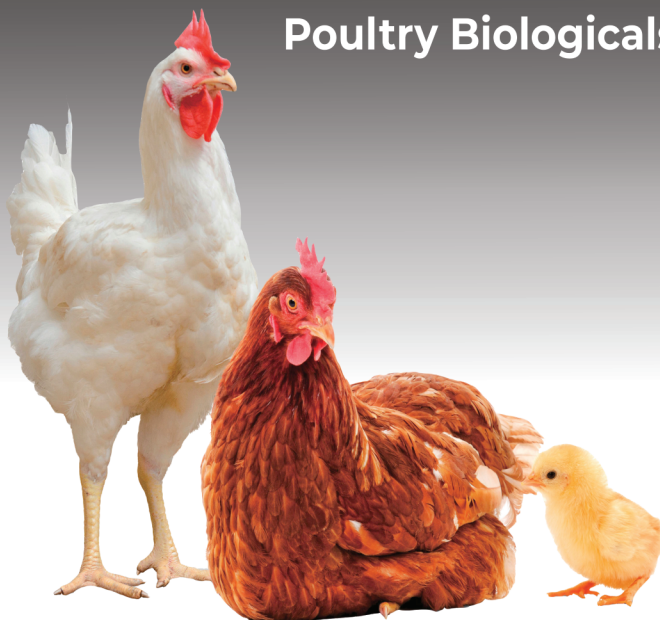
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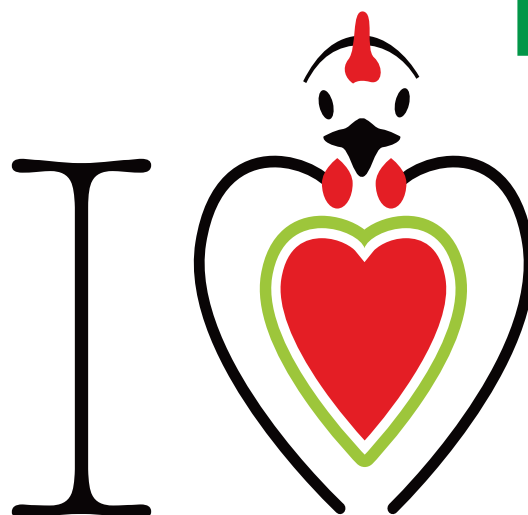


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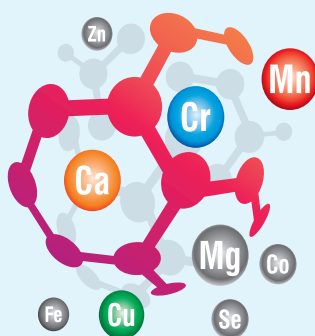
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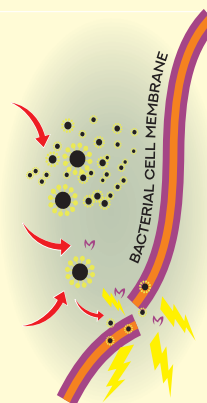
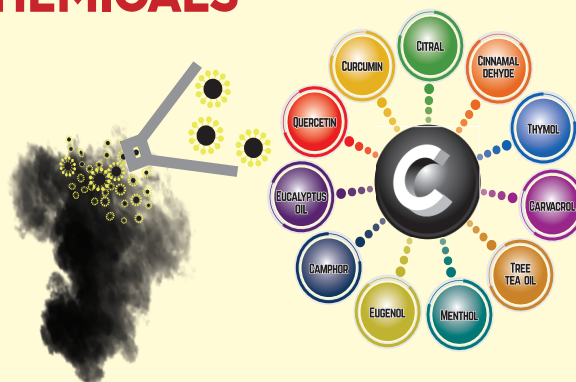
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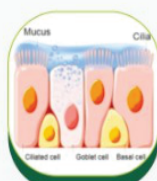
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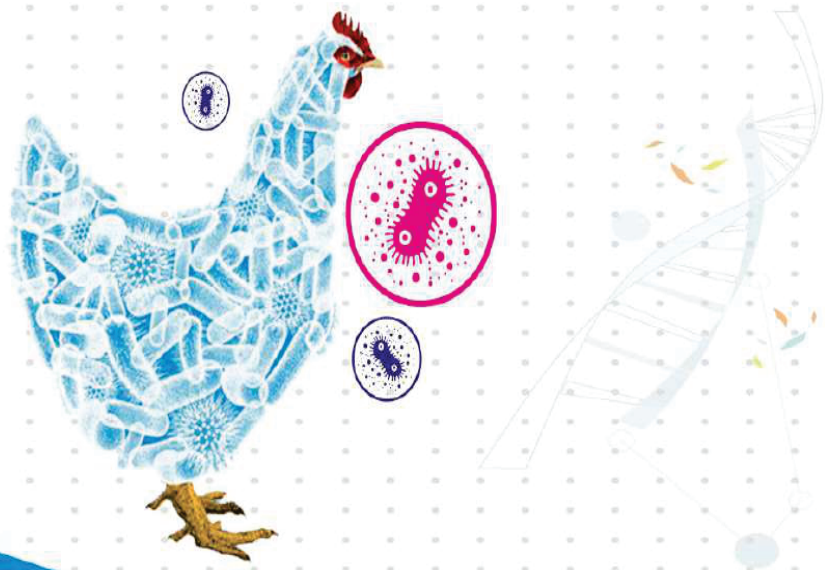
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CONTENTS

No.	Title and Authors	Page no.
Theme 1 : Existing and Emerging Diseases of Poultry		
L1	Integrated Control of H9N2 Low Pathogenic Avian Influenza S. Nagarajan, Manoj Kumar, C. Tosh, Aniket Sanyal	1
L2	Challenges of emerging concurrent viral infections in poultry: Focus on Newcastle Disease Virus and Avian Metapneumovirus in India H.K. Muniyellappa	5
L3	Avian Respiratory Disease Complex: Insights for Indian Poultry Production and Global Trends N.R. Bulbule and M.M. Chawak	14
L4	Control of Infectious Bronchitis in poultry with reference to QX-like serotypes <u>Ganesh Darban</u>	18
O1	Transmission of highly pathogenic avian influenza H5N1 (clade 2.3.4.4b) virus between ducks and chickens Kumar Gaurav, <u>Manoj Kumar</u> , Chakradhar Tosh, S. Nagarajan, H.V. Murugkar and Aniket Sanyal	19
O2	Molecular detection and comparative prevalence of <i>Mycoplasma gallisepticum</i> and <i>Mycoplasma synoviae</i> in commercial poultry flocks of India during 2024–2025 <u>Khardenavis S.U.</u> , Deshpande R.R., Agnihotri A.A., Bulbule N.R. and Chawak M.M.	20
O3	Investigations on <i>Lipeurus caponis</i> (wing louse) infestation in chickens of west-coast India <u>Shirish D. Narnaware</u> , Nibedita Nayak, Navina Naik, Sanjaykumar Udharwar and Amiya Ranjan Sahu	21
O4	Isolation, characterization, antibiogram and pathological studies of avian pathogenic <i>Escherichia coli</i> from respiratory affections in broiler chicken flocks Paras Saini, <u>Babu Lal Jangir</u> , Gulshan Narang, Rakshita Sharma, Manesh Kumar and Ramesh Kumar	22
O5	Pathological and molecular studies on certain viral respiratory infections in commercial broiler chickens Payal Nirmal, Vitthal Dhaygude, Namdeo Bulbule, Uma Tumlam, Sameer Jadhav, Chandan H.U., Amit Kumare and <u>Anushka Todmal</u>	23
O6	Association of Avian Metapneumovirus and Infectious Bronchitis virus in commercial broiler chickens with respiratory signs	24



	<u>Ravi Sonwane</u> , Pradip Doiphode, Myeongseob Kim, Deepak Koli and Shyam Vane	
07	Pathology of selective respiratory bacterial infections in birds <u>Rathod P.R.</u> , Gangane G.R., Kolekar A. K., Sawant R.J., Khambayatkar M.A. and Jallela A.	25
08	Gross and histopathological findings of Histomoniasis in a deshi chicken Chandan H.U, <u>Vitthal S Dhaygude</u> , Chandrashekar S Mote, Prajakta Dethe, Surabhi Bhandekar, Amith Kumare and Anushka Todmal	26
09	Case report on occurrence of Caecal Coccidiosis in indigenous Sonalika breed of poultry Supriya Shukla, Nidhi Shrivastava, G.P. Jatav, Rashmi Choudhary, <u>Ritesh Verma</u> , Vijay Yadav, Rahul Asware, Amol Tripathi, Pranjali Patel, Pratik Nighut, Amar Karamunge, Sakshi Kashikar	27
010	Prevalence and molecular characterization of avian pathogenic <i>Escherichia coli</i> in Kadaknath chickens from Mhow region <u>Pranoti Sharma</u> , Nidhi Shrivastava, Supriya Shukla, Kajal Kumar Jadhav, Daljeet Chhabra, Rakhi Gangil, Ravi Sikrodiya, G.P. Jatav, Mahesh Trivedi	28
011	Pathology and molecular confirmation of Avian Metapneumovirus and associated co-infections in chickens Sharada Garudi, <u>Bhupesh Kamdi</u> , Ranjit Ingole, Sudhakar Awandkar, Megha Kaore, Sunil Hajare & Nitin Kurkure	29
P1	Prevalence and characterization of Avian Pathogenic <i>Escherichia coli</i> (APEC) and its isolation from commercial broilers and breeder flocks for vertical transmission <u>Ranjith Reddy</u> , Dinesh C Pathak, Santosh S Swami, Suman P	30
P2	Pathological and Molecular Investigations of an Outbreak of Infectious Laryngotracheitis in a broiler poultry flock <u>S.G.Chavhan</u> , R.C.Kulkarni, B.M.Kondre, S.P.Awandkar, V.G.Rajput and K.K.Yernale	31
P3	Molecular and clinico-pathological investigation of Fowl Adenoviral inclusion body hepatitis and gizzard erosion outbreaks from Central India Sukirti Sharma, Madhuri Hedau, Megha Kaore and P. M. Sonkusale	32
P4	Prevalence and characterization of Avian Pathogenic <i>Escherichia Coli</i> (APEC) and its isolation from commercial broilers and breeder flocks for vertical transmission <u>Ranjith Reddy</u> , Dinesh C Pathak, Santosh S Swami, Suman P	33
P5	Studies on the prevalence and pathology of colibacillosis in broiler birds in North Eastern plain zone of Uttar Pradesh. <u>Arunima Singh</u> , Debasish Niyogi, Dharam Prakash Srivastava, K.K Tripathi	34
P6	Lameness in broiler breeders: Field investigation of bacterial spondylitis, spondylolisthesis and valgus-varus deformities	35

	<u>Deshmukh V.V.</u> , Kinge G.S., Bulbule N.R. and Chawak M.M.	
P7	Re-emerging fowl pox virus – Isolation, identification, and differential diagnosis in commercial birds during 2024-2025 <u>Nagargoje S. B.</u> , Agnihotri A. A., Deshmukh V. V., Bulbule N. R., Chawak M. M.	36
P8	Avian reovirus isolation associated with nephritis cases in commercial broiler chickens <u>Kopnar P. D.</u> , Khardenavis S. U., Deshmukh V.V., Bulbule N. R., Chawak M. M.	37
P9	Integrated clinical, haematological and molecular approach to the management of Avian Malaria in wild birds Priyal V. Chauragade, <u>P.V. Chauragade</u> , S.M. Kolangath, M.R. Kate, G.R. Bhojne, S.V. Upadhye, R.K. Fulsunge	38
P10	Characterization of H9N2 avian influenza viruses isolated from Bhopal, Madhya Pradesh <u>Kumbhar Anuradha</u> , H. V. Murugkar, Manoj Kumar, C. Tosh, Kumar Gaurav, Aniket Sanyal and S. Nagarajan	39
P11	Molecular Studies of <i>Ornithobacterium rhinotracheale</i> (ORT) in Chickens <u>Londhe P.D.</u> , Gangane G.R, Rathod P.R, Yeotikar P.V., Jonpelliwar K.A, Khambayatkar M.A, Jallela A Swant R.J. and Kolekar A.K.	40
P12	A Rare Report on Proventricular Tetrameriosis in a Three-Month-Old Desi Chicken <u>Anushka Todmal</u> , Vitthal S. Dhaygude, Chandrashekhar S. Mote, Amit Kumare	41
P13	Systemic Aspergillosis in a Kakariki Parakeet (<i>Cyanoramphus novaezelandiae</i>): A Case Report Abin Jithu Paul, <u>Bharat Chapke</u> , Pradeep M., Krithiga K., Ajith Jacob George and Hamza Palekkodan	42
P14	Pathology and molecular characterization of Avian enteric viruses in chickens <u>Sonkusale P.M.</u> and Ghosh R.C.	43
P15	Pathology, incidence and molecular characterization of chicken parvovirus in desi chickens of Chhattisgarh <u>P.M. Sonkusale</u> , R.C. Ghosh and Vivek Kumar	45
P16	Pathology and molecular diagnosis of Velogenic Viscerotropic Newcastle Disease Virus in desi chicken of Chhattisgarh <u>Prashant M. Sonkusale.</u> , Ratan C. Ghosh and Vivek Kumar	46
P17	Pathological studies and molecular detection of Salmonellosis in desi chickens of Nagpur Region Prashant M. Sonkusale, Chaitanya S, Sukirti Sharma, Megha Kaore and Madhuri Hedau	47

P18	Pathology and molecular detection of Salmonellosis in commercial chickens of Chhattisgarh <u>P. M. Sonkusale</u> , R.C. Ghosh, Devesh Kumar Giri and Vivek Kumar	48
P19	Isolation, identification and prevalence of poultry Coccidiosis in Maharashtra <u>Shivaji Mehatre</u> , Swapnil Kubal, Milind Chawak and Sanjay Gavkare	49
P20	Pathology and molecular characterisation of important respiratory pathogens of chicken Arul Pandiyan, <u>Megha Kaore</u> , Mahesh Dahake, Madhuri Hedau, Nitin Kurkure	50
P21	Pathological studies on coccidiosis in broiler chickens <u>Neeraj</u> , Sikander and Babu Lal Jangir	51
P22	Pathological and molecular observations of mid-intestinal coccidiosis due to <i>Eimeria necatrix</i> infection in desi chickens <u>K.K.Yernale</u> ¹ , B.M.Kondre ¹ , R.C.Kulkarni ¹ , S.G.Chavhan ¹ , S.P.Awandkar ² and V.G.Rajput ¹	52
P23	Pathological and molecular studies on Tetrameriosis in desi chickens <u>V.G.Rajput</u> ¹ , S.G.Chavhan ¹ , R.C.Kulkarni ¹ , B.M.Kondre ¹ , S.P.Awandkar ² and K.K.Yernale ¹	53
Theme 2: Vaccine, Variants and Vaccination Strategies		
L1	Avian Vaccines: Updates and applications – “From hatchery to harvest” <u>Sanjay K. Gavkare</u>	56
L2	Enhancing <i>Mycoplasma gallisepticum</i> vaccine delivery and disease control in commercial chicken operations Robin R. Achari	59
O1	A comprehensive molecular investigation and evolution of the Infectious Bronchitis virus in India reveals the region-specific mutations in the receptor-binding domain of S1 gene <u>Asok Kumar M</u> , Megha Sharma, Faslu Rahman AT, MV Srinivas, Gaurav Sharma, Anshuk Sharma, Umesh Kumar Singh and RVS Pawaiya	63
O2	Comparative evaluation of two different immune complex Infectious Bursal disease vaccines in commercial broiler chickens <u>Deepak Koli</u> , Pradip Doiphode, Myeong Seob Kim, Ravi Sonwane, Shyam Vane and Prashant Sonkusale	64
P1	Magniplex V877 : Novel IBD immunocomplex vaccine -Boon for sustainable poultry production <u>Srivastava A.K.</u> , Godase Raghavendra, Panday Amit, Kumaresan, S, Dixit R, Gujanti , Shiva., Soundatkar A, Gangurde Bhushan, Nair Renjith	65
P2	Provac 4: Augmenting breeders titers for a better livability and immunity for broiler health: An effort to reduce abuse of antibiotics addressing the issue of AMR for one health <u>Srivastava A.K.</u> , Godase Raghavendra, Panday Amit, Kumaresan, S, Dixit R, Gujanti , Shiva., Soundatkar A, Gangurde Bhushan, Nair Renjith	66

P3	Assessing the efficacy of a novel recombinant New Castel disease - Procerta HVT -ND against the virulent New Castle disease in commercial broilers <u>Srivastava A.K.</u> , Godase Raghavendra, Panday Amit, Kumaresan, S, Dixit R, Gujanti , Shiva., Soundatkar A, Gangurde Bhushan, Nair Renjith	67
P4	Review of vaccination strategies against Infectious Coryza in poultry <u>Ranjitsingh Ghadage</u> , Amol Pawar, Nilesh Pansare, Mukti Walse, Swapnil Kubal, D B. Bhandari, Gurudutt Joshi, Milind Kolhe, Santosh Deshpande , Sanjay Gavkare	68
P5	Safety study of experimental recombinant HVTND vaccine: One dose, repeated dose and over dose in birds <u>Umesh Kulthe</u> , Swapnil D. Kubal, Pooja Sathe, Gurudutt Joshi, Mangesh Shirke and Sanjay Gavkare	69
P6	Safety study of recombinant HVTND vaccine: Spread of recombinant rHVTND vaccine virus from vaccinated birds to non-vaccinated birds and non-target species <u>Umesh Kulthe</u> , Swapnil D. Kubal, Pooja Sathe, Gurudutt Joshi, Amol Shingade, Mangesh Shirke and Sanjay Gavkare	70
P7	Dissemination of experimental recombinant HVTND vaccine in birds <u>Umesh Kulthe</u> , Swapnil D. Kubal, Pooja Sathe, Gurudutt Joshi, Amol Shingade, Preeti Choudhary, Mangesh Shirke and Sanjay Gavkare	71
P8	Study of interaction of recombinant HVTND vaccine with other live vaccines <u>Umesh Kulthe</u> , Swapnil D. Kubal, Pooja Sathe, Gurudutt Joshi, Amol Shingade, Mangesh Shirke and Sanjay Gavkare	72
P9	Recombinant vaccines: A boon for poultry entrepreneurs <u>Onkar Kashikar</u> , Subhash Mule, Swapnil Kubal, Mangesh Shirke, Hanumant Thete, Sanjay Gavkare	73
P10	Chicken Infectious Anaemia (CIA) vaccine: Protection against hidden offender <u>Ranjitsingh Ghadage</u> , Amol Pawar, Dattatraya Ombase, Nilesh Pansare, Mukti Walse, Amol Shingade, D B. Bhandari, Milind Kolhe, Santosh Deshpande, Sanjay Gavkare	74
P11	Circulation of variants of Infectious Bronchitis virus of GI-13 and GI-24 lineage from Central India <u>Megha Kaore</u> , Arul Pandiyan, Mahesh Dahake, Madhuri Hedau, Nitin Kurkure	75
P12	Enhancing vaccine safety and antigen preservation through Beta-Propiolactone (BPL) based viral inactivation: An alternative to formaldehyde inactivation in poultry vaccine production <u>Amol Pawar</u> , Vishal Shelke, Nilesh Pansare, Ranjitsingh Ghadage, Mukti Walse, D Bhandari, Gurudutt Joshi, Milind Kolhe, Santosh Deshpande, Sanjay Gavkare	76

P13	Vengem 9, first indigenous low pathogenic Avian Influenza vaccine inactivated (H9N2) <u>Nilesh Pansare</u> , Amol Pawar, Ranjitsingh Ghadage, Mukti Walse, Swapnil Kubal, D B. Bhandari, Preeti Choudhary, Milind Kolhe, Santosh Deshpande , Sanjay Gavkare	77
P14	Innovations in poultry vaccine research: India's leading edge Amol Shingade, <u>Preeti S. Choudhary</u> , Pratik Kulkarni, Santosh Deshpande, Swapnil Kubal, D.B Bhandari, Gurudutt Joshi, Sanjay Gavkare	78
P15	The importance of SPF-eggs in manufacturing and testing of poultry vaccines <u>Onkar Kashikar</u> , Subhash Mule, Swapnil Kubal, Mangesh Shirke, Hanumant Thete, Kuhoo Singh, D. Shreyas	79
Theme 3: Diagnostics, Digital Technologies and Artificial Intelligence		
O1	Design and development of low cost and user-friendly electric stunner for meat animals including poultry birds <u>C. Ramakrishna</u> , Girish Patil S., Vikram R., Kobu Khate, Jayanta Chamuah, Harshit Kumar and S. B. Barbuddhe	82
P1	Global prevalence of natural infection of Chicken Infectious Anemia Virus (CIAV) in poultry through a systematic review, meta-analysis and AI powered literature mining <u>Christophert Oluwole Akakpo</u> , Syed Junaid Ali, Ashitha M, Yagavi Ravindran, Sangita Venkataraman, Madhuri Subbiah	83
P2	Serological pattern of IBD ELISA titres in broiler parents and its corresponding broiler chicks in relation to MABs Chandankar S.D., Satputey P.G., Aulwar B.S., Kinge G.S., <u>Bulbule N.R.</u> and Chawak M.M.	84
Theme 4: Antimicrobials, AMR and Alternatives to Antimicrobials		
L1	Poultry pathogens and antimicrobial resistance: A One Health imperative for sustainable food systems <u>Deepak B. Rawool</u> , Jess Vergis, S.B. Barbuddhe	86
L2	Nano-Intervention in poultry pathophysiology: Precision nutrients for higher productivity <u>Lavleen Kumar Gupta</u>	91
O1	Synergistic combination of herbal extracts, essential oils & probiotic: A novel tool against antimicrobial resistance, strengthening poultry health, farmers economy and public food safety. <u>Atul D. Deshmukh</u> , R. Nagaraja Reddy	92
O2	Potential of cinnamaldehyde and citral essential oil as a possible antimicrobial against E. coli infection in broiler chickens Mayuri Chelkar, <u>Madhuri Hedau</u> , Megha Kaore and N. V. Kurkure	93
P1	Antimicrobial efficacy of cinnamaldehyde and citral essential	94

	oil mixture against Avian Pathogenic <i>E. Coli</i> infection in broiler chickens Mayuri Chelkar, Madhuri Hedau, <u>Anushka Bhadauria</u> , Megha Kaore and N. V. Kurkure	
P2	Antimicrobial effect of metal nanoparticles on <i>E. coli</i> induced pathology in broiler chickens Shipra Pand, <u>Madhuri Hedau</u> , Megha Kaore, N. V. Kurkure	95
Theme 5: Food Safety and Foreign Trade Policy		
L1	Food Safety and Government Policies <u>S. B. Barbuddhe</u> and Deepak B. Rawool	98
L2	Role of avian health professionals in poultry sector <u>D. K. Dey</u>	104
Theme 6: GI Tract Health and Disease Management		
L1	Pathophysiological insights and strategic modulation of gut health for enhanced productivity in chickens <u>Megha Kaore</u> and Nitin Kurkure	114
L2	Gut microbiota modulation through essential oils: Impact in poultry health <u>Madhuri Hedau</u> and Nitin Kurkure	119
01	Effect of pomegranate (<i>Punica granatum</i>) peel powder and eucalyptus (<i>Eucalyptus globulus</i>) leaves powder on gut microbial study and comparative economics of broilers Bheru Lal Choudhari, <u>Kavita Shende</u> , Monika Joshi, M. S. Meel, Harendra Rajoriya, Shruti Garg, Swati Umap	123
02	Evaluating the effect of dietary supplementation of Tulsi leaf and Ginger powder as feed additives on carcass and gut microbes of broilers <u>Kavita A. Shende</u> , Rajesh K. Dhuria, Monika Joshi, M.S. Meel, Harendra Rajoriya, Bherulal Chaudhary and Shruti Garg	124
03	Comparative efficacy and performance of antimicrobial gut health additives fed to broiler chicken <u>Lonkar V.D.</u> , Palanisamy K ² , Patil R. B., Kadam A.S., Jadhav S.N., Tumlam U.M., Mote C.S	125
04	Studies of carbon dot encapsulated essential oils on performance and gut health of broiler chicken Dipasree Das, M.R. Jawale, <u>M.D. Kolhe</u> , Shital Chopde, A. P. Dhok, N.V. Kurkure, Megha Kaore, Darshana Bhaisare, Shweta Lende, P.E. Taksande, N.S. Shivshette, Madhuri Jagdale, Vidya Aglave	126
05	Studies of dietary inclusion of rice DDGS through different growth phases on performance and gut health of broiler chicken G.A. Butle, A. P. Dhok, <u>N.S. Shivshette</u> , Shital Chopde, M.R. Jawale, N.V. Kurkure, P.M. Sonkusale, Darshana Bhaisare, Shweta Lende, P.E. Taksande, M.D. Kolhe, Madhuri Jagdale, S.R. Deshmukh	127
06	Gut pathology and molecular characterization of virulent <i>C. Perfringens</i> from necrotic enteritis in cockrels	128

	<u>Ashwini Nerikar</u> , Madhuri Hedau, Mrunalini Budhe, Anushka Bhadauria, Vaishnavi Kadam and N. V. Kurkure	
P1	Effect of dietary β-mannanase on gut health of broilers fed with low energy Kawde V.S., <u>Lonkar V.D.</u> , Kadam A.S. Jadhav S.N., Tumlam U. M., Pawar A. A and Kale U.G.	129
P2	Studies of phytosome conjugated essential oils on performance and gut health of broiler chicken Jaishri Chaurasia, Shital Chopde, <u>Madhuri Jagdale</u> , M.R. Jawale, A. P. Dhok, N.V. Kurkure, Madhuri Headau, M.M. Kadam, Shweta Lende, P.E. Taksande, N.S. Shivshette, M.D. Kolhe, N.S. Ghuge	130
P3	Effect of different combinations of different feed additives on pathology of <i>Eimeria</i> infection in broilers <u>Vaishnavi Kadam</u> , Madhuri Hedau, Megha Kaore, P. M. Sonkusale and N. V. Kurkure	131
Theme 7: Metabolic, Toxic, Nutritional Diseases and their Management		
L1	Toxicity and Inclusion Body Hepatitis (IBH) in poultry: An overlooked link in field outbreaks <u>M.M. Chawak</u> and N.R. Bulbule	134
O1	Phytochemical profiling and anti-gout efficacy of <i>Zingiber officinale</i> and <i>Piper betle</i> in broiler chickens Hemlata Popere, R. S. Ingole, <u>Shraddha Dandale</u> , B. P. Kamdi, S. W. Hajare and S. J. Manwar	137
O2	Safety study of novel quercetin phytosomes in broiler chicken by implementing OECD guideline <u>Umap Swati</u> , Somkuwar A. P., Mahajan N. M., Kurkure N. V., Korde J.P., Kadam M.M., Limsay R.P. and Sawarkar A. R.	138
O3	Occurrence of visceral gout in a broiler poultry farm <u>M. Mondal</u>	139
O4	Protective effect of <i>Allium sativum</i> supplementation against experimentally induced <i>Escherichia coli</i> infection in Broiler chickens Bhagwat P. N., <u>Rathod P.R.</u> , Gangane G.R., Kolekar A. K., Sawant R.J., Khambayatkar M.A., and Jallela A.	140
O5	Effect of microencapsulated essential oil and organic acid in diets on performance of <i>Escherichia coli</i> challenged broilers <u>Akshali Jadav</u> , S.J.Manwar, M.R.Wade and R.S.Ingole	141
O6	Assessment of novel probiotic supplementation on growth performance and economics of broiler production in challenged dietary condition	142

	<u>K. K. Khose</u> , M. G. Nikam, G. R. Gangane, S. N. Rindhe, R. N. Waghmare, N. M. Karad, V. M. Shelke and Satyam Sharma.	
O7	Amelioration of induced Fatty liver syndrome in broilers by supplementation of Fortimas with or without herbs <u>Anushka Bhadauria</u> , Madhuri Hedau, Megha Kaore, P. M. Sonkusale and N. V. Kurkure	143
O8	Impact of dietary inclusion of organic zinc and chromium on physiological response of broiler chickens exposed to cold stress <u>Anisha Ali</u> , Sheikh Zaid Danish Abdul Mateen, Gowher Gull Sheikh, Boddupalli Ayesha, Anand S. Rana, P. D. Bhole	144
O9	Enhancing heat stress resilience in broiler chickens using <i>Withania somnifera</i> mediated zinc oxide nanoparticles N.A. Yadav, R.S. Ingole, <u>Sayali Fulmali</u> , B.P. Kamdi and S. W. Hajare	145
P1	Beneficial role of Indian Bay leaf powder (IBP) supplementation on environmental thermal stress-induced deleterious in White Pekin ducks <u>Aditya Agrawal</u> , P. P. Jena, G. R. Jena, S.K. Mishra, Dharendra Kumar and R. C. Patra	146
P2	Surveillance and risk assessment of Mycotoxin contamination in poultry feed samples during 2024–2025 <u>Agnihotri A.A.</u> , Lanje Rahul., Kinge G.S., Bulbule N.R. and Chawak M.M	147
P3	Pathology of experimentally induced subacute and sub-chronic Ziram toxicity in Kadaknath chickens <u>Kursangmit Lepcha</u> , Supriya Shukla, Nidhi Shrivastava, Daljeet Chhabra, Rashmi Choudhary, Ritesh Verma	148
P4	A case study on heat stress in poultry <u>Poornima Gumasta</u> , Narendra Kumar, Mahmuda Malik, Diptimayee Sahoo	149
P5	The potential risks of high-dose probiotic administration: Investigating bacterial translocation and mortality in neonatal chicks <u>Pradnya Sawlikar</u> , R. D. Fulsundar, Ashwini Nerikar, Shreya Bhojane, Wiqar Khan, Prashant Sonkusale	150
P6	A post-mortem study on testicular pathology of poultry T. K. Alin Moideen, <u>M. Pradeep</u> , Hamza Palekkodan, Ajith Jacob George	151
P7	Haematobiochemical changes in experimentally induced heat stress in broiler birds <u>Patil A. A.</u> , Rathod P.R., Gangane G.R., Yeotikar. P. V., Bhagwat P.N., and Jogdand B. M.	152
P8	Gross and histopathological studies on Ovarian Adenocarcinoma in a 7-year-old Turkey bird: A case report	153

	Chandan H.U, <u>Chandrashekhar S. Mote</u> , Vitthal S. Dhaygude, Vijaysinh Lonkar, Amit Kumare, Anushka Todmal, Prajakta Dethe, Surabhi Bhandekar	
P9	A rare case of Adrenal Teratoma in chicken <u>Mayuri Kalwaghe</u> , B.P. Kamdi, R.S. Ingole, P.M. Rakh, V.S.Dahatonde	154
P10	Comparative evaluation of novel probiotics in broiler diets on intestinal micrometry, ISI score, carcass traits and meat organoleptic properties under dietary challenge conditions <u>K. K. Khose</u> , M. G. Nikam, G. R. Gangane, S. N. Rindhe, R. N. Waghmare, N. M. Karad, V. M. Shelke and Satyam Sharma	155
P11	Effect of dietary supplementation of prebiotic and acidifier powder on comparative economics of broilers <u>Harendra Singh Rajoriya</u> , Kavita Shende, Bheru Lal Choudhari, Shruti Garg, Mahendra Singh Meel, Monika Joshi	156
P12	Growth performance and nutrient utilization in broiler chickens supplemented with zinc and <i>Saccharomyces cerevisiae</i> Dayamand Sahu ¹ , <u>Kiran Kumari</u> ² , Archana ³ , Prasanna Pal ² & M K Gendley	157
P13	Impact of supplementation of peppermint and eucalyptus essential oils on the growth performance of Japanese quails <u>Shraddha Kharvi</u> , R. K. Sharma, Jyoti Palod and Ripusudan Kumar	158
P14	Histomorphological changes in intestine of broiler in normal and <i>Saccharomyces cerevisiae</i> fermented fed product Boddupalli Ayesha, <u>Anisha Ali</u> , U. P. Mainde, N. C. Nandeshwar, R. Y. Charjan, Anand S. Rana and P. D. Bhole	159
Theme 8: Immunosuppression and Immunomodulation		
L1	Immunosuppression and immunomodulation in poultry <u>Babu Lal Jangir</u> , Deepika Lather and Vikas Nehra	162
O1	Monitoring of Chicken Infectious Anemia in commercial poultry flocks by PCR, serology and histopathology in 2025 <u>Deshpande R.R.</u> , Agnihotri A.A., Deshmukh V.V., Bulbule N.R. and Chawak M.M.	171
O2	Prevalence studies on endogenous and exogenous Avian Leukosis virus circulating in Kadaknath birds of farm <u>Rana D.S.</u> , Prince L.L.L., Edigi P, T.R. Kannaki, Vaghadevi, Reddy M.R.	172
O3	Effect of dietary yeast-derived mannan oligosaccharide and beta-glucans (1-3 1-6) on performance, intestinal morphology, redox status, immune response, and gut microbiota in broilers <u>Megha Kaore</u> ¹ , Madhuri Hedau ¹ , Samiksha Suroshe ¹ , Rohan Fulsunder ¹ , Mahesh Gupta ² , Jyoti Nandardhare ³ , C. S. Saraf ³ , Melina Bonato ⁴ , N. V. Kurkure	173
O4	Immunomodulatory effect of zinc nanoparticle conjugated with <i>Tinospora cordifolia</i> in Infectious Bursal Disease virus induced infection in broilers	174

	<u>Madhuri Hedau</u> , Mahesh Dahake, Megha Kaore and N. V. Kurkure	
P1	Analysis of bone marrow in Chicken Infectious Anemia affected commercial chicken P. Mounika, <u>V. Samatha</u> , Ch. Sudha Rani Chowdary, G Srividya and V Rama Devi	175
P2	Comparative evaluation of plain and phytosome-conjugated cinnamaldehyde essential oils on immunity and gut health of broilers Ashwini A. Kondhare, Mukund M. Kadam, Darshana B. Bhaisare, Shilpashree V. Shinde, Madhuri S. Hedau and <u>Abhidhnya V. Shyamkuwar</u>	176
P3	Prevalence of Marek's disease and molecular characterization of MEQ gene of MDV circulating in India <u>T. Vagdevi</u> , Parvati Patil, E. Priyanka, Diwakar singh Rana, T. R. Kannaki and M. R. Reddy	177
P4	Visceral lymphoproliferative lesions associated with Marek's disease in layers <u>Amit Kumare</u> , Chandrashekhar S. Mote, Vitthal S. Dhaygude, M. M. Chawak, N. R., Anushka Todmal, Chandan H.U., Prajakta Dethe, Surabhi Bhandekar	178
P5	Pathology and molecular characterisation of re-emerging immunosuppressive viral pathogens in chickens Chaitanya S., <u>Sonkusale P. M.</u> , Sukirti Sharma., Kadam Vaishnavi C., Anushka Bhadauria., Hedau M. S., Kaore M. P and Kurkure N. V.	179
P6	Pathology and molecular characterization of Chicken Infectious Anemia virus in Nagpur region, India Chaitanya S., <u>Sonkusale P.M.</u> , Hedau M.S., Kaore M.P. and Kurkure N.V.	180
P7	Pathology of Chicken Infectious Anemia with concurrent infections in chickens of Chhattisgarh <u>P. M. Sonkusale</u> , Vivek Kumar, R. C. Ghosh	181
Theme 9: Biosecurity and Biorisk Management		
P1	Biosecurity measures for disease prevention in avian holding facilities Priyal V. Chauragade, <u>P. V. Chauragade</u> , S.M. Kolangath, M.R. Kate, G.R. Bhojne, R.K. Fulsunge	184
Theme 10: Young Scientist Masters		
Y1	Efficacy of green synthesized nano silver with entrapped cinnamaldehyde on chicken infectious anemia virus induced pathology in cockerels <u>Samiksha Suroshe</u> , Rohan Fulsundar, Megha Kaore, Madhuri Hedau, P.M. Sonkusale, D. B. Rawool and N.V. Kurkure	186
Y2	Assessment of embryonated chicken eggs as a model in unraveling Infectious Bronchitis virus pathogenesis and host immune response	187

	<u>Divya Yadagiri</u> , Asok Kumar M., Rahul Singh, Suraj Dhankar, Deva R., Megha Sharma, Umesh Kumar Singh	
Y3	Occurrence, pathology and molecular characterization of <i>Salmonella</i> spp in backyard and farmed chickens <u>Isha P. Akare</u> , R. S. Ingole, B. P. Kamdi, S. W. Hajare, R. P. Kolhe and Uma Tumlam	188
Y4	Pathological studies of induced subacute ochratoxicosis in Kadaknath chickens and amelioration with <i>Saccharomyces boulardii</i> <u>Ritesh Verma</u> , Supriya Shukla, G.P. Jatav, A.K. Jayraw, Rashmi Choudhary, Kursangmit Lepcha, Vijay Yadav	189
Theme 11: Young Scientist Doctoral		
Y1	Evaluating the effect of herbal choline fortified with milk thistle (<i>Silybum marianum</i>) on growth, liver function and gut health in broilers <u>Gaikwad S. S.</u> , Desai D. N., Ranade A. S., Kurkure N. V., Kaore M. P., Gole M. A., Sawane M. P., Zende R. J., Jagadale S.D.	192
Theme 12: Case Study		
P1	Case report of <i>Eimeria brunetti</i> in commercial layers <u>Shivaji Mehatre</u> , Swapnil Kubal, Milind Chawak and Sanjay Gavkare	194
P2	Field trial of phytogenic extracts of <i>Tribulus terrestris</i> (Gokhru) to reduce ascites and improve renal health in broilers Megha Kaore, C.S. Saraf, Jyoti Nandardhane, Anju Waghmare	195
P3	Handling of respiratory infections in broiler breeders Dr Avinash Dhawale	196

Theme 1

*Existing and Emerging
Diseases of Poultry*

INTEGRATED CONTROL OF H9N2 LOW PATHOGENIC AVIAN INFLUENZA

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Introduction

H9N2 low pathogenic avian influenza (LPAI) virus has emerged as one of the most economically significant zoonotic pathogen affecting poultry that can also cause sporadic human infections. It is a segmented, negative sense single strand RNA virus belonging to Type A influenza virus under Orthomyxoviridae family. It has become enzootic in poultry populations across large parts of Asia, the Middle East, and North Africa. The primary hosts include chickens, turkeys and quail with ducks, pigeons, pheasants serving as secondary hosts. The H9N2 virus infections often result in substantial production losses and despite its low pathogenicity, H9N2 poses significant challenges due to persistent circulation, economic losses, zoonotic spillover, and its role as a gene donor for highly pathogenic and zoonotic influenza viruses such as H5N1 and H7N9. It also represents a major public-health concern due to repeated zoonotic infections.

Integrated Control Strategy

The epidemiology of H9N2 is depends on multiple interacting factors, including intensive poultry production, live bird markets (LBMs), vaccination-driven antigenic drift, and human–animal interfaces. Conventional single-measure control strategies such as vaccination alone have proven insufficient to interrupt evolution and transmission of the H9N2 virus. An integrated approach that combine biosecurity, strategic vaccination, surveillance, market interventions, and One Health coordination offers the most realistic and effective approach for long-term management.

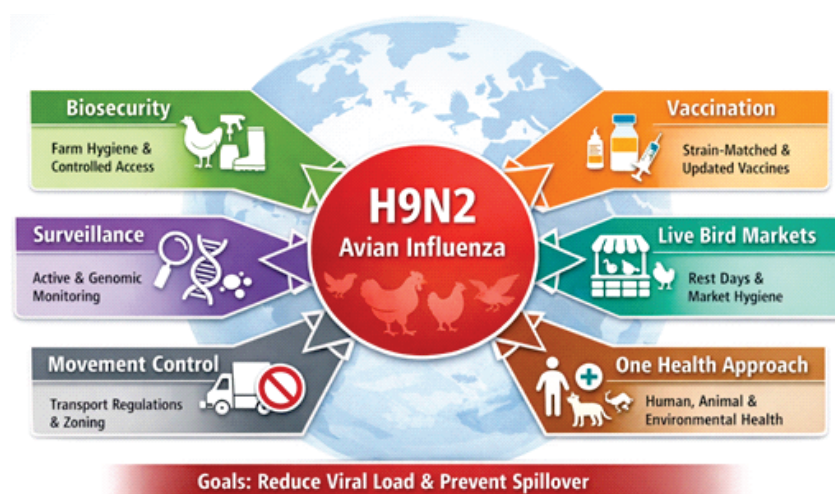


Fig. 1. Integrated control strategy for H9N2 avian influenza virus

The role of various components of integrated control strategy are as below:

Component	Role
Biosecurity	Core prevention
Vaccination	Disease impact reduction
Surveillance	Early detection & evolution tracking
LBMs control	Transmission interruption
Genomic monitoring	Pandemic risk mitigation
One Health coordination	Spillover prevention

A. Biosecurity:

Biosecurity is the most important and cost-effective control measure. H9N2 spreads efficiently through direct contact between birds, aerosols in high-density poultry housing, indirect transmission via fomites (equipment, vehicles, clothing etc.) and live bird markets and poultry movement. Farm-level practices such as all-in/all-out production, controlled entry, use of PPE, and separation of species significantly reduce virus introduction and spread. Even in vaccinated flocks, strong biosecurity is essential to prevent infection and amplification. Live bird markets play a critical role in maintaining and amplifying H9N2 viruses. The potential biosecurity interventions in live bird markets include market rest days (weekly/monthly), overnight depopulation, species segregation, regular cleaning and disinfection and progress towards centralized slaughter systems.

B. Vaccination:

Vaccination support, not replace, biosecurity for control of H9N2 avian influenza virus. An integrated vaccination strategy should include antigenic matching with circulating field strains, adequate antigen dose and booster schedules and periodic vaccine updates based on surveillance data. Inactivated H9N2 vaccines are widely used in endemic regions to reduce clinical disease and production losses. Increasing interest in recombinant virus vectored vaccines

Advantages) of LPAI H9N2 Vaccination

1. Stimulates Immune Response - Vaccinated birds often develop robust antibody responses reducing the severity of disease after exposure.
2. Reduces Clinical Signs and Virus Shedding - Under experimental conditions, inactivated vaccines have been shown to significantly reduce virus shedding and clinical sickness in vaccinated poultry compared to unvaccinated ones, decreasing overall viral load in a flock.
3. Helps Prevent Economic Losses - By reducing disease impact (e.g., respiratory symptoms, egg production drop), vaccination can lessen economic losses in the poultry industry due to LPAI H9N2.
4. Combined With Other Vaccines - Bivalent vaccines (e.g., H9N2 + Newcastle disease) can provide protection against multiple diseases with one administration, improving practicality and coverage.

Demerits (Limitations & Concerns) of LPAI H9N2 Vaccination

1. Does Not Always Prevent Transmission - Studies show that the commonly used inactivated H9N2 vaccine may not sufficiently block virus transmission within a flock, even when birds have high antibody levels.
2. Possible Immune Escape and Viral Evolution - Long-term use of the same vaccine strains may apply immune pressure on the virus, leading to antigenic drift or escape mutants limiting the effectiveness of the vaccine

3. Strain Specificity - Vaccines based on one regional or specific virus strain might be less effective against divergent or evolving H9N2 viruses circulating in the field unless updated.
4. Doesn't Guarantee Full Protection Alone - Vaccination might reduce severity but not eliminate risk completely. Biosecurity, surveillance, and farm management practices must still be maintained to reduce outbreaks.
5. DIVA (Differentiating Infected from Vaccinated Animals) Challenges - Traditional inactivated vaccines can make it hard to distinguish vaccinated birds from naturally infected ones using routine tests unless special marker (DIVA) vaccine strategies are used

C. Surveillance and Early Detection:

Surveillance is critical for early detection and control. Active surveillance involves routine sampling from farms and live bird markets, while passive surveillance relies on disease reporting. Molecular diagnostics like real time RT-PCR enable rapid identification and response to circulating H9N2 viruses. Genomic surveillance provides deeper insights into virus evolution. Whole-genome sequencing helps detect reassortment events, monitor antigenic drift, and identify markers of mammalian adaptation. This information is vital for vaccine strain selection/updating and assessing pandemic risk.

D. Management of Co-Infections

Under field conditions, H9N2 rarely occurs as a single infection. Co-infection is a key determinant of H9N2 disease expression and impact. The interaction between H9N2 and other pathogens amplifies clinical severity, prolongs viral shedding, compromises vaccination efficacy, and increases zoonotic risk. Co-infection with bacterial, viral, or immunosuppressive agents may lead to significant mortality ranging from 10-60% in poultry, increased viral shedding and duration of infection. In India, more than 10% mortality has been recorded in poultry farms having both H9N2 virus and *E. coli* infections. Co-infection with IBDV and Marek's disease virus might suppress humoral and cellular immunity and immune dysregulation resulting in reduced vaccine-induced immunity. Co-infected flocks act as long-term reservoirs resulting in environmental contamination and poultry workers in co-infected flocks face greater zoonotic risk. The control strategies targeting co-infection include integrated disease management combining vaccinations and antimicrobial treatment, biosecurity and improvement of farm management practices.

E. One Health and Public Health Integration:

H9N2 is not only an animal health issue but also a public health concern. H9N2 viruses possess molecular features associated with mammalian adaptation and have caused sporadic human infections, particularly among poultry workers and LBM vendors. The first reported human infection with avian influenza A (H9N2) occurred in 1998 in southern China. Since 2015, a total of about 99 laboratory-confirmed human H9N2 infections have been reported to the World Health Organization (WHO), including two deaths. Most of these cases have been reported from China, Cambodia and Vietnam. Most of the human cases have been traced to direct contact with infected poultry or contaminated environments. Clinical presentations in humans are generally mild respiratory illness, though severe clinical cases have also been reported. India has reported two human H9N2 infections reported to the WHO so far. The First Case was a 17-month-old boy from Maharashtra reported in 2019 and the second case was a child from West Bengal reported in May, 2024. Integrated control must therefore include occupational health measures (PPE, health monitoring), Seasonal influenza vaccination for poultry workers and intersectoral collaboration between veterinary, medical, and environmental authorities. The One Health approach ensures early detection and reduces pandemic risk.

F. Policy, Governance, and Capacity Building

Strong policy support is essential for integrated control. Sustainable integrated control requires strong veterinary infrastructure, mandatory reporting and standardized surveillance, quality control of vaccines, farmer education and incentive mechanisms and regional and international data sharing. Policy support is essential to translate scientific knowledge into effective field implementation.

Indian scenario:

The H9N2 virus was first reported in India in 2003 from Barwala region of Haryana and Punjab. Since then, it has been reported in many parts of the country. In August 2022, the Department of Animal Husbandry & Dairying (DAHD), Ministry of Fisheries, Animal Husbandry & Dairying, Government of India, issued a formal policy allowing vaccination against Low Pathogenic Avian Influenza (H9N2) in the country. The policy specifies the candidate vaccine strain A/chicken/India/22213/2006 (H9N2) as the reference strain for vaccination, which is a G1-lineage virus selected based on antigenic and phylogenetic relevance to field isolates. Based on this policy, commercial use of indigenous H9N2 vaccines developed by ICAR-National Institute of High Security Animal Diseases (NIHSAD), Bhopal has been approved. The vaccine technology has been transferred to four poultry vaccine manufacturers to strengthen supply and availability. To align with global recommendations for continuous monitoring of vaccine performance in endemic situations, a national Post-vaccination surveillance is mandated under the policy to assess serological response, vaccine matching with circulating field strains and potential antigenic drift.

Conclusion:

Although H9N2 is classified as low pathogenic, it has a high epidemiological and public health impact. H9N2 cannot be controlled by single interventions. Persistent circulation, economic losses, and zoonotic risk make integrated control essential. However, several challenges like vaccine mismatch, informal poultry sectors, limited diagnostic capacity, economic constraints and resistance to market restructuring remain. Future control efforts should focus on improved DIVA vaccines, expanded genomic surveillance, and restructuring of live bird markets. Strengthening One Health governance and regional cooperation will be key to sustainable control and pandemic prevention. A coordinated approach is essential to protect poultry health, livelihoods, and global health security.

CHALLENGES OF EMERGING CONCURRENT VIRAL INFECTIONS IN POULTRY: FOCUS ON NEWCASTLE DISEASE VIRUS AND AVIAN METAPNEUMOVIRUS IN INDIA

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Emerging concurrent viral infections in poultry are frequently recognized as a major threat to poultry production due to their ability to enhance disease severity, complicate diagnosis, and reduce the effectiveness of vaccination programs. NDV and aMPV frequently co-circulate within intensive poultry systems and synergistically exacerbate disease severity resulting in high morbidity, severe respiratory and nervous, increased mortality and marked production losses. Co-infection enhances viral replication, induces extensive tissue damage, and complicates clinical and pathological diagnosis due to overlapping symptoms. The immunosuppressive effects and immune evasive strategies triggered by these viruses elevating the risk of vaccine failures in the field. Gaps in biosecurity, presence of wild birds in poultry farms, uncontrolled bird movement, multi-age rearing, and limited monitoring and surveillance and molecular diagnostics contribute to disease persistence in India. Integrated management strategies such as optimized nutrition, stress free environment, strengthened biosecurity, continuous surveillance, improved diagnostics, optimized genotype matched vaccination programs are essential to mitigate the escalating economic and health impacts of concurrent viral infections in poultry. This paper highlights the impact of concurrent viral infections, contributing factors, pathogenesis and practical solutions for controlling NDV–aMPV co-infection in the Indian poultry sector.

Keywords: Newcastle disease virus, avian metapneumovirus, co-infection, respiratory disease, diagnostics, poultry vaccines, India

Introduction

Poultry production has been one of India's fastest-growing agribusiness sectors expanded rapidly, supporting food security, employment, and income generation. However, with the growing density and intensive poultry operations co-infection with multiple viruses such as Avian Influenza Virus (AIV), Newcastle Disease Virus (NDV), Infectious Bronchitis Virus (IBV), Infectious Bursal Disease Virus (IBDV), Chicken Infectious Anemia Virus (CIAV), Marek's Disease Virus (MDV) Fowl adenovirus infection (FAdV), Avian fowlpox virus (AFPV) Infectious Laryngotracheitis virus (ILT) and Avian Metapneumovirus Virus (aMPV) in different combinations have become quite frequent. NDV remains enzootic despite routine vaccination, while aMPV is increasingly detected across different types of poultry farms. Co-infection involving both viruses has resulted in more severe disease, high mortality and prolonged egg production losses leading to huge economic losses. Furthermore, complex diagnostic procedures are the current features of NDV and aMPV coinfection scenario. Therefore, understanding challenges specific to NDV–aMPV co-infection is crucial for poultry veterinarians and decision-makers in India.

Impact of concurrent viral infections

The concurrent viral infections are of serious concerns as they create a more severe, complex, and unpredictable disease scenario, higher mortality rates, and greater economic losses than a single viral infection. The specific impacts vary depending on the viruses involved and their interactions.

Common concurrent viral combinations reported

Viral combinations	Adverse effects / outcome
CAV and Marek's Disease Virus (MDV)	Multiple pathogens infecting a bird simultaneously often resulting in more intense pathological changes and worse clinical outcomes than either infection alone. In many cases, viruses interact synergistically, where the presence of one virus enhances the replication or pathogenicity of another. Immunosuppression causes more intense pathological changes, including severe organ enlargement and neoplastic lesions and high mortality. Outcome: Immunosuppression an exacerbated disease severity, secondary infections and greater economic losses
CAV and Fowl Adenovirus (FAdV)	This combination can lead to severe diseases like Inclusion Body Hepatitis (IBH) and Hydropericardium Syndrome (HPS, or Angara disease), often resulting in very high mortality. Outcome: Immunosuppression and exacerbated prolonged disease severity, secondary infections and greater economic losses.
IBDV and NDV or aMPV	IBDV has an immunosuppressive effect, leading to lower antibody titers against the concurrent viruses (NDV and aMPV), making control more challenging. Outcome: Immunosuppression and exacerbated prolonged disease severity, secondary infections and greater economic losses.
CAV and IBV.	Severe emaciation, poor growth and high mortality in young chicks. Outcome: Immunosuppression and exacerbated prolonged disease severity, secondary infections and greater economic losses.

FAdV and Avian Leukosis Virus (ALV)	Dual infections with different subgroups of ALV potential genetic recombination and emergence of new, more pathogenic strains. Outcome: Immunosuppression, exacerbated disease severity, prolonged mortality, reassort into novel strains and greater economic losses.
Fowl pox Virus (FWPV) and Infectious Laryngotracheitis Virus (ILT)	These respiratory viruses can co-infect, requiring reliable diagnostic tests to identify both agents simultaneously. Outcome: Exacerbated disease severity, secondary infections, high mortality, drop in egg production and greater economic losses.
IBV and AIV H9N2	Outcome: Exacerbated disease severity, prolonged viral shedding and enhanced pathogenicity due to potential viral interactions increased mortality, drop in egg production, secondary infections and greater economic losses and emergence of highly pathogenic strains.
NDV and AIV (H9N2/H5N1)	Outcome: High mortality rates and a significantly longer duration of disease compared to ND alone, drop in egg production often complicated by secondary bacterial infections and greater economic losses.
MDV, NDV and aMPV	Outcome: Exacerbated disease severity including higher mortality rates, longer duration of disease with usually nervous signs, drop in egg production, reassort into novel strains and greater economic losses.
AIV subtypes (H7 /H9/H5)	Antigenic drift and genetic reassortment result in novel H and N combinations with high transmissibility to affect wider range of hosts.

Contributing factors: Several factors facilitate occurrence of concurrent infections

- 1) High Stocking Density: Intensive farming conditions promote the rapid spread of infectious agents.
- 2) Overcrowding: Inadequate spacing causes stress leading to immune suppression and unexpected infections.
- 3) Immunosuppression: Immunosuppression caused by various immunosuppressive factors and agents results in unexpected poor protective immunity facilitates access to multiple infections

- 4) Inadequate immunity to current vaccines: Inadequate protection levels of antibodies promote concurrent infections
- 5) Viral Evolution: Antigenic drift and the emergence of new pathogenic, more virulent strains make control challenging.
- 6) Poor Biosecurity practices: Inadequate biosecurity measures and the movement of infected birds or contaminated equipment, movement of unauthorised personnel facilitate transmission.
- 7) Mixed Species Farming: Rearing different bird species (chickens ducks and turkeys) under one roof increases the opportunities for cross-species transmission and the emergence of novel strains/ concurrent infections.

Overview of NDV and aMPV

NDV causes a devastating and highly contagious disease known as Newcastle disease by generating digestive, neurological and respiratory symptoms. While aMPV is attributed to the diverse clinical presentations ranging from respiratory distress to reproductive issues such as loss of egg pigmentation and prolonged decreased egg production.

Table. 1 Classification and Characteristics of NDV and aMPV

Characteristics	NDV	aMPV
Order	Mononegavirales	Mononegavirales
Family	Paramyxoviridae	Pneumoviridae
Subfamily	Avulavirinae	Pneumovirinae
Genus	Orthoavulavirus	Metapneumovirus
Species	Newcastle Disease Virus (NDV)	Avian metapneumovirus (aMPV)
Genome Type	Single, negative-sense, non-segmented RNA	Single-stranded, negative-sense RNA.
Gene Order	Six genes 3'-NP-P-M-F-HN-L-5'	Eight genes encoding eight proteins 3'-leader-N-P-M-F-M2-SH-G-L-trailer-5'
Structure:	Enveloped virus	Enveloped virus
Encoded Proteins and Functions	N(Nucleoprotein): Encapsulates the viral RNA. P(Phosphoprotein): Essential for transcription and replication, stabilizing the L protein.	Nucleocapsid (N) Phosphoprotein (P), Matrix protein (M), Fusion protein (F), Second Matrix protein 2 (M2, producing M2-1 and M2-2),

	M (Matrix Protein): Involved in virus assembly. F (Fusion Protein): Mediates viral entry into host cells and is a key virulence factor. HN(Hemagglutinin-Neuraminidase): Binds to host cells and facilitates release. L (Polymerase): The large catalytic subunit for RNA synthesis (replication and transcription).	Small Hydrophobic protein (SH), surface glycoprotein (G), and RNA-dependent large (L) polymerase protein. P Gene Editing: The P gene undergoes RNA editing (insertion of non-templated G residues) to produce different mRNAs, including P, V, and W proteins. F Protein Cleavage: The F (fusion) protein has a cleavage site (RKKR↓F) that is characteristic but doesn't always fit the standard furin cleavage site, yet it's cleaved efficiently in cell culture.
Strains and Subtypes:	Classes: Class I (mostly avirulent) and Class II (most virulent/pathogenic strains). Genotypes: Class II is further broken down into many genotypes (e.g., GI, GII, GIII... up to GXV+) based on genetic analysis. Pathotypes: Based on disease severity, strains are grouped as lentogenic (low virulence), mesogenic (medium), and velogenic (highly virulent, further split into viscerotropic and neurotropic).	Six subtypes: aMPV-A, aMPV-B, aMPV-C, aMPV-D, GuMPV-B25, and PAR-05
Primary tissue tropism	Trachea, CNS, gut lymphoid tissue	Turbinates, trachea, oviduct.

Hosts and Transmission: NDV has been infecting wide range of domestic and wild birds worldwide. Birds of 241 species from 27 orders are reported to be susceptible to NDV. While aMPV has been detected in different orders of wild birds such as Anseriformes, Columbiformes, Falconiformes, and Psittaciformes as asymptomatic carriers of the virus. Such carrier state in wild birds as reservoirs and vectors contribute to the persistence and spread of the virus across different environments and poultry farms. Chickens of all ages and productive systems are susceptible to both NDV and aMPV infection.

NDV belongs to Paramyxoviridae and affects multiple organs including respiratory and nervous systems. Velogenic strains dominate Indian outbreaks. aMPV from Pneumoviridae primarily affects upper respiratory tissues and the reproductive tract.

Infected chickens and other domestic and wild birds may be sources of NDV. Infected birds shed virus in exhaled air, respiratory discharge, and feces. Virus may also be present in all parts of the carcass and in eggs laid during acute vNDV infections. NDV transmission occurs through the movement of infected birds, poultry products, movement of people and contaminated equipment or litter. While in case of aMPV wild birds as reservoirs contributing for the spread of the virus across different environments and poultry farms. AMPV affects mainly ciliated epithelial cells of the upper respiratory tract, transmission is primarily airborne by aerosolized respiratory secretions horizontally and can spread in a flock through contact with secretions, aerosols, contaminated feed, water, and bedding materials.

Pathogenesis: Variations in the hemagglutinin- neuraminidase (HN) and fusion (F) structural proteins of NDV lead to differences in the strains, affecting the virus's ability to infect and cause disease. The replication of NDV viruses vary and specific to type of pathotype. ND virus strain replicate in the lymphoid tissues such as the spleen, bursa of Fabricius, thymus and gut-associated lymphoid tissues resulting in splenic necrosis, sinusoidal thrombi in liver, conjunctivitis, lymphoid necrosis, and depletion, encephalitis, acute haemorrhagic lesions throughout the gastrointestinal tract and pancreatitis in chickens.

Lentogenic strains cause subclinical infections with mild respiratory or enteric diseases and are considered low- virulence strains. Mesogenic strains cause respiratory signs with lower mortality rate. Whereas velogenic strains cause severe neurological and respiratory signs with high mortality rates and specific histopathological changes. The vasogenic pathotype can be further classified into two subtypes: velogenic viscerotropic (VVNDV), which causes acute haemorrhagic lesions throughout the gastrointestinal tract, and velogenic neurotropic (VNNDV), which can lead to high mortality in chickens, with clinical signs ranging from respiratory distress and diarrhoea to neurological disorders and sudden and or high mortality.

Clinical signs and Lesions: Clinical signs of NDV in chickens include lethargy, inappetence, respiratory distress, clear mucus discharge from the mouth, watery greenish diarrhea, and show nervous signs including tremor, twisted necks (torticollis), and opisthotonus prostration, sudden death, up to 100% morbidity and mortality, decreased egg production. Poorly protected vaccinated birds may develop neurologic signs 10–14 days after infection and may recover. Clinical findings of infection with neurotropic velogenic NDV in chickens include respiratory signs may accompany neurologic signs such as tremors and convulsions paralysis of wings and legs, torticollis, circling with clonic spasms, complete paralysis, up to 100% morbidity and 50% mortality (up to 90% in young birds). Hemorrhages of the proventricular mucosa and intestinal serosa are accompanied by multifocal necrotic hemorrhagic areas in cecal tonsils and Peyer's patches of mucosal surface of the intestine. Oedema, haemorrhages and necrosis in spleen and thymus. Congestion and hemorrhage of the trachea and lungs. Egg yolk peritonitis with atrophied or haemorrhagic follicles in laying birds. Hyperaemia and mild multifocal petechial hemorrhages can be observed in the meninges of the encephalon in infection of chickens.

aMPV-A and aMPV-B subtypes are associated with mild to serious respiratory symptoms accompanied by neurological abnormalities in chickens. aMPV infected birds can develop. Swelling of the peri and infraorbital sinuses resulting in swollen head syndrome is common feature in chickens. Frothy eyes, nasal discharge, torticollis and opisthotonos due to ear infection are common. The mortality rate is rarely > 2%; it ranges between 0.4% and 50%. Egg production and quality are frequently affected in broiler breeders and commercial layers. Gross lesions include induced after experimental infection are due to rhinitis, tracheitis, sinusitis, and air sacculitis. Serous to turbid mucus can occur in the nasal cavity, nasal turbinates, trachea, and infraorbital sinuses. Egg peritonitis, ovary and oviduct regression, folded shell membranes in the oviduct, and misshapen eggs. Some infected birds lack gross lesions in cases of aMPV infection. Loss of cilia, increased glandular activity, congestion, and mild mononuclear infiltration of the submucosa are the most pronounced microscopic lesions are found in the upper respiratory tract including the secondary bronchi mucosa or nasal turbinates. Harderian and lacrimal glands can also show lymphocytic infiltration and formation of lymphoid follicle-like structures in the interstitial tissue and around the secondary collecting ducts.

Diagnosis of NDV and aMPV in Poultry

The clinical signs and gross lesions due to virulent strains of NDV infection are not pathognomonic and therefore laboratory confirmation is required for diagnosis. The laboratory tests involve virus isolation, detection of vNDV RNA and NDV-specific antibodies

Viral isolation: The virus can be isolated in 9 to 11-day-old SPF embryonated chicken eggs from oropharyngeal or cloacal swabs or tissues from infected birds by inoculation through the allantoic cavity. The isolated Virus is confirmed by HAHI test with NDV antiserum or by detection of NDV RNA by real-time RT-PCR. Real-time RT-PCR can be used for detection of viral RNA directly from clinical samples. Intracerebral pathogenicity index, or genomic sequencing and identification of a specific amino acid motif at the cleavage site of the fusion protein (F) precursor (F0) are used for identifying different pathotypes.

Serological assays: Hemagglutination-inhibition or Enzyme linked immunosorbent assay (ELISA) of paired serum samples indicates NDV infection. Neutralization test, Immunofluorescence assay, and Immune colloidal gold technique are also used for detecting NDV specific antibodies.

Molecular diagnosis: Reverse transcription polymerase chain Reaction (RT-PCR), Real-time qRT-PCR and Loop-mediated isothermal amplification (LAMP) techniques are being used. Nucleotide sequence analysis is performed in Reference laboratories to detect genetic differences for comparison of isolates from different outbreaks and to identify the source of those infections.

aMPV infection in chickens may not always show obvious respiratory symptoms and the infection goes unnoticed on the affected farm, essentially require proper diagnosis.

Virus isolation: Virus isolation from infected chickens is always difficult than turkeys. Tracheal and choanal swabs are preferred samples for virus isolation. Tracheal organ cultures of turkey or chicken embryos, or from 1- to 2-day-old chicks are the most sensitive for primary isolation. Six to eight day old embryonated chicken or turkey eggs also used for isolation through yolk sac route and identified by electron microscopy, virus neutralization test, or molecular techniques. Antigen detection tests, including immunofluorescence and immunoperoxidase assays on both fixed and unfixed tissues, have also been developed for aMPV.

Molecular diagnosis: Reverse transcription PCR (RT-PCR) assays and reverse transcription quantitative real-time PCR (RT-qPCR) tests are useful to detect the virus in respiratory swabs and samples on Fast Technology Analysis (FTA) cards for molecular diagnosis. Positive samples are to be

characterized by gene sequencing. The high genetic heterogeneity of the gene encoding the G protein makes it suitable for strain characterization and epidemiological investigation.

Serological testing: Serological diagnosis of aMPV infection with commercial ELISA kits is common as the virus isolation procedure has several constraints. Both acute and convalescent serum samples (paired serum samples from affected flocks) should be analysed. Protein N shows a high degree of identity across subtypes A, B, and D is suitable for serological assays to detect aMPV. While for subtype C the homologous antigen should be used for efficient detection of subtype C.

Controlling Strategies

An integrated approach focusing on strict biosecurity, comprehensive vaccination programs, and sound flock management practices is required for the effective control of concurrent viral infections in poultry.

A. Strict Biosecurity is paramount to preventing the introduction and spread of viruses. Measures include:

(i) "All-in, All-out" policy: Rearing single-age flocks and completely cleaning and disinfecting facilities between batches of birds.

(ii) Restricting movement: Controlling the movement of personnel, vehicles, and equipment between farms or sheds

(iii) Sanitation: Regular and thorough cleaning and disinfection of houses, feeders, waterers, and all equipment.

(iv) Pest control: Managing rodents, wild birds, and insects to minimize disease transmission vectors.

B. Vaccination and sero-monitoring: Vaccination is one of the most effective ways to increase a bird's resistance to disease and reduce the severity of infection and virus shedding.

(I) Follow a vaccination schedule appropriate for the specific local diseases and types of production.

(ii) Update the vaccine and vaccination schedule with the emergence of newer strains

(iii) Ensuring vaccines are stored and administered correctly to ensure efficacy.

(iv) Regular Sero monitoring of the flock's antibody levels to ensure the vaccination program is providing adequate protection.

C. Management & Supportive Care:

(i) Minimize stress: Providing a clean environment with proper ventilation, optimal temperature, and adequate space can reduce stress, which otherwise compromises the immune system.

(ii) Nutritional support: A balanced diet with essential vitamins, antioxidants, prebiotics and mucolytic enzyme and antimicrobial lysozyme can enhance the birds' immune response.

(iii) Plant extracts and essential oils that show antiviral capabilities and immunomodulatory effects to help birds fight against infections.

Conclusion

Accumulated serological data of aMPV infection in layer, broiler and breeder flocks across various states in the country suggests the establishment of infection. Concurrent NDV and aMPV infections represent a growing threat to the Indian poultry industry. The heightened disease severity, vaccine performance issues, and diagnostic complexity demand proactive, integrated control strategies. Improving molecular surveillance, appropriate vaccination, and strict compliance of biosecurity measures at the farm level will be crucial for the prevention and control of either individual or concurrent viral infections and their impacts.

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AVIAN RESPIRATORY DISEASE COMPLEX: INSIGHTS FOR INDIAN POULTRY PRODUCTION AND GLOBAL TRENDS

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Abstract

Respiratory Disease Complex (RDC) remains one of the most persistent and economically devastating health challenges in modern poultry production worldwide, causing annual global losses exceeding \$1 billion. Unlike single-pathogen infections, RDC is a multifactorial syndrome involving synergistic interactions among viral, bacterial, immunosuppressive, and environmental factors, leading to severe respiratory disease, poor performance, and increased mortality. In India, year-round circulation of Infectious Bronchitis Virus (IBV), Newcastle Disease Virus (NDV), Low Pathogenic Avian Influenza (H9N2), Mycoplasma species, and secondary Escherichia coli infections—exacerbated by climatic stress, high stocking density, and immunosuppression—poses a constant production constraint. Globally, emerging NDV genotypes, novel IBV variants, re-emerging Infectious Laryngotracheitis (ILT), expanding High Pathogenic Avian Influenza (HPAI; H5N1 & H5N8), Low Pathogenic Avian Influenza (LPAI; H9N2), and rising antimicrobial resistance complicate control efforts. Mitigation demands integrated strategies, including robust biosecurity, region-specific vaccination, early molecular diagnosis, immunosuppression control, and optimized farm management.

1. Introduction

Respiratory diseases rank among the leading causes of economic loss in the poultry industry, especially in intensive production regions like India, China, Southeast Asia, and the Middle East. Historically, outbreaks were treated as single-agent infections. However, field observations and advanced diagnostics reveal that most involve multiple interacting pathogens.

This multifactorial condition, known as Respiratory Disease Complex (RDC), features synergistic effects of viral, bacterial, mycoplasmal, immunosuppressive, and environmental factors that amplify disease severity beyond individual infections. With poultry intensification, shortened cycles, climate variability, and global trade, RDC threatens sustainable production at national and international scales. Annual economic impacts include reduced feed efficiency (5–15%) and mortality spikes (up to 20% in affected flocks).

2. Components of the Respiratory Disease Complex

2.1 Viral Pathogens

Viruses initiate respiratory damage and predispose birds to secondary infections. Key contributors include:

- Infectious Bronchitis Virus (IBV): Features multiple serotypes/variants (e.g., Mass, 4/91, QX, nephropathogenic, enteropathogenic), damaging tracheal epithelium, kidneys, and reproductive organs; often causes vaccine mismatch.
- Newcastle Disease Virus (NDV): Lentogenic, mesogenic, and velogenic strains persist in under-vaccinated flocks.
- Avian Influenza Virus (H9N2 – LPAI): Endemic in Asia, including India; synergizes with bacterial pathogens for severe outcomes.
- Infectious Laryngotracheitis Virus (ILT): Re-emerging, often via vaccine revertants.
- Avian Metapneumovirus (aMPV): Increasingly reported globally, with sporadic Indian detections.
- Avian Pox Virus (Wet Pox / Diphtheritic Form): Wet pox lesions in the larynx and trachea cause caseous plaques and obstruction, predisposing birds to secondary bacterial infections (E. coli, Mycoplasma). This significantly worsens Respiratory Disease Complex (RDC) and may be confused clinically with ILT or severe IBV.

2.2 Bacterial and Mycoplasmal Pathogens

These agents exacerbate severity and losses:

- Avian Pathogenic Escherichia coli (APEC): Common secondary invader causing airsacculitis, septicemia, and mortality.
- Mycoplasma gallisepticum (MG) and Mycoplasma synoviae (MS): Endemic in layers/breeders, leading to chronic respiratory issues and productivity drops.
- Ornithobacterium rhinotracheale (ORT): Emerging in select regions.

2.3 Immunosuppressive Factors

Immunosuppression amplifies RDC by impairing vaccine responses and immunity. Subclinical Infectious Bursal Disease (IBD) damages the bursa of Fabricius, reducing humoral immunity without overt signs. Chicken Infectious Anemia Virus (CAV) depletes T-lymphocytes and suppresses bone marrow in young birds. Fowl Adenovirus (FAdV) worsens dysfunction, especially in inclusion body hepatitis cases. Tropical mycotoxicosis from poor feed storage induces chronic suppression and vaccine failures. Marek's Disease further dysregulates immunity, enabling persistent respiratory infections. These "silent drivers" transform mild cases into severe outbreaks.

2.4 Environmental and Management Stressors

Stressors predispose birds to RDC: Ammonia levels >25 ppm erode epithelium and impair clearance. Dust and poor ventilation elevate pathogen loads and irritation. Heat/cold stress suppresses immunity; poor litter boosts ammonia and microbes. High density and multi-age farming heighten transmission.

3. Global Scenario

RDC has evolved into a transboundary issue, driven by intensification, trade, climate change, and viral evolution. Pathogen spectra vary by region: In North America, Europe, and Australia, IBV variants and ILT (via revertant vaccines) dominate despite strong biosecurity. In Asia, the Middle East, and North Africa, endemic H9N2 synergizes with Mycoplasma/bacteria, fueled by live markets and backyard systems. aMPV rises in Europe, South America, and the Middle East, causing swollen head syndrome.

Global challenges include antimicrobial resistance in *E. coli* from overuse, climate-altered seasonality, and trade-facilitated spread. Coordinated surveillance and harmonized strategies are essential.

4. Indian Scenario

In India, RDC occurs year-round, peaking in winter (December–February; cold stress, poor ventilation) and monsoon (July–September; humidity, wet litter). High-density areas (Maharashtra, Telangana, Andhra Pradesh, Tamil Nadu, Haryana, West Bengal) are hotspots. Key features: Co-circulating IBV variants (Mass, Variant-2, QX-like); H9N2 outbreaks with *E. coli* mortality; endemic MG/MS in layers/breeders; rising ILT; NDV in under-immunized flocks. Challenges include lax biosecurity, high ammonia/dust, antibiotic overuse, and subclinical immunosuppression eroding vaccine efficacy.

5. Key Pathogen Genotypes in RDC

Table 1: Worldwide and Indian Genotypes/Serotypes of Major RDC Pathogens

Pathogen	Worldwide Genotype / Serotype	Genotypes Reported in India	Predominant in India
NDV	Class II Genotypes I–XXII	XIII, VII, II	XIII.2.2
LPAI (H9N2)	Lineages G1, G9, Korean	Lineage G1	Lineage G1 (Western)
HPAI	H5Nx (Clade 2.3.4.4 a–h)	H5N1 (2.3.2.1a), H5N8/H5N1 (2.3.4.4b)	Clade 2.3.2.1a (H5N1)
IBV	Genotypes GI–GVI; Lineages 1–32	GI–Lineage 1, 13, 19, 24	GI–Lineage 24 (Nephropathogenic IBV)
ILT	CEO-like, TCO-like, recombinant field strains	Vaccine-derived (CEO, TCO-like)	Vaccine-derived (CEO/TCO)
Fowl Pox Virus (FPV)	Avipoxvirus Clades A–C (host-associated)	Clade A (Fowlpox-like); REV-inserted strains	Clade A (Fowlpox virus)
Avian Metapneumovirus (aMPV)	Subtypes A, B, C, D	Limited data; subtype B– like suspected	Not well established / sporadic

6. Diagnostic Approach for Respiratory Disease Complex (RDC)

Table 2: Stepwise Diagnostic Protocol for RDC

Diagnostic Step	Test/Examination	Purpose/Key Findings
Gross Pathology	Necropsy	Airsacculitis, tracheal hemorrhage, kidney swelling, lung consolidation
Molecular Screening	qRT-PCR (quantitative reverse transcription PCR; for IBV, NDV, AI, H9N2, ILT, aMPV)	Rapid viral detection

Virus Confirmation	Virus isolation (embryonated eggs/cell culture)	Viable virus confirmation; strain typing
Mycoplasma Detection	MG/MS PCR	Identifies MG/MS involvement
Secondary Bacterial Assessment	Bacterial culture & sensitivity	Guides targeted antimicrobial therapy
Immune Status Evaluation	Serology (ELISA, HI)	Assesses vaccine response/exposure
Tissue-Level Confirmation	Histopathology	Distinguishes viral, bacterial, toxic, immunosuppressive lesions

7. Prevention and Control

RDC control integrates biosecurity, vaccination, and management. Implement ventilation/ammonia control (<20 ppm), all-in/all-out systems, hygiene, and downtime. Vaccinate against IBV (Mass + 4/91 or Variant-2), NDV (LaSota + R2B), H9N2 (region-specific, if permitted), MG/MS, and ILT in high-risk areas; bolster with IBD/CAV programs. Optimize brooding, density, nutrition, mycotoxin binders, and stress reduction for immune health. Prioritize antimicrobial stewardship to combat resistance.

8. Future Directions

Controlling Respiratory Disease Complex (RDC) requires integrated, forward-looking strategies. Enhanced molecular surveillance and next-generation sequencing can track emerging viral variants and vaccine escape. Climate-resilient housing, improved ventilation, and litter management will reduce environmental triggers, while global data-sharing and immunosuppression monitoring enable proactive outbreak prevention. Together, these approaches support evidence-based, sustainable control of RDC in poultry.

9. Conclusion

RDC is a dynamic, multi-agent challenge shaped by production shifts, climate, and mutations. Indian and global industries must adopt holistic strategies beyond single-pathogen focus. Strengthened biosecurity, tailored vaccination, immunosuppression management, early diagnosis, and best practices can mitigate RDC, enhancing poultry health and profitability.

CONTROL OF INFECTIOUS BRONCHITIS IN POULTRY WITH REFERENCE TO QX-LIKE SEROTYPES

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Infectious bronchitis virus (IBV) QX Serotype (GI-19) is currently the fastest-spreading IBV variant worldwide, posing a major threat to poultry production. This genotype exhibits broad tissue tropism, affecting the respiratory tract, kidneys, and reproductive organs, and is strongly associated with nephritis, false layer syndrome, cystic oviducts, egg drop, and poor egg quality. Infection during early life or before sexual maturity results in permanent reproductive damage, leading to significant and irreversible economic losses. The rapid global spread of IB QX is driven by viral genetic variability, recombination in the S1 gene, prolonged shedding, multi-age farming systems, and limited cross-protection from conventional vaccines such as Mass and 793B. Additionally, IB QX infection predisposes flocks to secondary infections, increasing mortality and treatment costs. Diagnostic challenges and ELISA interpretation further complicate field control. Evidence indicates that homologous, genotype-matched vaccination provides superior protection of the upper respiratory tract and oviduct, as reflected by improved ciliostasis scores and production performance. VAKSIMUNE® IB QX, a live attenuated homologous vaccine with high genetic homology to circulating field strains, demonstrates enhanced efficacy in controlling IB QX and QX-like viruses. Strategic vaccination, combined with biosecurity and flock management, is essential to minimize production losses, sustain egg output, and improve farmer profitability.

TRANSMISSION OF HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1 (CLADE 2.3.4.4B) VIRUS BETWEEN DUCKS AND CHICKENS

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The cross-species transmission potential of the recently emerged clade 2.3.4.4b H5N1 highly pathogenic avian influenza virus (HPAIV) is not fully understood. A H5N1 virus (A/duck/India/11CA04/2022) belonging to clade 2.3.4.4b isolated from ducks during 2022 in Kerala was used to study the interspecies transmission between ducks and chickens. A group of 4–5-week-old eight domestic ducks were inoculated intranasally with 6log₁₀ EID₅₀ H5N1 virus. Oral and cloacal swabs were collected every day and tested for the presence of viral RNA through RT- qPCR. Upon the first detection of virus in swabs, a naïve group of eight SPF chickens (CR1-first contact) were introduced. The CR1 chickens were swabbed and tested for viral RNA presence. Upon detection of shedding of virus in CR1 chickens, the ducks were removed, and a group of naïve eight SPF chickens (CR2-second contact) were introduced. Similarly, eight naïve SPF chickens (CR3-third contact) were cohoused with confirmed shedding CR2 group after removing CR1. The birds were observed every 24 hours for any clinical signs. The sera samples of all the surviving chickens were collected at the end of the experiment (14th day of contact) to assess seroconversion. The results revealed that the virus transmitted efficiently from ducks to chicken, but not as efficiently between chickens. Shedding of the virus was confirmed in all the ducks and chickens in the CR1 group in oropharyngeal swabs on 1 dpc and 2 days post contact respectively. Only one chicken in the CR2 group shed virus on third day post contact. There was no shedding of virus in the CR3 group. The shedding of the virus was highest in the ducks group, followed by chickens of the CR1 group and the CR2 group. Three chickens in the CR1 group died on the third day post contact, and the remaining five birds died 4th day post contact. There was only one mortality in the CR2 group at fourth day post contact. The clinical signs in chickens were dullness, cyanotic comb and shank and respiratory distress. None of the survived chickens seroconverted against H5N1 virus. This study provides an important insight regarding transmission potential of clade 2.3.4.4b H5N1 virus from ducks to chickens and chickens to chickens.

MOLECULAR DETECTION AND COMPARATIVE PREVALENCE OF *MYCOPLASMA GALLISEPTICUM* AND *MYCOPLASMA SYNOVIAE* IN COMMERCIAL POULTRY FLOCKS OF INDIA DURING 2024–2025

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The present study was undertaken to determine and compare the prevalence of *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) infections in commercial poultry flocks across different regions of India during 2024 and 2025 using molecular diagnostic tools. Field samples comprising tracheal and choanal swabs were collected from commercial chicken flocks across five zones—East (EZ), North (NZ), South (SZ), and West (WZ). DNA was extracted and tested using species-specific PCR assays for MG and MS detection. The prevalence rates were calculated and compared between the two years. A total of 6,329 samples (2024) and 5,873 samples (2025) were analyzed. In 2024, MG and MS positivity was 9.06% and 4.31%, respectively. In 2025, MG and MS positivity were 9.31% and 5.63%, showing a marginal increase in both infections. Zone-wise comparison revealed the highest MG prevalence in SZ (13.24% in 2024; 11.99% in 2025) and the highest MS positivity in SZ (1.16% in 2024; 6.73% in 2025), indicating regional and temporal variations. The persistence and slight rise in PCR positivity highlight ongoing field-level infection pressure despite existing vaccination and biosecurity measures. The study emphasizes the need for continuous molecular monitoring and improved vaccine strategies aimed to minimize shedding and vertical transmission with stringent biosecurity and single age group rearing systems should be followed to achieve sustainable mycoplasma control in commercial poultry.

INVESTIGATIONS ON *LIPEURUS CAPONIS* (WING LOUSE) INFESTATION IN CHICKENS OF WEST-COAST INDIA

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In the present study, we recorded outbreak of wing louse infestation in ornamental and improved indigenous chicken varieties of an organized poultry farm located at North Goa during the winter season. The wing louse was found to be attached to underside of the wings between the feather barbs on the primary or secondary wing feathers. White clumps of louse's eggs (nits) were found attached at the base of feathers in breast and thigh region in addition to active louse populations. The affected birds showed signs of feather loss, irritation, annoyance, restlessness, injure themselves by scratching and feathers plucking, anaemia, decrease egg production, irritation and itching. Morphologically lice were dark brown coloured, elongated, had dorsoventrally flattened body, wingless, about 2mm in length and body structure resembled to *Lipeurus caponis* when observed under stereo microscope. For molecular characterization, the total genomic DNA extracted from whole lice was used to amplify the mitochondrial COI (Cytochrome Oxidase Subunit I) gene using universal primers. The PCR product obtained was sequenced, and the gene sequence was submitted to GenBank. Based on the phylogenetic analysis of the COI gene, of the present study's sequences were clustered with *L. caponis* hosted by Indian peacocks of China and Jungle fowl of the UK.

ISOLATION, CHARACTERIZATION, ANTIBIOGRAM AND PATHOLOGICAL STUDIES OF AVIAN PATHOGENIC *ESCHERICHIA* *COLI* FROM RESPIRATORY AFFECTIONS IN BROILER CHICKEN FLOCKS

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Avian Pathogenic *Escherichia coli* (APEC) is one of the most important etiological agents causing severe respiratory affections in the broiler chickens. The present work was conducted with objectives of isolation, characterization, antibiogram and pathomorphological studies of APEC in broiler chickens suspected for respiratory affections. The representative samples of trachea, lungs, air sacs and/or heart were collected from 29 different chicken flocks suspected for respiratory affections presented to Departments of Veterinary Pathology, Veterinary Public Health and Epidemiology of Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar and Government Veterinary Hospital, Jind for disease diagnosis were included in this study. All the carcasses were subjected for the detailed necropsy. The bacterial isolates were identified as *Escherichia coli* (*E. coli*) based on specific colony characteristics followed by Gram staining and biochemical analysis. A total of 33 *E. coli* isolates were found to be APEC by pentaplex PCR assay targeted for detection of virulence genes (*iroN*, *ompT*, *hlyF*, *iss*, and *iutA*). In-vitro antibiotic sensitivity test performed on these isolates against 19 different types of antibiotics belonging to nine different classes. These isolates exhibited highest resistance towards moxifloxacin (93.94%), followed by tetracycline (87.88%), ciprofloxacin (78.79%), and levofloxacin (75.76%). On gross examination, vascular changes and presence of catarrhal exudate in trachea, varying degree of pneumonic changes in lungs, air sacculitis with cloudy appearance of air sacs and fibrinous pericarditis were observed. Histopathologically, mild to moderate tracheitis characterized by deciliation and mucosal thickening, pneumonic changes, air sacculitis and fibrinous pericarditis with infiltration of mixed type of inflammatory cells viz. heterophils, lymphocytes, macrophages and plasma cells were observed. In conclusion, pentaplex PCR was found to be very useful for characterizing the *E. coli* isolates as APEC. An alarming antibiotic resistance towards third or fourth generation antibiotics warrant judicious use of antibiotics to prevent antimicrobial resistance, public health hazard and thus fulfilling the 'One Health Concept' in its true sense.

PATHOLOGICAL AND MOLECULAR STUDIES ON CERTAIN VIRAL RESPIRATORY INFECTIONS IN COMMERCIAL BROILER CHICKENS

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Respiratory diseases continue to pose a major challenge to the poultry industry, leading to substantial mortality and severe economic losses. Despite strict vaccination and control measures, the new viral strains and co-infections complicate the disease management. In the present study, we screened 100 flocks with respiratory lesions for the presence of low-pathogenic avian influenza virus (LPAIV), infectious bronchitis virus (IBV), Newcastle disease virus (NDV), avian metapneumovirus (aMPV), and fowl adenovirus (FAdV). A total of 31 flocks, which represents 31% of the total samples screened (including trachea, lungs, and spleen), tested positive for one or more of these viral pathogens. Detection was performed using PCR, RT-PCR, and Real-Time RT-PCR techniques. Out of 100 flocks, 3 flocks (3%) were found positive for LPAIV, 9 flocks (9%) for NDV, 5 flocks (5%) for IBV using Real time RT-PCR, and 10 flocks (10%) were found positive for FAdV using PCR. Notably, none of the samples of 100 flocks were tested positive for aMPV. Total 27 % samples exhibited mono-infection whereas only 4 % had co-infection of the tested viral pathogens. The concurrent presence of IBV and LPAIV was identified in 2 % of the samples while NDV and IBV co-infection was found in 1 % of flocks. Similarly, the co-occurrence of the IBV and FAdV was detected in 1 % of the screened flocks. The phylogenetic analysis was carried for the positive samples for LPAI, NDV and FAdV. Phylogenetic analysis indicated that LPAI positive F35 is the evolutionary precursor of LPAI positive F36 from present study, and Indian isolates formed a closely related cluster while isolates from other countries diverged significantly. Phylogenetic analysis placed F34 (NDV positive) in the same subclade as a recent 2024 Jammu isolate (PO510125.1), indicating recent circulation and shared evolutionary lineage, whereas, FAdV positive flock (L1 F Shirwal 22) exhibited over 99.88% similarity with Indian isolates from Jammu, Pantnagar, and Nagpur, as well as a Pakistan strain, suggesting minimal genetic variation.

ASSOCIATION OF AVIAN METAPNEUMOVIRUS AND INFECTIOUS BRONCHITIS VIRUS IN COMMERCIAL BROILER CHICKENS WITH RESPIRATORY SIGNS

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Respiratory disease complexes in commercial broiler chickens are frequently multifactorial, leading to increased disease severity and significant economic losses. Avian metapneumovirus (aMPV) and infectious bronchitis virus (IBV) are important viral agents associated with respiratory disease and head swelling in poultry. The objective of this study was to evaluate the association between aMPV exposure and IBV infection in commercial broiler flocks exhibiting respiratory complaints. Six commercial broiler farms with comparable clinical signs, including respiratory distress, facial edema, and head swelling, were investigated. All flocks were vaccinated at day one with a live IBV vaccine, while no aMPV vaccination was practiced. In three farms, molecular testing of organ samples and choanal swabs confirmed IBV infection, whereas aMPV and Newcastle disease virus were not detected. Serological testing for both aMPV and IBV was performed in all six farms using commercial ELISA kits. Serological analysis revealed consistently moderate to high IBV antibody levels in all farms, with geometric mean titers (GMTs) ranging from 212 to 1571 and maximum titers reaching up to 7274, consistent with vaccination and possible field virus exposure. In contrast, one farm was seronegative for aMPV, with no detectable antibodies. The remaining five farms showed low to moderate aMPV antibody levels, with GMTs ranging from 55 to 174 and partial flock seropositivity. Without aMPV vaccination, the detection of aMPV antibodies indicates natural field exposure. Despite negative molecular detection of aMPV, serological evidence of exposure in clinically affected flocks suggests prior or concurrent aMPV infection that may have been missed due to the transient nature of viral shedding. The concurrence of high IBV antibody titers and aMPV seropositivity supports the hypothesis that aMPV may act as a contributing or predisposing factor, exacerbating IBV-associated respiratory disease. In conclusion, this study demonstrates respiratory disease complexes in commercial broiler and highlights the limitations of relying solely on molecular diagnostics. Integrated diagnostic approaches incorporating serology are essential for accurate assessment of pathogen interactions and for improving respiratory disease control strategies in commercial broiler production.

PATHOLOGY OF SELECTIVE RESPIRATORY BACTERIAL INFECTIONS IN BIRDS

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Respiratory bacterial diseases are a significant cause of mortality and economic loss in poultry production systems. This study, conducted at the Department of Veterinary Pathology, aimed to investigate the prevalence and pathological characteristics of respiratory bacterial infections in poultry birds over a one-year period, from April 1, 2022, to March 31, 2023. A total of 167 poultry carcasses from 49 different farms were examined, consisting of 78 broilers (46.7%), 71 indigenous birds (42.5%), and 18 layer birds (10.8%). Necropsy examinations were performed based on prominent respiratory lesions, followed by pathological and laboratory evaluations. Tracheal, nasal, and lung swabs, along with affected tissue samples, were screened for both individual and mixed bacterial infections. Of the 56 suspected samples, 19 (33.9%) were confirmed with single bacterial infections. Infectious coryza was the most prevalent disease (21.4%), followed by *Ornithobacterium rhinotracheale* (7.1%), *Mycoplasma synoviae* (3.6%), and *Mycoplasma gallisepticum* (1.8%). Mixed infections were found in 7 samples (12.5%), with *Mycoplasma synoviae* in combination with *Ornithobacterium rhinotracheale* being the most common (5.4%). Age-based analysis revealed increased susceptibility in broilers up to one month of age, indigenous birds beyond two months, and layer birds after 17 weeks of age. Moreover, *Escherichia coli* was detected in 26 samples (31.7%), indicating colibacillosis as the most widespread respiratory bacterial infection. Gross and histopathological examinations revealed lesions such as tracheitis, air sacculitis, fibrinous pneumonia, pericarditis, and perihepatitis, which varied depending on the etiological agent. The study underscores the importance of pathological diagnosis in understanding respiratory bacterial infections and stresses the need for enhanced disease surveillance, biosecurity protocols, and timely therapeutic interventions to mitigate outbreaks in poultry populations.

GROSS AND HISTOPATHOLOGICAL FINDINGS OF HISTOMONIASIS IN A DESHI CHICKEN

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Histomoniasis, commonly known as blackhead disease, is a protozoan infection caused by *Histomonas meleagridis*, primarily affecting gallinaceous birds and resulting in significant morbidity and mortality. Although the disease is well documented in turkeys, reports in indigenous (Deshi) chickens are comparatively limited. The present study describes the pathological findings of histomoniasis in a Deshi chicken presented for the necropsy to the Department of Veterinary Pathology, KNPCVS, Shirwal. A thorough necropsy was performed and the gross lesions were noted. The liver showed the pale, circular, depressed, focal necrotic spots (target like lesions). The ceca showed the severe thickening of the mucosal walls and filled with thick, cheesy exudate in the lumen. The representative tissue samples were taken in 10% NBF for histopathology. The histopathological examination of liver revealed the presence of focal areas of necrosis in the parenchyma. Congestion and haemorrhage in the periportal areas. Severe infiltration of MNCs and few heterophiles in the sinusoidal areas. The trophozoites of the *Histomonas* spp. observed in the necrotic areas. Histopathological examination of the cecum demonstrated severe necrotizing typhlitis with extensive mucosal destruction, infiltration of inflammatory cells. There was the presence of the trophozoites of *Histomonas* spp. in necrotic areas of caecum. Genomic DNA extracted from liver and cecal tissues was subjected to polymerase chain reaction (PCR), which confirmed the presence of *Histomonas meleagridis*. Based on clinical history, gross and microscopic lesions, the case was diagnosed as histomoniasis. This report highlights the pathological features of histomoniasis in Deshi chickens and emphasizes the need for early diagnosis and effective biosecurity measures to prevent economic losses in backyard and free-range poultry production systems.

CASE REPORT ON OCCURENCE OF CAECAL COCCIDIOSIS IN INDIGENOUS SONALIKA BREED OF POULTRY

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Cecal coccidiosis is a highly pathogenic and economically important protozoan disease of poultry, predominantly affecting young chicks and leading to significant mortality under intensive rearing conditions. Incidence in indigenous breed of chicken like kadaknath, Aseel, Chittagong, etc. The present study documents the clinico-pathological and histopathological findings of a confirmed field case of caecal coccidiosis in chickens. Clinically, affected chicks exhibited depression, anorexia, ruffled feathers, progressive weakness, and prostration, followed by death. Enteric manifestations were prominent, characterized by yellowish-green to blood-stained diarrhea, while respiratory distress with gasping was observed in a subset of birds, likely secondary to systemic compromise. A systematic postmortem examination was performed on a dead and moribund bird. Gross pathological examination revealed lesions predominantly localized to the ceca, which were markedly distended, thickened, and dark red to hemorrhagic. The cecal lumen was filled with large blood clots, necrotic debris, and caseous cores firmly adherent to the mucosa. Mild congestion of the liver and spleen was also noted. Histopathological evaluation of formalin-fixed cecal tissues revealed haemorrhagic caecal enteritis characterized by epithelial sloughing, coagulative necrosis, hemorrhage, edema, and dense infiltration of mononuclear inflammatory cells. Numerous developmental stages of *Eimeria tenella*, including schizonts, merozoites, gamonts, unsporulated oocysts, were demonstrated within epithelial cells and deeper layers of the cecal mucosa. Parasitological examination of mucosal scrapings and fecal samples revealed both sporulated and unsporulated oocysts of *E. tenella*, confirming the diagnosis. The study underscores the severe pathological impact of cecal coccidiosis and highlights the importance of integrated control strategies encompassing hatchery hygiene, farm-level biosecurity, litter management, nutritional modulation, and judicious use of anticoccidial drugs and vaccination to mitigate disease burden in poultry production systems.

PREVALENCE AND MOLECULAR CHARACTERIZATION OF AVIAN PATHOGENIC *ESCHERICHIA COLI* IN KADAKNATH CHICKENS FROM MHOW REGION

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Colibacillosis caused by avian pathogenic *Escherichia coli* (APEC) is a significant poultry disease worldwide, causing substantial economic losses. Kadaknath, an indigenous Indian chicken breed valued for its meat quality and medicinal properties, however lacks adequate information on APEC prevalence and molecular characteristics. This study aimed to determine the prevalence of colibacillosis in Kadaknath birds from the Mhow region and to identify virulence-associated genes in the prevalent serotypes. A total of 150 Kadaknath carcasses irrespective of age and sex were necropsied from farms located in and around Mhow. Samples from organs showing gross lesions were collected and enriched in brain heart infusion broth. Isolation was performed on MacConkey and EMB agar. Suspected *E. coli* isolates were confirmed through Gram staining and biochemical tests. Serotyping was conducted to identify prevalent APEC serogroups. Molecular detection of five virulence genes (*iss*, *tsh*, *papC*, *sitA*, and *iucC*) was performed using PCR in predominant serotypes. Necropsy revealed swollen liver (22.66%) as the most frequent lesion, followed by intestinal affection (20%), splenomegaly (14.66%) and congested lungs (13.33%). Thirty-seven *E. coli* isolates were confirmed, establishing a prevalence of 24.66%. Serotyping identified 15 different serogroups, with O8 being most prevalent (21.4%), followed by O78 (14.28%), O84 (10.71%), O7 (10.71%), and O115 (7.1%). All five virulence genes were detected in serotypes O8, O78, O7, and O84, with amplicon sizes of 762, 642, 541, 205 and 608 bp for *iss*, *tsh*, *iucC*, *papC*, and *sitA*, respectively. The study revealed a significant prevalence of colibacillosis (24.66%) in Kadaknath chickens. The presence of multiple virulence genes in predominant serotypes O8, O78, O7 and O84 indicates their high pathogenic potential, necessitating targeted control measures for effective disease management in this valuable indigenous breed.

PATHOLOGY AND MOLECULAR CONFIRMATION OF AVIAN METAPNEUMOVIRUS AND ASSOCIATED CO-INFECTIONS IN CHICKENS

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Avian metapneumovirus (aMPV) is a respiratory pathogen in chickens that is responsible for the economic losses due to impaired performance, reproductive failure and increased susceptibility to secondary infections. Despite its global distribution, aMPV remains underdiagnosed and underreported in several countries, including India, where very few seroprevalence studies are available. Although, Maharashtra is one of the leading states in India in eggs and chicken meat production, there are no reports of the disease occurrence from state. This study aimed to investigate natural respiratory disease outbreaks in chickens, focusing on the molecular detection of aMPV, allied co-infections and their associated pathological manifestations in the affected chickens. A total of 42 poultry flocks exhibiting respiratory symptoms were examined in this study. Reverse transcription-PCR (RT-PCR) targeting the aMPV nucleoprotein gene revealed 19.04% flock-level positivity. Co-infection with other respiratory pathogens, such as *Mycoplasma gallisepticum*, *Ornithobacterium rhinotracheale*, *Avibacterium paragallinarum*, *Escherichia coli*, Infectious bronchitis virus, and Infectious laryngotracheitis virus were frequently detected, highlighting the complex etiology of respiratory disease in poultry. Gross and histopathological lesions ranged from tracheitis and air sacculitis to necrotizing pneumonia and pericarditis, with markedly exacerbated severity in co-infected birds compared to single-infected birds. These findings underscore the need for improved molecular surveillance, accurate diagnosis, and integrated disease control strategies targeting both viral and bacterial respiratory pathogens.

PREVALENCE AND CHARACTERIZATION OF AVIAN PATHOGENIC *ESCHERICHIA COLI* (APEC) AND ITS ISOLATION FROM COMMERCIAL BROILERS AND BREEDER FLOCKS FOR VERTICAL TRANSMISSION

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The study aimed to determine the prevalence and serogroup characterization of Avian Pathogenic *Escherichia coli* (APEC) isolated from commercial broilers and breeder flocks, with special focus on identifying vertical transmission of pathogenic strains/serotypes. A cross-sectional field study was conducted between 2024–2025 across 43 farms in both breeder and broiler flocks showing colibacillosis-like lesions to assess the distribution of *E. coli* O-serogroups and their possible transfer from breeders to progeny. A total of 154 samples (liver, pericardium, air sacs, yolk sac, and ovary) were collected from affected birds. Bacterial isolation was performed using MacConkey and EMB agar, and colonies showing lactose fermentation and metallic sheen were subjected to Gram staining and IMViC biochemical tests. A subset of 56 isolates was serotyped at the National Salmonella and *E. coli* Center (CRI, Kasauli) to determine O-serogroups. Out of 154 samples, 112 (72.7%) were confirmed as *E. coli*. Thirteen O-serogroups were identified, with O88 (48.1%) being the most prevalent, followed by O7, O34, and O52 (5.5% each) and O78 (3.7%), O11 (3.7%), O111 (3.7%).

Notably, O88 was detected in both breeder and day-old chick isolates from the same flocks in Tamil Nadu, providing strong evidence of vertical transmission. Typical postmortem findings included fibrinous pericarditis/perihepatitis, air sacculitis, and black proventriculus in early chick mortality cases. The predominance of *E. coli* O88 serotype in both breeders and their progeny suggests a vertical route of transmission in poultry flocks challenging the historical dominance of O78/O2. This finding highlights the need for serotype-specific monitoring and vaccine development, as current *E. coli* vaccines confer mainly homologous protection.

PATHOLOGICAL AND MOLECULAR INVESTIGATIONS OF AN OUTBREAK OF INFECTIOUS LARYNGOTRACHEITIS IN A BROILER POULTRY FLOCK

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An outbreak of infectious laryngotracheitis (ILT) was recorded in a broiler poultry flock of birds aged 31 to 36 days, located in Jalkot Tahsil, Latur District of Maharashtra State, India, during August 2025. A mortality of 200 birds (4%) was recorded within a span of 6 days (from the 31st to the 36th day of age) in a flock of 5,000 birds. The affected birds exhibited clinical signs including severe dyspnea, coughing, rales, gasping, expectoration of bloody mucus, nasal and ocular discharge (conjunctival swelling) and mortality. Post-mortem examination of the affected broiler birds revealed lesions primarily localized in the upper respiratory tract, consisting of the presence of frank blood and blood clots throughout the tracheal lumen, as well as, in a few cases, the presence of cheesy yellow plugs at the laryngeal opening and upper third of the trachea, often leading to complete occlusion of the airway. The tracheal tissues from ILT affected birds showed variable type of histopathological lesions comprising catarrhal tracheitis, necro-haemorrhagic tracheitis and fibrino-necrotic tracheitis in birds with advanced stages of disease. The PCR detection targeting the p32 gene of Gallid alphaherpesvirus 1 from tracheal tissue samples amplified a distinct band at the expected size (588 bp); the specific amplicon confirmed the presence of Gallid alphaherpesvirus 1 (Infectious Laryngotracheitis Virus). In conclusion, the characteristic clinical signs, gross and histopathological lesions, and molecular detection confirmed the diagnosis of infectious laryngotracheitis (ILT) in a broiler poultry flock.

MOLECULAR AND CLINICO-PATHOLOGICAL INVESTIGATION OF FOWL ADENOVIRAL INCLUSION BODY HEPATITIS AND GIZZARD EROSION OUTBREAKS FROM CENTRAL INDIA

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Fowl adenovirus (FAdV) serotypes are associated with variety of pathological conditions in poultry that inflicts substantial economic losses in affected flocks. In Central India, poultry flocks including broilers, cockerels, desi birds were experiencing disease outbreaks. Therefore, the present study was planned to explore on molecular detection of FAdV and associated pathology from field outbreaks in Central India. Total of 114 samples were collected from 50 poultry flocks comprising of broilers, cockerels, broiler breeders and desi birds from Central India during 2023 to 2024. 14% positivity was revealed by PCR when samples were subjected for amplification of partial hexon gene. Out of 7 flocks, 5 flocks had mono-infection of FAdV (71.42%) while 2 flocks had co-infection with Infectious Bursal disease, Chicken Infectious Anaemia, New Castle Disease and coccidiosis. Average mortality within positive flocks ranged from 0.2-4%. Grossly, the deceased birds showed pale, enlarged liver with petechial and ecchymotic hemorrhages, flabby heart, mottled kidneys, haemorrhages in pancreas and discoloration, ablation of koilin layer and mucosal erosions in gizzard. Microscopically, basophilic intranuclear inclusion bodies in the hepatocytes with multifocal necrosis and cellular infiltration were the most striking lesions in liver. Degenerated tubular epithelial cells and interstitial nephritis, erosion and loss of the keratinoid layer with necrosis of glandular epithelial cells and basophilic intranuclear inclusions in gizzard were evident. This study provides critical insights into molecular detection and pathology associated with recent outbreaks of FAdV infections in chickens from Central India.

PREVALENCE AND CHARACTERIZATION OF AVIAN PATHOGENIC *ESCHERICHIA COLI* (APEC) AND ITS ISOLATION FROM COMMERCIAL BROILERS AND BREEDER FLOCKS FOR VERTICAL TRANSMISSION

Ranjith Reddy, Dinesh C Pathak, Santosh S Swami, Suman P
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A total of 154 samples (liver, pericardium, air sacs, yolk sac, and ovary) were collected from affected birds. Bacterial isolation was performed using MacConkey and EMB agar, and colonies showing lactose fermentation and metallic sheen were subjected to Gram staining and IMViC biochemical tests. A subset of 56 isolates was serotyped at the National Salmonella and *E. coli* Center (CRI, Kasauli) to determine O-serogroups.

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Thirteen O-serogroups were identified, with O88 (48.1%) being the most prevalent, followed by O7, O34, and O52 (5.5% each) and O78 (3.7%), O11 (3.7%), O111 (3.7%).

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STUDIES ON THE PREVALENCE AND PATHOLOGY OF COLIBACILLOSIS IN BROILER BIRDS IN NORTH EASTERN PLAIN ZONE OF UTTAR PRADESH.

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Broiler production constitutes a key segment of the poultry industry in India, where the demand for poultry meat continues to rise. Despite this growth, broiler farms remain highly vulnerable to infections caused by *Escherichia coli* (*E. coli*), a bacterium commonly found in the intestinal tract of birds and their surrounding environment. Certain pathogenic strains can lead to elevated mortality, particularly in young chicks. The present study aimed to determine the serotype prevalence, gross pathological lesions and histopathological alterations associated with *E. coli* infection in broiler chickens reared in the North Eastern Plain Zone of Uttar Pradesh. A total of 677 naturally dead broiler chickens 372 from Basti, 217 from Bahraich, and 88 from Siddharth Nagar were collected from 12 private farms between October 2023 and September 2024. Seven isolates obtained from organs such as the heart, liver, lungs and intestine were submitted for serogrouping. Tissue samples from suspected birds were collected aseptically and fixed in 10% buffered formal saline for further analysis. Gross lesions observed included hepatomegaly and cardiac enlargement enveloped by thick yellowish-white serofibrinous membranes, intestinal haemorrhages with catarrhal inflammation and marked pulmonary congestion. Histopathological examination revealed severe hepatic congestion, haemorrhage, hepatocellular necrosis, villous necrosis in the intestine, myocardial infiltration and pulmonary emphysema with oedema. Bacteriological examination revealed characteristic pink colonies on MacConkey agar and a metallic sheen on EMB agar. The highest prevalence of *E. coli* infection was recorded in Basti (53.49%), followed by Bahraich (42.85%) and Siddharth Nagar (39.77%). Serotyping identified O8 as the predominant serogroup, particularly in heart isolates, while one isolate was untypeable and another classified as rough. The detection of serogroup O8 known to share virulence traits with human ExPEC emphasizes its zoonotic significance and raises important public health concerns regarding contaminated poultry meat and antimicrobial resistance.

LAMENESS IN BROILER BREEDERS: FIELD INVESTIGATION OF BACTERIAL SPONDYLITIS, SPONDYLOLISTHESIS AND VALGUS-VARUS DEFORMITIES

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Lameness in broiler breeders represent a major welfare concern and a significant cause of economic loss, adversely affecting growth, mobility, and overall flock performance. Rapid growth rates, high body weight and management-related factors predispose birds to skeletal and spinal disorders, leading to pain, deformities and reduced movement. The present study summarizes detailed investigations conducted at the Poultry Diagnostic and Research Centre (PDRC), Pune, to identify the major causes of lameness in broiler breeder flocks. Based on clinical examination, post-mortem findings, and laboratory investigations, three major conditions were identified as predominant causes of lameness. Bacterial spondylitis caused by *Enterococcus* spp. was frequently observed, particularly during the brooding phase between 3 and 6 weeks of age. Enterococci, which are normal inhabitants of the intestinal tract, were found to enter systemic circulation following compromise of the intestinal barrier, often associated with gut health disturbances. These bacteria showed a predilection for cartilage and bone, especially the caudal thoracic vertebrae (T5-T7), with the T6 vertebra being particularly susceptible due to mechanical stress and micro-trauma during rapid skeletal growth. The highly vascularized growth plates in young, fast-growing birds further predisposed these sites to bacterial colonization, with a higher prevalence observed in male birds due to higher body weight and load on joints. Spondylolisthesis was identified as another important cause of lameness, characterized by hock-sitting posture with legs extended forward. Affected birds remained alert with normal feed and water intake. Compression of the spinal cord at the posterior end of the T5 vertebra was observed, resulting from spinal malformation rather than infectious causes, as nodular lesions typical of bacterial spondylitis were absent. Valgus-Varus Deformities (VVD) were also commonly recorded, predominantly as valgus deformities involving lateral deviation of the distal tibia. These deformities often affected one leg and were associated with damage to the distal tibial growth plate, leading to outward rotation of the feet and thickening of the hock joint due to altered tendon positioning. Rapid growth rate, limited exercise in cage systems and increased mechanical stress were identified as important contributing factors. The findings indicate that bacterial spondylitis, spondylolisthesis, and valgus-varus deformities are the major causes of lameness in broiler breeders and may act as predisposing factors for one another. Rapid growth, particularly in males, and limited physical activity significantly increase susceptibility. Management strategies such as controlled growth through low-energy diets, extended dark periods during early life, improved gut health, and adequate exercise are essential to reduce the incidence of skeletal deformities and lameness in broiler breeder flocks.

RE-EMERGING FOWL POX VIRUS – ISOLATION, IDENTIFICATION, AND DIFFERENTIAL DIAGNOSIS IN COMMERCIAL BIRDS DURING 2024-2025

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Fowl pox is a re-emerging viral disease of poultry caused by viruses belonging to the genus Avipoxvirus. The disease has significant economic importance worldwide, resulting in production losses due to reduced egg production in layers, poor growth performance in broilers, and occasional mortality. Clinically, fowl pox occurs in two forms: the cutaneous form, which is mainly associated with mechanical transmission by biting arthropods, and the diphtheritic form, which involves the mucous membranes of the oral cavity, pharynx, and larynx. The present study was undertaken to isolate, identify, and characterize fowl pox virus involved in recent outbreaks. PCR amplification, histopathology, and serological assays are widely used tools for diagnosis and epidemiological monitoring of fowl pox virus. In this study, nodular tissues from suspected birds were collected and processed for virus detection. Samples were inoculated into 11-day-old specific pathogen-free (SPF) embryonated chicken eggs for virus propagation. Three serial passages were performed, and viral growth was assessed on the chorioallantoic membrane (CAM). CAMs showing characteristic pock lesions were harvested for DNA extraction and further molecular confirmation. Affected birds, including commercial broilers, layers, and breeders, exhibited fibrino-necrotic lesions on the mucous membranes of the upper respiratory and digestive tracts (diphtheritic form) along with proliferative nodular skin lesions (cutaneous form). Histopathological examination revealed epithelial hyperplasia, degeneration, and numerous intracytoplasmic inclusion bodies. Between January 2024 and November 2025, 153 samples were suspected of fowl pox virus infection, of which 40 were selected for virus isolation. Virus propagation on CAM yielded a positivity rate of 57% (23/40), characterized by membrane thickening and opaque pock-like lesions. Molecular confirmation by conventional PCR targeting the P4b gene produced a 1200 bp amplicon. A few isolates were also positive for reticuloendotheliosis virus (REV) by PCR targeting the LTR gene, indicating integration of REV sequences into the fowl pox virus genome, which may alter tissue tropism and contribute to outbreaks in vaccinated flocks.

Continuous monitoring of fowl pox virus in commercial poultry is essential. Strengthening biosecurity, improving hygiene, implementing effective vector control, and optimizing vaccination strategies are critical for disease control. Based on field observations, vaccination with pigeon pox vaccine at 3–4 weeks followed by fowl pox vaccine at 6 weeks is recommended for layers and breeder flocks.

AVIAN REOVIRUS ISOLATION ASSOCIATED WITH NEPHRITIS CASES IN COMMERCIAL BROILER CHICKENS

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Avian reovirus (ARV) remains an important pathogen of poultry and continues to cause significant economic losses to the global poultry industry. The virus is widely prevalent and capable of producing a broad spectrum of clinical conditions, including tenosynovitis/arthritis, immunosuppression, enteric disease, hepatitis, myocarditis, malabsorption, runting–stunting syndrome, and renal involvement. Despite routine vaccination practices, the continuous evolution and genetic diversity of ARV strains complicate effective disease control. The present study was undertaken to investigate the occurrence, molecular characteristics, and genotypic diversity of ARV associated with nephritis in commercial broiler flocks in India during 2024–2025. A total of 500 tissue samples, including kidney, liver, heart, lungs, trachea, spleen, and bursa, were collected from commercial broiler flocks aged 10 to 37 days. Affected flocks exhibited nephritis, hydropericardium, visceral gout, hepatitis, hepatomegaly, respiratory distress, and mortality ranging from 4% to 18%. Initial direct PCR screening of field samples was negative for ARV. Following inoculation into primary chicken embryo liver cell cultures and three to five serial passages were given. Virus isolation was achieved in 22 samples (4.4%) showing typical cytopathic effects, including syncytia formation within 24–48 hours, monolayer degeneration, and giant cell formation, were observed. Viral isolates were confirmed by RT-PCR and sequencing of the sigma C (σ C) gene. Sequence analysis revealed the presence of multiple genotypic clusters among the isolates, indicating co-circulation of genetically distinct ARV strains in commercial broiler flocks. The study confirms the continued circulation of ARV in Indian poultry and highlights its association with nephritis and systemic disease. The detection of different genotypic clusters emphasizes the limitations of existing vaccination strategies and underlines the need for continuous molecular surveillance and the use of appropriate live attenuated and inactivated vaccines in breeder flocks to ensure effective maternal immunity and minimize economic losses.

INTEGRATED CLINICAL, HAEMATOLOGICAL AND MOLECULAR APPROACH TO THE MANAGEMENT OF AVIAN MALARIA IN WILD BIRDS

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The present study reports the clinical management of avian malaria in five rescued wild birds belonging to three different species- one Painted Stork (*Mycteria leucocephala*), two Peahens (*Pavo cristatus*), and two Black-headed Ibises (*Threskiornis melanocephalus*). All birds were presented to the Transit Treatment Centre, Nagpur in a dull, inactive, and lethargic condition, unable to stand on their phalanges and refusing to feed. Haemato-biochemical analysis and peripheral blood smear examination revealed the presence of *Plasmodium* spp. and *Haemoproteus* spp., confirming a diagnosis of avian malaria accompanied by anaemia. Additionally, polymerase chain reaction (PCR) was performed for molecular confirmation and sex determination. Based on the diagnostic findings, birds were treated with chloroquine (@25 mg/kg) and primaquine (@0.8 mg/kg) as antiprotozoal therapy, along with supportive care including hand feeding and fluid supplementation for 15 days. Gradual improvement in activity, appetite, and posture was observed, leading to complete recovery in all birds. This case underscores the importance of early diagnosis, prompt therapeutic intervention, and supportive management in the successful rehabilitation of wild birds affected by avian malaria.

CHARACTERIZATION OF H9N2 AVIAN INFLUENZA VIRUSES ISOLATED FROM BHOPAL, MADHYA PRADESH

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Avian influenza viruses (AIVs) are major pathogen affecting poultry causing severe economic losses to the poultry industry. In this study, we characterized four H9N2 avian influenza viruses isolated during the surveillance for the prevalence of AIVs in poultry units, mixed farms, and LBMs in rural and urban Bhopal, Madhya Pradesh. The viruses were typed as H9N2 avian influenza virus by reverse transcription polymerase chain reaction. The HA gene of the four H9N2 viruses were sequenced by Sanger's method. Molecular analysis indicated presence of leucine (L) at position 234 which showed that these viruses preferentially bind to human-like α 2, 6 sialic acid receptor. The HA cleavage site had a monobasic motif 336KSSR/GLF342 (H9 numbering) site indicating low pathogenicity to poultry. Some specific mutations such as 93E, 128R and 183E are specifically present in the H9N2 viruses isolated from Bhopal which are not found in H9N2 viruses reported earlier. The percent identity matrices of sequences was generated through MEGA12 software. While two H9N2 viruses viz.

(A/chicken/India/09EN93/2025_MP and A/chicken/India/09TI08/2025_MP) had nucleotide homology of 99.94%, the other two H9N2 viruses viz. (A/chicken/India/09EN02/2025_MP and A/chicken/India/09EN03/2025_MP) shared a homology of 99.99% between them. The percent homology between the two groups of H9N2 viruses was 98.46%. The HA gene phylogenetic tree showed that all the four isolates belonged to G1-Western lineage and grouped with H9N2 viruses isolated from India, Bangladesh, Pakistan, Nepal, Iran, Israel and UAE with a bootstrap value of 88%. The unique mutations and difference in specific amino acids between the H9N2 viruses isolated in this study might be due to antigenic drift induced probably due to vaccination and hence continuous surveillance is required for monitoring the evolution of H9N2 viruses in India.

MOLECULAR STUDIES OF ORNITHOBACTERIUM RHINOTRACHEALE (ORT) IN CHICKENS

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Key words: Ornithobacterium rhinotracheale (ORT), 16S rRNA gene, PCR

Ornithobacterium rhinotracheale (ORT) is an emerging respiratory pathogen of global significance in the poultry industry. It is a highly contagious disease affecting the respiratory tract of chickens, which culminates in considerable economic detriment to the poultry sector.

A study on Pathology of Ornithobacterium rhinotracheale infection in chickens was conducted by using molecular method which included confirmation and characterization of Ornithobacterium rhinotracheale through PCR and 16S rRNA gene sequence analysis, respectively.

All suspected ORT chicken's tracheal tissue samples underwent genomic DNA extraction followed by polymerase chain reaction (PCR) using a temperature gradient thermocycler targeting the 16S rRNA gene, thereby amplifying a 784 base pair fragment for the detection of Ornithobacterium rhinotracheale (ORT).

Sequence analysis of positive samples was conducted utilizing gene-specific primers. BLAST analysis of the resultant 16S rRNA sequences against GenBank database demonstrated a genetic similarity exceeding 99% between the ORT isolates identified in this study and previously characterized ORT strains globally.

Phylogenetic analysis of 16 S rRNA gene sequences of all isolates (ORT1 and ORT2) was performed to study evolutionary relationships by incorporating 18 GenBank sequences within MEGA 12 software. Phylogenetic tree in the present study distinctly indicated that all isolates exhibited a close evolutionary relationship amongst themselves. They clustered within a well-supported clade and showed strong similarity and close evolutionary relationship with reference strains of turkey and avian species from the Poland and USA (MW298697.1 and CP094846.1) respectively, reinforcing their identity as Ornithobacterium rhinotracheale.

A RARE REPORT ON PROVENTRICULAR TETRAMERIOSIS IN A THREE-MONTH-OLD DESI CHICKEN

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Abstract

Tetramere spp. is a genus of parasitic roundworms that infects chickens, turkeys, pigeons, guinea fowls, ducks, pheasants, quails as well as numerous wild birds. *T. americana*, *T. crami*, *T. fissispina*, and *T. pattersoni* are reported commonly in poultry worldwide. *T. mohtedai* has been reported as a new Indian spp. recently in 2017. It causes proventriculopathy characterized by signs like green feces (excess bile), weight loss, anemia, and occasionally fatality. The infection with *Tetramere* spp. are not observed as frequent like other gastrointestinal roundworms of poultry and are considered to be rare. The present case describes tetrameriosis in a three-month-old desi poultry bird presented for necropsy exhibiting weakness and retarded growth in the flock of 200 desi birds. The gross examination of the proventriculus revealed multiple small, red, nodular parasitic lesions embedded within the proventricular glands. On closer inspection, the globular, blood-filled roundworms of *Tetrameres* spp. were found deeply embedded within the glandular tissue of the proventricular wall. Microscopic examination of impression smears and tissue sections from the proventriculus confirmed the presence of adult *Tetrameres* parasites along with numerous characteristic eggs. Histopathological examination of proventricular tissue revealed degenerative and focal areas of necrosis of proventricular glands accompanied with congested vasculature and areas of hemorrhage, and infiltration of inflammatory cells. The presence of both adult parasites and embryonated eggs confirmed the diagnosis of tetrameriosis. The condition likely resulted from ingestion of intermediate hosts such as earthworms, ants, cockroach or grasshoppers, encountered in free-range desi poultry systems. This case highlights the importance of routine parasitological examination and appropriate management practices in backyard poultry to prevent occurrence of tetrameriosis that can adversely affect growth and productivity of desi birds.

Keywords: Tetrameriosis, Desi chicken, backyard poultry.

SYSTEMIC ASPERGILLOSIS IN A KAKARIKI PARAKEET (*CYANORAMPHUS NOVAEZELANDIAE*): A CASE REPORT

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ABSTRACT

A two-year-old kakariki parakeet with a history of anorexia and no apparent signs of disease was presented to the Department of Veterinary Pathology, CVAS, Pookode, Kerala, for postmortem examination. On examination, mild pectoral muscle atrophy with white to yellowish fibrinous adhesions between the proventriculus and lungs was observed. A yellowish-white spongy membrane covered the proventriculus and liver, with focal black discolouration, and the lungs were oedematous. Tissue samples were collected and fixed in 10% neutral buffered formalin for histopathological examination. Microscopic examination with H&E staining revealed that the fibrinous adhesions were identified as organised mycotic plaques composed of spongy mycelial networks and distinct fruiting bodies and marked infiltration of mononuclear cells in the liver and lungs. Special staining with PAS demonstrated proliferating fungal hyphae. Culture on Sabouraud Dextrose Agar yielded characteristic velvety, powdery colonies, definitively identified as *Aspergillus* species via Lactophenol Cotton Blue staining. This case demonstrates that aspergillosis can grossly mimic fibrinous bacterial disease; thus, histopathology and culture are critical for definitive diagnosis in subclinical cases.

Keywords: Aspergillosis, Kakariki parakeet, Histopathology, Mycotic plaques, PAS staining.

PATHOLOGY AND MOLECULAR CHARACTERIZATION OF AVIAN ENTERIC VIRUSES IN CHICKENS

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Enteritis is one of the important diseases of chickens characterized by diarrhoea, poor weight gain and in some flocks, high mortality. Enteric viruses are the etiological agents leading to a variety of health disturbances in commercial chickens around the world which include FAdV- I, ChPV, AReoV, ARtV, CAsTV and ANV. The causes of enteric disease have never been definitely established because they are complex and polymicrobial and similar disease signs can likely be caused by different pathogens. Further, there is a lack of documented information and no baseline data is available regarding the prevalence of enteric viruses in Chhattisgarh. Considering the fact, present study was carried out to study the prevalence of enteric viruses associated with enteritis along with molecular characterization and pathology of enteric viruses in natural and experimental cases of chickens. A total of 414 necropsies were performed during the full course of present work. Intestinal tissue samples from enteritis affected birds were collected during necropsy and stored at -20°C for molecular work. Tissue samples of small intestine, caeca, proventriculus, pancreas, liver, thymus, bursa and spleen were also collected from enteritis associated chickens and preserved in 10% buffered formalin for histopathological study. Intestinal tissue samples found positive for enteric viruses were also subjected to PCR amplification to rule out complications with *E. coli* and *Salmonella* spp. infection. Further, detected enteric viruses with single infection by PCR/RT-PCR assay, were subjected to oral inoculation in commercial broiler chicks on 7th day of age to study its pathogenicity. Overall prevalence of FAdV-I, ChPV and AReoV in four districts of Chhattisgarh was recorded to be 25.84%, 36.37% and 12.07% respectively. Highest prevalence of FAdV-I was found in Rajnandgaon (28.43%) followed by Balod (27.45%), Raipur (26.13%) and Durg districts (23.70%). Prevalence of ChPV infection was higher in Balod (41.17%) followed by Raipur (36.36%), Durg (35.84%) and Rajnandgaon district (35.29%). Further, highest prevalence of AReoV infection was found in Balod (15.68%) followed by Raipur (15.50%), Durg (11.56%) and Rajnandgaon district (10.78%). A total of 4.11%, 11.83% and 3.14% cases were found positive for single FAdV-I, ChPV and AReoV infection respectively. A total of 16.91%, 0.72% and 4.11% of cases were found positive for FAdV-I + ChPV and FAdV-I + AReoV, ChPV + AReoV combined infection respectively. Moreover, combined infection of FAdV-I + ChPV+AReoV was detected in 4.34% of cases. Out of 187 confirmed cases of enteric viruses, 23 (12.99%) and 25 (13.36%) cases were found positive for *Salmonella* spp. and *E. coli* infection respectively. For molecular detection of FAdV-I, ChPV, AReoV and ARtV, the hexon (897 bp), NS (561 bp), sigma C (972 bp) and NSP4 (630bp) gene were targeted for amplification by PCR/RT-PCR. Sequence of 362

bp and 473 bp of polymerase gene was targeted for detection of CASTv and ANV respectively by RT-PCR. Three isolates of FAdV-I belongs to genotype D which showed 99.17-100% homology with other nucleotide sequences of FAdV-I available in GenBank among which two isolates showed highest similarities with the isolates from Pantnagar, India and one isolate showed highest similarities with the isolate from Canada. Further, another two isolates belongs to the genotype E of FAdV-I which had 99.02-99.76% identity with other nucleotide sequences available in GenBank and showed highest similarities with isolates from South Africa. Six isolates of ChPV revealed 98.05-99.43% homology with other nucleotide sequences of ChPV available in GenBank. Four isolates from Chhattisgarh showed highest similarities with the isolates from Brazil. Furthermore, another two isolates showed highest similarities with isolates from Poland and China. Two isolates of AReoV revealed 93.53-97.51% homology with other nucleotide sequences of AReoV available in GenBank and showed highest similarities with the isolates from Japan. Gross lesion in chickens naturally affected with enteric viruses showed emaciation, prominent keel bones, pale breast muscle, catarrhal enteritis, typhlitis, enlarged, swollen and rounded proventriculus, paleness or congestion in liver and atrophy of pancreas, thymus, bursa and spleen during necropsy. Microscopically, intestine showed enterocyte desquamation, vacuolar degeneration and necrosis of epithelium of villi, distension of crypts and infiltration of lymphocytes. Proventriculus showed haemorrhages and infiltration of lymphocytes. Pancreas showed degenerative and necrotic changes in acini, lymphocytic infiltration and fibrous tissue proliferation. Liver showed fatty changes, necrotic and degenerative lesions in hepatocytes alongwith congestion, hemorrhages and lymphocytic infiltration. Lymphoid organs showed depletion of lymphocytes. The severity of the lesions was more in AReoV affected birds followed by FAdV-I and ChPV infected birds. Pathogenicity testing revealed significantly decreased growth performance, symptoms of diarrhoea, dehydration, vent pasting, partially digested food material in faeces and ruffled feathers. Lesions of catarrhal enteritis, typhlitis, proventriculitis, paleness of liver and atrophy of pancreas, thymus, bursa and spleen were evident. Severity of was more in AReoV challenged birds followed by FAdV-I and ChPV inoculated birds.

PATHOLOGY, INCIDENCE AND MOLECULAR CHARACTERIZATION OF CHICKEN PARVOVIRUS IN DESI CHICKENS OF CHHATTISGARH

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Enteritis is one of important disease of chickens characterized by diarrhoea, poor weight gain and in some flocks, high mortality. Chicken Parvovirus (ChPV) is one of the important viral agents involved in enteric disorders. The present study was aimed to detect the ChPV associated with enteritis in desi and kadaknath chickens of Chhattisgarh. Total of 151 desi and kadanath birds (1-18 weeks old) died due to enteritis with symptoms of diarrhea, vent pasting, ruffled feathers and poor growth were subjected to necropsy. Intestinal tissue samples collected from 151 birds were subjected to PCR assay for detection of ChPV by amplifying 561 bp sequence of NS gene. ChPV was detected in 31.78% (48/151) of intestinal samples collected chickens of Durg, Rajnandgaon, Balod and Raipur district of Chhattisgarh which suffered from enteritis. Lesions consisted of catarrhal enteritis, dilated, inflamed or pale intestinal wall with watery and gas filled contents, atrophy of immune organs and pancreas. Sequences of NS gene of ChPV showed 98.37%-99.19% nucleotide similarities with other previously published sequences. Phylogenetic analysis revealed that detected Indian isolate is grouped with Poland 1 and USA 2 isolates. The results indicated the presence of ChPV in Indian chicken flocks and its close association with enteritis.

PATHOLOGY AND MOLECULAR DIAGNOSIS OF VELOGENIC VISCEROTROPIC NEWCASTLE DISEASE VIRUS IN DESI CHICKEN OF CHHATTISGARH

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Newcastle disease (ND) is an economically important viral disease of birds caused by Avian Paramyxovirus type 1. In the present study, mortality of 56.50 to 74.50% due to ND in four desi chickens farms located in Durg district of Chhattisgarh was noticed which were not vaccinated against NDV. The gross and histopathological lesions were suggestive of viscerotropic velogenic form of ND. Tissue samples of trachea and lung collected from 18 desi birds belonging to four desi flocks were confirmed as ND by RT-PCR amplification of 535 bp of fusion protein gene of NDV. In the present study, velogenic viscerotropic NDV was confirmed in all four desi flocks by gross and microscopic examination and RT-PCR assay.

PATHOLOGICAL STUDIES AND MOLECULAR DETECTION OF SALMONELLOSIS IN DESI CHICKENS OF NAGPUR REGION

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Avian salmonellosis is an important disease causing serious impediment to the development of poultry industry especially in developing countries. During present study, mortality due to Salmonellosis in six desi chicken flocks belonging to Nagpur region of Maharashtra was noticed. Characteristic lesions of necrotic foci on liver, omphalitis and catarrhal enteritis were noticed in *Salmonella* spp. affected chickens. Colourless colonies on MLA, light pink colonies on BGA and jet black colonies on XLD agar confirmed the *Salmonella* spp. infection. Clinical samples of liver collected from six desi chicken flocks were further confirmed as *Salmonella* spp. by PCR amplification of 423bp of *InvA* gene. The lesions, cultural characteristics along with PCR amplification of *InvA* gene confirmed *Salmonella* spp. infection in desi chickens of Nagpur region.

PATHOLOGY AND MOLECULAR DETECTION OF SALMONELLOSIS IN COMMERCIAL CHICKENS OF CHHATTISGARH

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Avian salmonellosis is an important disease-causing serious impediment to the development of the poultry industry especially in developing countries. During the present study, mortality of 16 to 22% due to Salmonellosis in thirteen, 1-2 weeks old commercial chicken flocks located at Durg, Rajnandgaon, and Raipur district of Chhattisgarh was noticed. Characteristic clinical signs of depression, anorexia, huddling, droopy wings, dehydration, laboured breathing, diarrhoea, and adherence of faeces to the vent were suggestive of Salmonellosis. Necrotic foci on the liver, omphalitis, and catarrhal enteritis were the characteristic lesions found in *Salmonella*-affected birds. Colorless colonies on MLA, light pink colonies on BGA, and jet-black colonies on XLD agar confirmed the *Salmonella* infection. Clinical samples of liver collected from thirteen commercial chicken flocks were further confirmed as *Salmonella* spp. by PCR amplification of 423bp of *InvA* gene. The clinical signs, lesions, and cultural characteristics along with PCR amplification of *InvA* gene confirmed *Salmonella* infection in commercial chickens of Chhattisgarh.

ISOLATION, IDENTIFICATION AND PREVALENCE OF POULTRY COCCIDIOSIS IN MAHARASHTRA

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The present study was conducted at PDRC, VHPL, Pune, from April 2023 to October 2025 to isolate, identify, and determine the prevalence of *Eimeria* species infecting poultry. Coccidiosis, caused by protozoan parasites of the genus *Eimeria*, develops in the intestinal tract of birds and occurs in both clinical and subclinical forms. Clinical coccidiosis is characterized by mortality, morbidity, diarrhoea, and bloody faeces, whereas subclinical infection results in poor weight gain and reduced feed conversion efficiency, leading to substantial economic losses. Faecal samples were collected from poultry house floors, and intestinal and caecal samples were obtained from affected birds during necropsy. Intestinal contents and mucosal scrapings were preserved in 2.5% potassium dichromate at room temperature. Oocysts were isolated using standard faecal flotation and purification techniques. Identification of *Eimeria* species was based on clinical signs, gross lesions, oocyst morphology, and PCR screening. A total of 194 samples were examined, of which 63.4% were positive for *Eimeria* infection, while 37.1% were negative. Species-wise prevalence revealed *Eimeria tenella* as the most prevalent species (54.1%), followed by *Eimeria acervulina* (44.0%), *Eimeria maxima* (43.2%), and *Eimeria mitis* (12.3%). The lowest prevalence was observed for *Eimeria brunetti* (2.57%) and *Eimeria necatrix* (0.5%). The findings indicate that *E. tenella*, the most pathogenic species, predominates in poultry coccidiosis, followed by *E. maxima* and *E. acervulina*. These species are major contributors to production losses due to morbidity and mortality. The study highlights the need for targeted and species-specific coccidiosis control strategies to reduce economic losses and improve poultry productivity.

PATHOLOGY AND MOLECULAR CHARACTERISATION OF IMPORTANT RESPIRATORY PATHOGENS OF CHICKEN

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Respiratory diseases remain a great threat to the poultry industry as causing great mortality and significant economic losses. Despite the strict vaccination and treatment procedures, these pathogens remain a challenge to control due to the emergence of new strains of pathogens. The present study aimed to study the pathology and molecular characterization of important respiratory disease pathogens of chicken. In this present study total of 50 samples were collected from the birds, which showed clinical manifestations and gross lesions of respiratory diseases. All the collected samples are screened for viral diseases such as ND, IB, ILT, Fowl pox, and Fowl adenovirus infection; bacterial diseases such as colibacillosis, CRD, and ORT by RT-PCR/PCR. Out of 50 samples, 45 samples were positive for one of the screened pathogens concurrently or singly. Concurrent infection induced severe pathological lesions than mono-infection. Grossly, the trachea revealed mild to severe congestion, hemorrhages, and presence of exudates varying from catarrhal, mucopurulent to fibrinopurulent. Lungs revealed severe congestion and hemorrhages, and deposition of pleura over the surface of the lungs. Air sac revealed cloudiness, thickening, and caseous exudate. Histopathological examination revealed moderate to severe epithelial hyperplasia, necrosis of mucosal epithelial cells, lymphocytic infiltration, and exudate in the tracheal lumen. Examination of lungs showed thickening of interalveolar septa with fibrin, infiltration of heterophils, lymphocytes, and macrophages, and edema in parabronchi. Molecular characterization of NDV revealed the circulation of two different genotypes, genotype XIII and genotype VII. IBV viruses were grouped with 4/91 strain of IBV and belonged to GI 24 lineage. Molecular characterization of ILTV revealed the circulation of chicken embryo originated (CEO) related vaccine strain in unvaccinated flocks. FAdV virus belongs to serogroup D. Experimental study of these viruses needs to be done to study detailed pathogenesis of these pathogens.

PATHOLOGICAL STUDIES ON COCCIDIOSIS IN BROILER CHICKENS

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Coccidiosis is one of the most common parasitic diseases prevalent in the broiler chickens. It causes heavy economic losses due poor and stunted growth, reduced feed efficiency and even high mortality in severe outbreaks. The present study was undertaken to evaluate gross and histopathological features and molecular diagnosis of coccidiosis in the broiler chickens of 15 days. The birds had a history of dullness, reduced feed intake, blood-stained loose droppings and mortality. The detailed necropsy of chicken carcasses was performed. During the external examination, vent area revealed presence of blood-stained faecal material. Grossly, the birds revealed haemorrhagic enteritis and typhilitis. Caeca of affected birds were highly distended and filled with bloody exudate. Thickened mucosa, extensive haemorrhage and necrosis were also evident in intestine and caecum. Microscopic examination of intestinal mucosal scrapings revealed presence of coccidian oocysts. Histopathologically, necro-haemorrhagic enteritis and typhilitis were observed; there was marked infiltration of mononuclear cells including lymphocytes and macrophages along with few heterophils. Enterocytes revealed different developmental stages of coccidia. For molecular investigation, polymerase chain reaction (PCR) was performed by using primers specific for 18S rRNA gene of the *Eimeria* genus, which yielded a product of 450 bp. In conclusion, based on clinical signs, macroscopic and microscopic findings, and results of PCR assay, outbreak in broiler chicken flock was confirmed as coccidiosis caused by *Eimeria* species. This case study highlights the importance of PCR assay for confirmatory diagnosis of coccidiosis, which is essential for the effective disease control and management in broiler chickens.

PATHOLOGICAL AND MOLECULAR OBSERVATIONS OF MID-INTESTINAL COCCIDIOSIS DUE TO *EIMERIA NECATRIX* INFECTION IN DESI CHICKENS

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Carcasses of 120-day-old Desi birds reared on deep litter, with a history of bloody diarrhea and a mortality of 695 birds (13.90%) out of a flock size of 5000 birds within the last two days in the month of June (rainy season), were received for necropsy. The necropsy of the affected birds showed pale discoloration of the breast musculature (indicative of anemia). The jejunum revealed marked ballooning, and the serosal surface showed small, white spots, usually intermingled with rounded, bright- or dull-red spots of various sizes (a "salt and pepper" appearance). The jejunal wall was thickened, and the lumen showed the presence of hemorrhagic intestinal content. The histopathological examination of the sections of the affected jejunum revealed the presence of hemorrhagic enteritis, along with various developmental stages of *Eimeria* spp. The PCR detection targeting the Internal Transcribed Spacer 1 (ITS-1) gene of *Eimeria necatrix* amplified a distinct band of expected size confirming the presence of *Eimeria necatrix* infection in the affected birds. In conclusion, the clinical signs, gross and histopathological lesions, and molecular findings supported the case diagnosis for mid-intestinal coccidiosis due to *Eimeria necatrix* infection.

PATHOLOGICAL AND MOLECULAR STUDIES ON TETRAMERIOSIS IN DESI CHICKENS

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Carcasses of 78 desi chickens from different backyard poultry owners in areas in and around Udgir city, Maharashtra, received for post-mortem examination during the period of January 2018 to July 2024 (6.5 years), formed the materials of the present study. Out of 78 post-mortem examinations of desi chickens conducted during the study period of 6.5 years, ten (n=10, 12.82%) birds were found infected by *Tetrameres* spp. roundworms. The age of the affected birds ranged from 1.5 months to 1 year. The affected birds showed clinical signs such as loss of appetite, drowsiness, stunted growth, emaciation, white mucoid diarrhea, and mortality. The affected flocks showed an average mortality of 6.41% within a span of 5-8 days. During necropsy, the proventriculi from the affected chickens showed a marked enlargement or rounded appearance and the presence of multiple reddish-black spots (1-2 mm in diameter) easily visible through the serosal as well as the cut surface of the proventricular wall, which corresponded to *Tetrameres* spp. gravid females. The histopathological examination of the affected proventriculi revealed the presence of globular or spherical shaped gravid female worms within the lumen of the proventricular glands. Marked pressure atrophy of the affected proventricular glands and compression of the lamina propria, stroma, and adjacent proventricular glands, as well as smooth muscle atrophy and marked fibroplasia around the parasitized glands, were also evident. The PCR detection targeting the 18S rRNA gene of *Tetrameres fissipina* amplified a distinct band at the expected size (721 bp); the specific amplicon confirmed the presence of *Tetrameres fissipina* infection in the affected birds. In conclusion, the clinical signs, gross and histopathological lesions, and molecular findings supported the case diagnosis for tetrameriosis.

Theme 2

*Vaccine, Variants and
Vaccination Strategies*

AVIAN VACCINES: UPDATES AND APPLICATIONS – "FROM HATCHERY TO HARVEST"

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Abstract

For the Indian poultry farmer, the margin between profit and loss is often determined by flock health. Viral diseases remain a critical threat, and while traditional vaccines have been foundational, their limitations are increasingly apparent. This paper presents a practical overview of modern avian vaccinology, tracing the path from first-generation vaccines to today's advanced platforms like recombinant vectors and immunocomplex technology. We emphasize that scientific innovation must be paired with precision application. The core of this update is the "Hatchery to Harvest" strategy, which leverages automation—from day-one subcutaneous autovaccinators and double-shot applicators to in ovo systems—to ensure timely, accurate, and stress-free vaccine delivery. By integrating smart vaccine design with robust, safe adjuvants and foolproof application, we can build resilient flocks, improve farmer economics, and secure the future of the Indian poultry industry.

1.0 Introduction: The Economic and Biological Imperative

The sustainability of India's poultry sector hinges on effective disease prevention. Outbreaks of Newcastle Disease (ND), Avian Influenza (H9N2), and Infectious Bursal Disease (IBD or Gumboro) can devastate small and large farms alike. Conventional vaccines, while useful, face challenges in our context: circulating strains often differ from vaccine strains, maternal antibodies block early protection, and manual application leads to uneven coverage. This paper bridges the gap between modern vaccine science and on-farm reality. It focuses on practical updates—newer, safer vaccine types and the automated technologies to deliver them—that form a complete shield from the hatchery to the point of harvest.

2.0 Evolving Vaccine Platforms: Safer, Smarter and More Specific

2.1 First Generation: The Established Tools:

These are the live-attenuated and inactivated (killed) whole-virus vaccines.

- Examples: LaSota (ND), Intermediate IBD strains, inactivated emulsion vaccines.
- **Key Considerations:**
- **Safety in Inactivation:** The choice of inactivating agent (e.g., binary ethylenimine (BEL) over formalin) is crucial to preserve the virus's antigenic structure while ensuring complete safety, making the final product both safe and potent.

- **Adjuvants for Killed Vaccines:** The efficacy of inactivated vaccines depends heavily on safe and effective adjuvants. Modern oil-emulsion adjuvants (like water-in-oil formulations) are designed to create a depot effect, slowly releasing antigen to stimulate strong, durable immunity without causing excessive tissue reactions.

2.2 Second Generation: Precision-Engineered Protection

This generation moved beyond the whole virus to target the immune system with precision.

- **Subunit Vaccines:** Use purified viral proteins. They are very safe but require potent adjuvants.
- **Recombinant Vector Vaccines (A Major Leap):** A harmless virus (vector) like Herpesvirus of Turkeys (HVT) is engineered to carry and express a key gene from a pathogen (e.g., NDV's F gene).
- **The Power of Combination:** This technology enables multivalent combination vaccines. A single HVT vector can now carry antigens for MD, ND, and IBD, providing broad protection with one hatchery dose, simplifying logistics and reducing bird stress.
- **Immunocomplex Vaccines (A Brilliant Workaround for IBD):** This is a clever formulation, without a genetic modification. A live attenuated IBD virus is coated with specific antibodies.
- **Mechanism:** This "antibody shield" protects the vaccine virus from neutralization by maternal antibodies in the chick. The vaccine activates only when maternal antibodies wane naturally, ensuring perfect timing.
- **Application:** Products like IBD-ICx are designed for precise day-old subcutaneous administration or in ovo, making them a cornerstone of early hatchery protection.

2.3 Third Generation: The Genetic Frontier

- **mRNA Vaccines:** These instruct the bird's cells to produce the viral antigen internally. Their great advantage is speed: a vaccine can be rapidly designed against a new outbreak strain. While currently a strategic tool for high-value stock, they represent the future of rapid response.

3.0 The "Hatchery to Harvest" Application Revolution

Technology in the vial is useless without technology in the field. Modern application ensures uniformity, accuracy, and animal welfare.

3.1 Day-One Hatchery Automation: Building a Uniform Foundation

- **Subcutaneous Autovaccinators:** Before chicks leave the hatchery, automated systems deliver precise subcutaneous injections. These machines ensure consistent dose volume and injection depth, critical for vaccines like inactivated combinations or Marek's HVT vectors. This method is faster and less stressful than manual handling.
- **Advanced Applicators:** Modern systems like "double-shot" vaccinators can administer two different vaccines in one quick, automated pass, maximizing efficiency and coverage on day one.

3.2 In Ovo Vaccination: Advanced vaccination procedure for Broilers

- Following day-one procedures, in ovo vaccination (at day 18 of incubation) represents the pinnacle of preventative care. Automated injectors place the vaccine (e.g., HVT-ND-/ HVT-ND-IBD combinations or Immunocomplex IBD) directly into the embryonated egg, ensuring 100% flock coverage before hatch and priming the immune system early.

3.3 Use of Spray vaccination cabinets for vaccination of 1-day chicks in hatchery. Live vaccines like IB, ND are popularly used for priming of chicks before placing in farms. Accurate droplet size of spray and coverage of spray area across tray are critical factors for uniform vaccination.

3.4 On-Farm Applications

- **Drinking Water & Spray Vaccination:** For booster doses, success depends on water quality (free of chlorine, metals) and. The use of dedicated stabilizers and dyes is non-negotiable to monitor uptake.
- **The Role of Diagnostics:** Simple serological tests (like ELISA) allow farmers to check vaccine "takes" and monitor flock immunity, moving from a calendar-based to an evidence-based vaccination schedule.

4.0 An Indian Roadmap: Pragmatic Adoption of Technology

For new technologies to succeed in India, they must address local realities.

- **Indigenization of Strains:** The development and launch of indigenous H9N2 and NDV Genotype VII, Genotype XIII vaccines are vital to match circulating field challenges.
- **Overcoming Infrastructure Hurdles:** Prioritizing thermo stable formulations (lyophilized vaccines) and logistically simple combination vaccines reduces dependency on the cold chain and complex farm schedules.
- **The Farmer's Bottom Line:** Every innovation—from a trivalent / quadrivalent vaccine that saves three to four vaccine shots or an auto vaccinator that cuts labour—must ultimately improve the economic return for the farmer. The "Hatchery to Harvest" model shifts cost and complexity to the automated hatchery, delivering a more predictable, protected bird to the grow-out farm.

5.0 Conclusion: Integrating Science and Application for a Resilient Industry

The future of poultry health is not about choosing between advanced vaccines or advanced application; it requires both. The journey from conventional to immunocomplex and recombinant vector vaccines, delivered via precision auto-vaccinators and in ovo systems, creates a powerful synergy. For India, embracing this integrated "Hatchery to Harvest" approach is the key to transforming disease management from a constant challenge into a predictable, profitable component of farming. By mastering both the molecule and the method, we can build an industry that is resilient, sustainable, and economically vibrant.

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ENHANCING *MYCOPLASMA GALLISEPTICUM* VACCINE DELIVERY AND DISEASE CONTROL IN COMMERCIAL CHICKEN OPERATIONS

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Abstract

Mycoplasma gallisepticum (MG) is a major respiratory pathogen in poultry, causing significant economic losses globally. Current live MG vaccines, such as the ts-11 strain, are effective but face challenges in mass application, particularly in day-old chicks. Eye-drop (ED) vaccination is the gold standard for MG control but is labour-intensive and unsuitable for large-scale hatchery operations. To address these limitations, the University of Melbourne developed a second-generation live MG vaccine, Vaxsafe® MG (strain ts-304), which expresses high levels of the GapA surface antigen, enhancing cytoadhesin capabilities while maintaining the safety and efficacy of the Vaxsafe® MG strain ts-11 vaccine. This vaccine has been evaluated for day-old chick administration using various methods, including ED, coarse spray (CS), gel spray (GS), and intra-nasal delivery. Novel AI-guided vaccine delivery options have been prototyped to automate vaccine delivery, providing a precise, high-throughput solution for commercial poultry operations. This article explores advancements in MG vaccination, focusing on the integration of the advanced Vaxsafe® MG ts-304 vaccine and novel automated vaccine delivery options.

Introduction

Mycoplasma gallisepticum (MG) is a significant pathogen worldwide, known for causing respiratory disease and reducing egg production in chickens and turkeys[1]. While biosecurity measures, antimicrobials, and maintaining MG-free replacement flocks are considered effective control strategies, vaccines have also proven successful in managing MG outbreaks[2, 3]. For years, different MG vaccines have been used to limit infection spread, prevent disease symptoms, and reduce egg transmission to offspring. Vaccination programs support long-term eradication efforts by displacing virulent field strains from site[4, 5]. There are inactivated, live attenuated, and recombinant MG vaccines available, each differing in their effectiveness depending on the specific goals of the vaccination program.

The host's response to MG infection typically involves a strong lymphoproliferative reaction, with much of the tissue damage caused by this immune activity[4]. A key step in infection is

the bacteria adhering to cells in the upper respiratory tract; mucosal antibodies play an essential role in preventing colonization by blocking MG from attaching to the host's epithelial cells[6].

Apart from respiratory illness, MG can cause drops in egg production and lead to egg transmission. Though the exact mechanisms are not fully understood, systemic immune responses may help protect the reproductive system after the respiratory tract is colonized[4, 7].

Humoral antibody levels do not correlate strongly with protection against infection, but circulating antibodies offer some benefit, and cell-mediated immunity contributes to MG clearance and host resistance[8].

MG can persist in hosts for extended periods, even when the immune response is robust[4].

Live attenuated vaccines work by colonizing the upper respiratory tract, producing both local and some systemic immunity. These vaccines can reduce or prevent infection, mitigate

clinical signs, and stop egg transmission. They have also been used to displace harmful MG strains in commercial flocks[9]. A practical, long-term approach is to vaccinate chickens early

in life to establish immunity; however, large-scale delivery—especially to day-old chicks—is challenging. The eye-drop (ED) method is considered the standard for MG management, but its labour-intensive nature makes it impractical for high-volume hatcheries. Therefore, more efficient vaccine delivery systems are necessary for effective large-scale vaccination.

Development of Vaxsafe® MG ts-304 Vaccine

The Vaxsafe MG ts-304 vaccine, developed by the University of Melbourne, is a second-generation live MG vaccine closely related to the ts-11 strain[10, 11]. It continuously expresses high levels of the GapA surface antigen, enhancing its cytoadhesin capabilities and improving cell attachment[10, 11]. Studies have demonstrated that MG ts-304 is safe and effective for day-old chicks[3, 12, 13] and 3-week-old turkeys[11], providing protection against virulent MG challenge. However, manual ED vaccination is commercially unfeasible for large-scale operations, and mechanical ED vaccination poses challenges due to the precision required for dose-sensitive vaccines.

Challenges and opportunities in vaccine delivery

Mass application methods such as coarse spray (CS) and gel spray (GS) have been explored[13] but often result in suboptimal vaccination, uncontrolled disease cycling,

unintended horizontal transmission, and delayed onset of protection. The industry has

identified the need for a shift to hatchery-based vaccination to improve uniformity, reduce costs, enhance biosecurity, and accelerate immunity development. However, no live MG

vaccines are currently registered for administration at the day-old chick stage, and no devices exist for accurate, efficient, high-throughput individual delivery of respiratory vaccines to day-old chicks.

To address these challenges, Bioproperties partnered with a leading innovator in hatchery equipment to

develop a novel IPC machine for automated vaccine delivery[14]. The IPC

machine utilizes a reverse spray technique to administer the vaccine into the choanal cleft, a key site for colonization by live respiratory vaccines. Research comparing IPC and ED methods revealed comparable colonization levels of MG ts-304 in day-old chicks, with both methods outperforming CS and GS techniques[14]. The IPC machine demonstrated high throughput, precision, and uniform vaccine delivery, making it suitable for large scale commercial layer and broiler breeder operations.

Another alternative could be, TARGAN®'s innovative AI-guided chick vaccination system (www.targan.com) that utilizes micro-dose ocular delivery and advanced feature recognition to ensure precise and efficient vaccine administration. Currently this approach achieves over 95% accuracy and can vaccinate up to 100,000 chicks per hour for coccidiosis vaccinations, ensuring uniform coverage and improved flock immunity in comparison to conventional spray methods. This delivery method could be adapted for Vaxsafe® MG ts304 vaccine application.

Results and discussion

Studies showed that intra-nasal vaccination using the IPC machine resulted in consistent colonization and protection against MG challenge, with uniform excretion of the vaccine

among individuals. The IPC machine's ability to deliver live respiratory vaccines like MG ts- 304 efficiently and precisely addresses industry needs for improved biosecurity, reduced labor costs, and accelerated immunity in poultry flocks[14]. This innovation has the potential to transform MG control strategies in poultry farming, enhancing flock health and

contributing to the economic sustainability of the industry.

Both the micro-dose ocular and IPC methods provide accurate dosing like the gold standard ED vaccination method, while lowering production costs.

Conclusion

The Vaxsafe MG ts-304 vaccine, combined with the novel AI guided delivery options, offers a revolutionary approach to MG management in poultry. By enabling consistent, precise and uniform delivery of live respiratory vaccines to day-old chicks, this advancement ensures effective protection against MG, paving the way for improved disease control and productivity while reducing dependency on antimicrobial use in commercial poultry operations. The integration of advanced vaccine technology and AI guided automated delivery systems represents a significant step forward in the fight against MG, benefiting both poultry health and industry profitability.

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A COMPREHENSIVE MOLECULAR INVESTIGATION AND EVOLUTION OF THE INFECTIOUS BRONCHITIS VIRUS IN INDIA REVEALS THE REGION-SPECIFIC MUTATIONS IN THE RECEPTOR-BINDING DOMAIN OF S1 GENE

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Infectious Bronchitis (IB) is an acute contagious disease of poultry caused by the infectious bronchitis virus (IBV), genus Gammacoronavirus of the family Coronaviridae. The objective of this study was to characterize the genotypic and pathological features of IBV circulating in chickens in major poultry-producing states of India from 2022-2024. A total of 1685 representative carcasses from 272 IB-suspected flocks were examined with the help of conventional and advanced diagnostic techniques. All the flocks under study were vaccinated with live/inactivated IB vaccines. A total of 33 flocks (12.2%) out of 272 showed PCR positivity for IBV. A total of 22 field isolates were successfully adapted in embryonated chicken eggs. Clinical signs, gross and histopathological lesions relevant to a respiratory/renal/reproductive system ailment were apparent in all IB cases. Thirty selected S1 gene PCR products were sequenced, and phylogenetic analysis revealed that all the study sequences were clustered with the Genotype I-1 (GI-1). Three consistent mutations were identified in the S1 protein from Northern India isolates: Ile131Leu, Asn306Asp, and Gly376Arg. Similarly, all the IBV isolates from Southern India had a consistent Glu179Ala substitution. Interestingly, these mutations were associated with the marked differences in tissue tropism of IBV variants, identified by IHC and real-time PCR. S1 epitope analysis predicted one amino acid variation in a potential linear B-cell epitope in the IBV 376Arg variant, located within HVR3, alters the local charge, which may potentially influence antigenicity and vaccine efficacy, however confirmation will require neutralization assays and reverse-genetics experiments. This comprehensive study provides valuable insights into IBV evolution and its implications for disease control in the Indian poultry sector.

COMPARATIVE EVALUATION OF TWO DIFFERENT IMMUNE COMPLEX INFECTIOUS BURSAL DISEASE VACCINES IN COMMERCIAL BROILER CHICKENS

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Infectious bursal disease (IBD) is a highly contagious and immunosuppressive viral disease of poultry that causes significant economic losses globally. Immune complex (IC) vaccines are designed to ensure timely vaccine virus replication regardless of maternally derived antibody (MDA) levels. GUMBOHATCH® is a next-generation immune complex live attenuated vaccine against infectious bursal disease virus (IBDV), formulated using egg-origin IgY. This formulation provides complete coating of the vaccine virus, preserves vaccine potency, and minimizes the risk of vaccine-induced immunosuppression. The present study aimed to comparatively evaluate the immunogenicity and bursal safety of GUMBOHATCH® and a standard-formulated immune complex IBD vaccine under commercial field conditions in broiler chickens. A field-level comparative study was conducted on four commercial broiler farms divided into two groups (A and B), each comprising approximately 10,000 birds. Broilers in Group A were vaccinated with a commercially available standard-formulated immune complex IBD vaccine, while Group B received GUMBOHATCH®. Both vaccines were administered subcutaneously at a dose of 0.2 ml on day 1 at the hatchery. Serum samples were collected from 15 birds per farm on days 28 and 35 post-vaccination to determine IBD antibody titers using the IDEXX ELISA kit. Bursa samples collected on day 35 were fixed in 10% buffered formalin and examined histopathological. Bursal lesions were scored according to the European Pharmacopoeia 6.0 guidelines. No IBD-related clinical signs were observed in either group throughout the rearing period. On day 35, both groups exhibited comparable and protective geometric mean titers (GMTs) (Group A: 3374; Group B: 3367), indicating similar humoral immune responses. However, histopathological evaluation revealed marked differences in bursal integrity. Group A showed severe lesions with 76–100% lymphoid depletion, follicular necrosis, cyst formation, and extensive fibrous connective tissue proliferation, resulting in an average bursal score of 4.5. In contrast, Group B exhibited only 26–50% lymphoid depletion with minimal follicular necrosis and a lower bursal score of 3. These findings demonstrate that while both vaccines induce protective immunity, GUMBOHATCH® provides superior bursal protection and reduced immunosuppression compared to the standard immune complex IBD vaccine.

MAGNIPLEX V877 : NOVEL IBD IMMUNOCOMPLEX VACCINE -BOON FOR SUSTAINABLE POULTRY PRODUCTION

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Infectious Bursal Disease (IBD) is a significant immunosuppressive viral disease affecting poultry worldwide. Several vaccination strategies are available but despite robust vaccination programs and strict biosecurity measures, many poultry farms continue to face subclinical and clinical IBD infections, often leading to immunosuppression and secondary bacterial infections and viral outbreaks. To evaluate the efficacy of an IBD V877 Strain Immuno-complex vaccine in providing IBD protection to long lived birds when used at DOC at farm in combination with HVT +301/B1. The study was done on 20 commercial layer farms involving 150,000 birds. DOC were vaccinated with Magniplex and HVT-301/B1 @ 0.2ml SQ. IBD ELISA titers were measured at 30days, 60 days 90 days. In high challenge areas Intermediate plus IBD vaccine in DW recommended at 12-14 Days of age. Serum samples were tested with help of IDEXX Kits. The study demonstrated 95% protection from IBD across all 20 commercial farms, with no clinical or subclinical outbreaks reported and with all performance parameters in normal range . IBD-ELISA titers ranged up to 5000 IU 35 days, 8000 IU range in 60 days, up to 12000 IU in 90 Day in breeder layers. Only 1 farm was reported to have a mixed viral infection, PCR confirmed positive for CAV and LPAI and negative for IBD in this 1 farm. The results of this study demonstrate that co-administration of Magniplex-HVT-301/B1 at the day-old commercial layers, provides comprehensive protection against IBD and MD in these commercial layers and breeders chickens. It ensures optimal flock performance, reduced mortality and disease control. This vaccination strategy of giving V877 with HVT-301/B1 at day old age at farms can be considered as a reliable approach for sustainable poultry production in high IBD disease-prone areas. V877 IBD strain protects the birds from both variant and virulent IBD infections.

PROVAC 4: AUGMENTING BREEDERS TITERS FOR A BETTER LIVABILITY AND IMMUNITY FOR BROILER HEALTH: AN EFFORT TO REDUCE ABUSE OF ANTIBIOTICS ADDRESSING THE ISSUE OF AMR FOR ONE HEALTH

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Broiler health in poultry depends upon the level of humoral immunity they receive from their breeder parents. The decreased MDA level of DOC titers exposes it to many viral and other bacterial diseases. The immunosuppression caused by these diseases further exposes birds for other respiratory diseases like IB, ND, LPAI and HPAI. To monitor the health status, livability and immunity status of DOC broilers chicks received from the Breeders parent given Inactivated 4 way killed vaccine with Kimber ND Strain, Lukert-IBD, M-41-IBV and Reo 2408/1733 strains. To monitor the maternal derived antibodies in DOC for ND, IB, IBD and Reo Virus. 25 different broiler flocks whose parent received Provac 4 once or twice between the age of 20-40 weeks were monitored for FCR, Mortality, infections and antibiotic treatment. The serum samples were tested with help of IDEXX ELISA Kits. All 100% of Broiler flocks (25/25) have shown very high titers for ND because of Kimber mesogenic ND strain in the range of 7000 IU or higher, IBD MDA levels were 5000 IU or higher, IBV titers up to 3000 IU or higher and REO virus up to 6000 IU or higher. All 100 % flocks monitored till 35 days were extremely healthy with no clinical signs of any disease and normal mortality range varying from 3-5% and FCR up to 1.5 in all the flocks. It was concluded from this study that Broilers who emanate from Breeders who have received single or double shots of Provac 4- an inactivated killed vaccine not only gives excellent titers in breeders but also corresponding high MDA levels in Broilers and these high protective titers in broilers which give them a good immunity to start healthy, which further reduces infections and limits antibiotic usage for AMR issues for One Health.

ASSESSING THE EFFICACY OF A NOVEL RECOMBINANT NEW CASTLE DISEASE -PROCERTA HVT -ND AGAINST THE VIRULENT NEW CASTLE DISEASE IN COMMERCIAL BROILERS

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Newcastle disease (ND) remains a significant threat to the poultry industry worldwide, with variable disease prevalence across regions and farms. Vaccination is the primary control strategy, but current protocols using live attenuated and inactivated vaccines do not always provide consistent protection. Recombinant HVT-ND vaccines combined with live ND vaccines offer a promising alternative to improve immunity and flock performance. To collate the data from 2 different HVT -ND vaccinated broiler flocks in high Virulent ND challenge areas. The study was conducted from January to August 2025 across three commercial broiler farms with a study on total of 600,000 birds who received 2 different recombinant vaccine at hatchery and same live vaccine at the same age in farms in a high ND challenge areas. Serum samples were collected at day-old and 35 days of age and tested for ND antibody titers using IDEXX and ID Vet ELISA kits. Mortality rates and corrected feed conversion ratio (cFCR) were recorded and analyzed. All the groups including recombinant vaccine from competitors groups have shown good Fusion protein ELISA demonstrating protection against New Castle disease virus. The best protective F protein ELISA titres with maximum uniformity was in the group where Procerta HVT ND was used. The flock with better FCR and reduced mortality was also the one which received Procerta HVT ND. There was difference of 0.2% in mortality and 0.1 points in cFCR till age of 35 days between Poulvac Procerta HVT -ND and second best performer group given competitor recombinant HVT-ND vaccine. It was demonstrated that Poulvac Procerta HVT-ND at hatchery and 1 live ND vaccine at farm around 10 days is found to be one of the best of vaccination protocol for commercial broilers NDV protection in a high challenge areas with maximum ND protection and lowest mortality and optimum FCR in these flocks.

REVIEW OF VACCINATION STRATEGIES AGAINST INFECTIOUS CORYZA IN POULTRY

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In India, poultry industry is continuously growing field also resulting many emerging and re-emerging disease problems. In this challenging field situation, poultry flocks need to be protected against various circulating pathogens. Some of the pathogens also diversifying its pathogenicity ultimately resulting in development of new serovars. Infectious coryza caused by *Avibacterium paragallinarum* one of the important poultry disease causes massive economical losses poultry farmers. Ventri Biologicals Vaccine Division is manufacturing Infectious Coryza Vaccine Inactivated since 1987 using A, B, C serogroup till 2016. Since 2015 despite of complete vaccination program, cases of Infectious Coryza were observed. Poultry Diagnostic Research Centre, VH Group further studied confirmed the existing serovar as C2 and a new highly pathogenic serovar C3 was isolated within Serovar C which was not getting protective immune response against existing Serovar C2. In 2016, Ventri Biologicals Vaccine Division incorporated new serovar C3 along with existing A, B, C2 to cater the field situation, which ultimately resolved the situation and no further field cases of Infectious coryza were observed till today. It shows that there is very limited cross protection within Serovar C. However, recently evidence of 1 or 2 new serovars were observed in all three A, B, C serogroups in Israel, South Africa, South America, China and C4 in Australia. Therefore, we need to closely monitor all serogroups of infectious coryza to mitigate the infectious by incorporating it into the vaccine formulation to avoid further economic losses.

SAFETY STUDY OF EXPERIMENTAL RECOMBINANT HVTND VACCINE: ONE DOSE, REPEATED DOSE AND OVER DOSE IN BIRDS

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Vaccination remains the most effective preventive strategy against major poultry pathogens, thereby improving flock health and farmer income. The recombinant herpesvirus of turkey–Newcastle disease (rHVT-ND) vaccine provides broad protection against two economically important poultry diseases, Marek's disease (MD) and Newcastle disease (ND). The present study was undertaken to evaluate the safety of the rHVT-ND vaccine in poultry. Specific pathogen-free (SPF) embryonated eggs (18-day-old) and day-old SPF chicks were used for the safety evaluation. The study was categorized into three phases: Safety-1 (single dose), Safety-2 (repeated dose), and Safety-3 (overdose). In each phase, birds were divided into in ovo-vaccinated groups, day-old chick-vaccinated groups, and corresponding control groups. Uniform management conditions, including feed, environmental parameters, and husbandry practices, were maintained throughout the study. In the Safety-1 study, a single dose of vaccine was administered to in ovo embryos and day-old chicks. In the Safety-2 study, a repeated dose was administered at a 14-day interval, while in the Safety-3 study, birds received an overdose of the vaccine. Birds were monitored daily for clinical health. Serological evaluation for ND antibodies was performed using ELISA, along with assessment of feed conversion ratio, haematology, serum biochemistry, post-mortem examination, and histopathological evaluation of the liver, spleen, kidney, lungs, and heart. All evaluated parameters, including ELISA antibody titres, haematological and biochemical indices, feed conversion ratio, and histopathological findings, remained within normal and acceptable limits, with no vaccine-related adverse effects observed. The study concludes that the experimental recombinant HVT-ND vaccine is safe for use in poultry and plays a crucial role in effective immunization against Marek's disease and Newcastle disease.

SAFETY STUDY OF RECOMBINANT HVTND VACCINE: SPREAD OF RECOMBINANT rHVTND VACCINE VIRUS FROM VACCINATED BIRDS TO NON-VACCINATED BIRDS AND NON-TARGET SPECIES

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Vaccination is a cornerstone of poultry health management and plays a vital role in preventing economically important diseases. The recombinant herpesvirus of turkey–Newcastle disease (rHVT-ND) vaccine confers protection against Marek's disease and Newcastle disease. This study was undertaken to evaluate the potential transmission of the rHVT-ND vaccine from vaccinated birds to non-vaccinated birds of the same species and to non-target species. The study was conducted using day-old specific pathogen-free (SPF) chicks and non-target species, including pigeons and quails. Experimental groups were categorized as: (i) vaccinated and non-vaccinated birds of the same species and (ii) vaccinated birds and non-target species. Comparative safety studies were performed using vaccinated and control SPF chicks, while pigeons and quails were included to assess cross-species transmission. All groups were maintained under identical management conditions, including feed, environment, and husbandry practices. Vaccine safety and transmission were assessed using viremia assays, polymerase chain reaction (PCR), and enzyme-linked immunosorbent assay (ELISA). Viremia testing conducted on day 9 post-vaccination detected viral presence only in vaccinated chicks, whereas no viremia was observed in non-vaccinated birds or in peripheral blood leukocytes of non-target species. Feather quills, spleen, and white blood cells collected at weekly intervals were subjected to PCR analysis. Viral DNA was detected exclusively in samples from vaccinated birds, with no amplification observed in non-vaccinated or non-target species. ELISA performed on days 14, 28, and 42 post-vaccination demonstrated antibody titres within acceptable ranges (≥ 993), with mean titres of 1563.26, 4674.79, and 6654.87, respectively. No Newcastle disease–specific antibodies were detected in non-vaccinated or non-target species. The findings demonstrate that the rHVT-ND vaccine does not transmit from vaccinated to non-vaccinated birds or to non-target species and is safe and effective for use in poultry vaccination programs.

DISSEMINATION OF EXPERIMENTAL RECOMBINANT HVTND VACCINE IN BIRDS

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Vaccination is a key strategy for preventing infectious diseases in poultry by stimulating immune responses and reducing pathogen circulation. Evaluating dissemination safety is essential to ensure that vaccines do not spread beyond vaccinated birds under field conditions. This study assessed the dissemination safety of the recombinant herpesvirus of turkey–Newcastle disease (RHVT-ND) vaccine following administration via in ovo (18-day-old SPF eggs) and in vivo (day-old SPF chicks) routes, with appropriate control groups. Birds were vaccinated according to the recommended dose and monitored for clinical signs, mortality, behaviour, and general health. Samples, including feather quills, spleen, white blood cells, kidney, lungs, liver, proventriculus, bursa, cloacal swabs, and oropharyngeal swabs, were collected at multiple intervals up to 42 days post-vaccination and tested by PCR for the presence of vaccine virus. PCR results indicated HVT virus in feather quills, spleen, white blood cells, bursa, liver, and kidney at all sampling points. Lung samples were positive only on days 7 and 14, and proventriculus samples only on day 14. Cloacal and oropharyngeal swabs collected on days 28 and 42 were negative, confirming no environmental dissemination. Newcastle disease antibody responses were assessed using ELISA on days 14, 28, and 42 post-vaccination. Vaccinated birds demonstrated antibody titres above the acceptance threshold (≥ 993), whereas control birds showed no detectable antibodies. The study confirms that the rHVT-ND vaccine is safe regarding dissemination under recommended conditions and supports its use in routine poultry vaccination programs.

STUDY OF INTERACTION OF RECOMBINANT HVTND VACCINE WITH OTHER LIVE VACCINES

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Vaccination is a cornerstone of poultry health management, enhancing bird immunity, productivity, and disease prevention. When multiple vaccines are administered concurrently or sequentially, it is essential to assess interaction safety to ensure that combined use does not compromise bird health or immune responses. This study evaluated the interaction safety of the recombinant herpesvirus of turkey–Newcastle disease (RHVT-ND) vaccine when administered alongside other poultry vaccines. Birds were divided into four groups: Group 1 (RHVT-ND alone), Group 2a (RHVT-ND + Infectious Bronchitis [IB]), Group 2b (RHVT-ND + Infectious Bursal Disease [IBD]), Group 2c (RHVT-ND + Newcastle Disease [ND]), and Group 3 as an unvaccinated control. Birds were monitored for clinical signs, mortality, gross lesions, and adverse reactions, including Marek's disease–specific indicators such as emaciation, dehydration, bone marrow anaemia, and splenic tumors. No clinical signs or pathological lesions were observed in any group. Serological and viremia assessments were conducted to evaluate immune response and virus replication. Blood and serum samples collected on day 21 post-vaccination demonstrated cytopathic effects in peripheral blood leukocytes of all vaccinated groups, whereas no effects were observed in the control group. ELISA assays confirmed that antibody titres against IB, IBD, and ND in co-administered groups were within acceptable ranges. The study demonstrates that experimental RHVT-ND vaccine is safe for concurrent administration with other poultry vaccines, does not result in unintended interactions, and does not compromise immune responses, supporting its use in standard poultry vaccination programs.

RECOMBINANT VACCINES: A BOON FOR POULTRY ENTREPRENEURS

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Recombinant vector-based vaccines, particularly recombinant herpesvirus of turkey (RHVT), provide poultry entrepreneurs with significant economic advantages over traditional vaccines through single-dose hatchery administration that confers lifelong immunity against multiple pathogens including Marek's disease, Newcastle disease, and Infectious Bursal Disease, thereby minimizing labor-intensive field boosters and reducing disease transmission. In the Indian poultry sector, marked by high-density farming and outbreak vulnerability, RHVT vaccines evade maternal antibody interference, enhance flock performance via improved feed efficiency and body weights, and lower mortality alongside antibiotic dependency, fostering resilience and export compliance. This technology emerges as a transformative boon, supporting sustained profitability and sector growth amid expanding commercial operations.

CHICKEN INFECTIOUS ANAEMIA (CIA) VACCINE: PROTECTION AGAINST HIDDEN OFFENDER

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Gyrovirus a smallest DNA virus amongst poultry pathogens causes chicken infectious anaemia (CIA). CIA is an immunosuppressive disease affecting young chickens of both layer and broilers causes great economic losses in poultry industry globally. CIA infection leads to lower hematocrit value, thymus regression ultimately chicks become susceptible to secondary infection, reduced growth which leads to substantial economic losses in poultry production. Control relies on vaccination of breeder flocks, biosecurity and hygiene practices. CIA live vaccine, one of the key tools for prevention of viral infection. Ventri Biologicals Vaccine Division successfully developed CIA live and killed vaccines for poultry welfare. Vaccination of 2-3 weeks old chicks via subcutaneous route effectively reduced CIA cases of young broiler and layer chicks in the field. Also vaccination of parent birds with combination of live and killed vaccine gives protective immunity also transfers maternal antibodies to protect early chick infection. This would effectively replace circulating pathogenic virus with vaccine virus, ultimately reduces disease severity, secondary infection and economic losses.

CIRCULATION OF VARIANTS OF INFECTIOUS BRONCHITIS VIRUS OF GI-13 AND GI-24 LINEAGE FROM CENTRAL INDIA

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Infectious bronchitis is one of the devastating diseases affecting the poultry industry worldwide. Infectious bronchitis virus exists in many genotypic and antigenic forms, often challenging prevention and control. A total of 60 samples from commercial broiler and layer flocks suspected to have respiratory and renal lesions were collected from the Nagpur region of Maharashtra state. A total of six isolates of infectious bronchitis virus were characterized. Immunohistochemically, IBV antigen was detected in the cytoplasm of epithelial cells of kidneys and trachea. Phylogenetic analysis based on the S1 protein gene revealed the involvement of nephropathic strains belonging to the GI-13 and GI-24 lineage of IBV. These isolates had only 79.95 % nucleotide identity with currently using Mass vaccine strain (M41) in India.

ENHANCING VACCINE SAFETY AND ANTIGEN PRESERVATION THROUGH BETA-PROPIOLACTONE (BPL) BASED VIRAL INACTIVATION: AN ALTERNATIVE TO FORMALDEHYDE INACTIVATION IN POULTRY VACCINE PRODUCTION.

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Inactivation of viruses is a critical step in the production of inactivated poultry vaccines, directly influencing product safety, immunogenicity, tissue reactions and overall quality. Formaldehyde has traditionally been used as an inactivation agent; however, its prolonged inactivation time and the possibility of residual reactive compounds raise concerns regarding antigen modification and vaccine safety. These limitations have encouraged the evaluation of alternative inactivation agents. Beta-propiolactone (BPL) is a well-established viral inactivating agent known for its rapid action and minimal impact on antigenic structures. The present study was undertaken to evaluate the suitability of BPL as an alternative to formaldehyde for the inactivation of Newcastle Disease Virus (NDV), Avian Infectious Bronchitis (IBV) and Low Pathogenic Avian Influenza (H9N2) LPAI, for vaccine manufacturing. NDV, IBV and LPAI propagated in embryonated chicken eggs and further antigen was inactivated using BPL at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ under controlled temperature and continuous mixing. Critical process parameters validated like inactivation kinetics by assessment of complete virus inactivation and hemagglutination activity. The results demonstrated complete inactivation of NDV, IBV and LPAI within the validated inactivation period using BPL, with no residual live virus detected. Hemagglutination activity remained stable throughout the process, indicating preservation of antigenic integrity. Unlike formaldehyde, which may leave residual reactive compounds, Beta-propiolactone (BPL) undergoes the rapid hydrolysis under controlled temperature to form non-toxic degradation products, primarily β -hydroxypropionic acid (3-hydroxypropionic acid) and acrylic acid, thereby eliminating residual cytotoxicity. BPL based inactivation process gives consistent and reproducible across consecutive runs. Vaccine prepared using BPL inactivated antigen complies protective immunity against NDV, IBV and LPAI. This study establishes Beta-propiolactone (BPL) as an effective and reliable alternative to formaldehyde for virus inactivation in poultry vaccine manufacturing. Adoption of BPL-based inactivation offers advantages in terms of improved antigen preservation, absence of toxic residues, and enhanced process efficiency, ultimately supporting vaccine safety, quality and farmer confidence.

VENGEM 9, FIRST INDIGENOUS LOW PATHOGENIC AVIAN INFLUENZA VACCINE INACTIVATED (H9N2)

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Low Pathogenic Avian Influenza (LPAI) H9N2 remains an economically important disease of poultry, causing reduced growth performance, poor feed efficiency, drop in egg production, increased mortality in complicated infections, and higher medication costs. These production losses, along with increased management interventions, pose persistent challenges to effective disease control. Until recently, the unavailability of an indigenously produced LPAI vaccine in India restricted preventive approaches and preparedness efforts. Development of a safe, effective, and locally manufactured inactivated vaccine is therefore critical for strengthening poultry health management. The present work describes the first successful indigenous manufacturing effort in India for an inactivated LPAI (H9N2) poultry vaccine, undertaken under regulatory test license approval. Prior to initiation of test batches, critical process parameters and critical quality attributes were systematically optimized. Process development was carried out based on a technology transfer and virus seed obtained from the National Institute of High Security Animal Diseases (NIHSAD), Bhopal. The LPAI H9N2 virus was propagated in embryonated chicken eggs to achieve the desired virus titre and inactivated in strict bio-security level environment as per CGMP. LPAI virus inactivation using beta-propiolactone (BPL) was validated under controlled temperature ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$) and further hydrolysed at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ to nullify residual effect of BPL. Inactivation kinetics was evaluated through infectivity testing and hemagglutination assays to confirm complete viral inactivation and preservation of antigenic integrity. Complete and consistent inactivation of the virus was achieved within the validated inactivation period, with stable hemagglutination activity observed throughout the process. BPL underwent rapid hydrolysis in aqueous conditions to non-toxic degradation products, ensuring absence of residual cytotoxicity and supporting vaccine safety. The vaccine safety and efficacy was tested as per standard guidelines and technology transfer. This work represents a significant milestone as the Vengem-9 is the first indigenous manufacture of an inactivated LPAI (H9N2) vaccine in India, establishing a robust, safe, and regulatory compliant manufacturing platform. The successful development of the individual LPAI and combination vaccines with ND strains strengthens national capability in controlling avian influenza and supports sustainable poultry production and farmer stability and economy.

INNOVATIONS IN POULTRY VACCINE RESEARCH: INDIA'S LEADING EDGE

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The poultry industry faces continuous threats from a multitude of viral diseases, requiring a shift towards innovative recombinant technologies and advanced surveillance. Development of a diverse vaccine portfolio, including advanced platforms such as Immune complex vaccines, VLPs, Recombinant Vector Vaccines which target multiple diseases simultaneously. Central to these Research advancements is the integration of cutting-edge molecular tools for precise disease detection and monitoring. The deployment of Next-Generation Sequencing and q-PCR assays enables the accurate genotyping of strains. A critical component of modern management is the DIVA (Differentiating Infected from Vaccinated Animals) strategy, implemented via specific ELISA techniques. This approach ensures vaccine programme integrity, facilitates epidemiological tracking, and supports compliance with international export standards. Furthermore, Molecular analysis is utilised to enhance strain identification and refine vaccine efficacy. By leveraging state-of-the-art facilities and multi-disciplinary scientific teams, these innovations aim to accelerate timelines from design to market readiness. Despite technical, cost, and regulatory challenges, the integration of broad-spectrum vaccines with sophisticated diagnostics is essential for strengthening poultry health management and improving economic outcomes for producers globally.

THE IMPORTANCE OF SPF-EGGS IN MANUFACTURING AND TESTING OF POULTRY VACCINES.

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Specific Pathogen Free (SPF) eggs, produced by flocks free from designated avian pathogens as per European Pharmacopoeia standards (e.g., Avian leukosis, Newcastle disease, and infectious bronchitis), are indispensable for propagating egg-adapted viruses in poultry vaccine production. These embryonated eggs provide a natural, cost-effective biological system superior to cell lines, eliminating risks from animal-derived culture media contaminants like transmissible spongiform encephalopathies (TSE). In manufacturing, SPF eggs enable direct harvesting of allantoic fluid or embryos post-inoculation and incubation, bypassing trypsinization or washing steps required in cell cultures, which simplifies purification—often minimal for cost-sensitive poultry mass vaccination via non-parenteral routes like spray or drinking water. Primary cells such as chicken embryo fibroblasts (CEF) from SPF eggs support viruses adapted to cell culture, while live SPF chickens are used rarely for non-adapted strains due to welfare and logistical challenges; SPF eggs are critical for vaccine potency assays (e.g., live virus titration), extraneous agent detection, and serum neutralization, preventing false positives from maternal antibodies or contaminants in conventional eggs. Derived primary cells (CEF, chicken embryo kidney cells) facilitate in vitro validations under GMP/GLP, while SPF chickens enable essential in vivo safety (e.g., overdose, reversion to virulence) and efficacy trials (serology, challenges) to confirm immunity duration in target birds. Amid rising global poultry demands and emerging threats like avian influenza, SPF eggs remain irreplaceable despite resource-intensive production (isolated housing, feed/water sterilization), as no alternative substrate matches their safety, scalability, and regulatory compliance for live/inactivated poultry vaccines. Ongoing efforts to reduce in vivo tests via in vitro alternatives underscore SPF eggs' foundational role, balancing costs with superior vaccine purity and efficacy in modern biosecurity frameworks.

Theme 3

*Diagnostics, Digital Technologies
and Artificial Intelligence*



DESIGN AND DEVELOPMENT OF LOW COST AND USER-FRIENDLY ELECTRIC STUNNER FOR MEAT ANIMALS INCLUDING POULTRY BIRDS

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As per “The Prevention of Cruelty to Animals Act, 1960 (Act No 59 of 1960); Chapter III (3)”, poultry birds are allowed to be slaughtered (killed) for food purpose. However, slaughter of poultry birds should not inflict unnecessary pain or suffering. Further, as per “Food Safety and Standards (Licensing and Registration of Food Businesses) Regulations 2011; Part IV (See Regulation 2.1.2(1)(5); A. Slaughter House; 4.0 Slaughter; 4.1 Stunning”, it is mandatory to stun (making unconscious with low voltage electric current for few seconds for painless killing) the poultry birds before slaughter. At present, the electric stunners for poultry birds available in the market are expensive (about 1 lakh rupees) and not useful for small scale poultry meat shops who slaughter poultry birds manually in batches of about 5 birds. Hence, a low cost and user-friendly electric stunner has been designed, developed, tested and validated which costs about Rs. 4,000 only. The same stunner can be used for other meat animals also. Small scale poultry butchers can conveniently stun and slaughter poultry birds in batches of 5 birds with the newly designed electric stunner. The newly designed electric stunner consists of five plastic containers with holes at the bottom for resting the poultry birds, five plastic containers for collecting blood, a 2.5 ft X 4.0 ft plastic sheet equipped with handles for wall mounting, an electrical unit to dispense 70 to 100 V output, and two plastic clamps integrated with electric cables. Live poultry birds (1 to 5) are to be placed in the plastic containers so that the head and neck comes out from the hole at the bottom. Two plastic clamps are fastened above hock joint. Electric current (70 to 110V) is passed for 10 to 15 seconds depending on the breed and size of bird. Soon after passing electric current, birds will be in unconscious state in tonic phase i.e., contraction of all muscles. Birds are to be slaughtered before they enter clonic phase (violent movements) for painless killing. In view of non-availability of regular power supply in remote and rural areas, a technology on “Portable electric stunner for meat animals & poultry” has been developed (Design patent No. 431610-001; date 25-09-2024). Further, another technology on “Solar powered portable electric stunner for meat animals & poultry” has also been developed (Patent application No. 202531023134; date 15-03-2025). These technologies have already been commercialized to a firm in Hyderabad.

GLOBAL PREVALENCE OF NATURAL INFECTION OF CHICKEN INFECTIOUS ANEMIA VIRUS (CIAV) IN POULTRY THROUGH A SYSTEMATIC REVIEW, META-ANALYSIS AND AI POWERED LITERATURE MINING

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Chicken infectious anemia virus (CIAV) is an economically important pathogen in chickens causing anemia, immunosuppression, and mortality in young poultry worldwide. The first global quantitative prevalence of natural CIAV infection was studied through a systematic review and meta-analysis of 118 studies, including 29,892 samples tested by serological, molecular, or immunological methods. A random-effects meta-analysis with logit transformation of proportions was performed using R language and a pooled prevalence of 52.1% (95% CI: 45.4%–58.6%) with considerable heterogeneity ($I^2 = 98.5\%$) was estimated. Subgroup analyses revealed significant differences in the diagnostic method used ($Q_5 = 174.5$, $p < 0.001$) and in the study period ($Q_3 = 34.45$, $p < 0.001$). Meta-regression showed 37% of the inter-study variance. Additionally, an AI-powered tool, Kirumi, was designed to automate literature mining and the findings were validated against manually curated data, confirming its efficacy. Kirumi retrieved 1,913 CIAV-related records and confirmed 203 overlapping with the manually curated dataset, reinforcing the robustness of data collection. These results underscore the widespread nature of CIAV infection globally and demonstrate the value of integrating AI in epidemiological research, providing a foundation for improved surveillance and disease control in poultry production.

SEROLOGICAL PATTERN OF IBD ELISA TITRES IN BROILER PARENTS AND ITS CORRESPONDING BROILER CHICKS IN RELATION TO MABs

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Seroprevalence and related risk factors of infectious bursal disease, a viral infectious disease, primarily affecting young chicks, highly contagious, immunosuppressive viral infection and causing severe economic and production losses. The study has been carried out in broiler breeders by IBD ELISA titres in relation to maternal antibodies (MABs), age and sex. The sera sample collections of broiler breeder birds and its corresponding chicks from various age groups was used for the investigation and the existence of IBD titres was checked. An indirect enzyme-linked immunosorbent assay test (ELISA) approach was used to process these serum samples. Data has been compiled for the last 12 months during 2024 and 2025 from different broiler breeders. Early age of the birds (0-20 weeks) showed comparatively lower titres (Ranges from 50-3000). Middle aged (21-45 weeks) showed moderated type of titre values (Ranges from 3000-6000). Advanced aged birds (46 weeks and above) were showing elevated range of the titres (Ranges from above 6000). It has been also recorded the relatively higher titre values in males compared to females in all age group including MABs. Proper and timely vaccination of the birds contributed in an uniform of the titres (CV% less than 30). Exceptionally higher seroprevalence in the commercial broiler birds (Age 3-4 weeks) present study indicated a distribution of the disease and one of the potential threats for poultry production. So, chicken vaccinations and improved management system should be warranted in order to control the diseases effectively. Proper vaccination curtailed the risk of IBD prevalence in the off-springs by showing elevated titres in the advanced ages.

Theme 4

*Antimicrobials, AMR and
Alternatives to Antimicrobials*

POULTRY PATHOGENS AND ANTIMICROBIAL RESISTANCE: A ONE HEALTH IMPERATIVE FOR SUSTAINABLE FOOD SYSTEMS

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Introduction

Antimicrobial resistance (AMR) has become one of the defining public health threats of modern times, progressively eroding the effectiveness of antimicrobial therapies essential for safeguarding human and animal health. The rapid emergence and global dissemination of drug-resistant pathogens now compromise infection control, clinical outcomes, and food system resilience across interconnected human, animal, and environmental sectors. Current estimates indicate that AMR contributes substantially to global mortality, with the heaviest burden borne by low- and middle-income countries (LMICs), where inadequate regulation of antimicrobial use, limited diagnostic capacity, and environmental contamination accelerate resistance development (Ho et al., 2025).

The drivers of AMR extend far beyond clinical settings. Intensive food production systems, particularly poultry farming, have emerged as critical reservoirs and amplifiers of drug-resistant bacteria and resistant genes. Intensive poultry production is characterised by high stocking densities, rapid turnover, and antimicrobial use for disease prevention and productivity enhancement. These conditions favour the selection and persistence of antimicrobial-resistant pathogens, including *Salmonella*, *Escherichia coli*, and *Campylobacter*, which can be transmitted to humans through food, occupational exposure, and environmental dissemination. The capacity of these organisms to exchange resistance determinants further magnifies the risk, reinforcing the interconnected nature of AMR across species and ecosystems. The poultry-pathogen interface thus represents a pivotal point in the global AMR continuum. Resistant bacteria originating in poultry systems do not remain confined to farms but circulate through processing environments, retail food chains, wastewater, and natural ecosystems, contributing to a shared resistome that affects both animal and human populations (Bustamante et al., 2025). These dynamics highlight AMR as a quintessential One Health challenge, driven by the convergence of agricultural practices, microbial ecology, and public health vulnerabilities.

Ensuring sustainable food systems in the face of AMR demands a paradigm shift in poultry health management. Reducing reliance on antimicrobials while maintaining productivity, animal welfare, and food safety requires integrated strategies that emphasise prevention, biosecurity, microbiome management, and the adoption of effective antimicrobial alternatives. Within this framework, innovative approaches- such as phytochemical compounds, biological interventions, and precision nutrition- offer promising avenues to disrupt pathogen transmission and limit resistance selection. This chapter examines the role of poultry production in the AMR landscape and explores emerging solutions aligned with One Health principles to support resilient and sustainable food systems.

Global expansion of poultry production and antimicrobial use

As LMICs transition toward higher income levels, demand for affordable, high-quality animal protein continues to rise steadily. Poultry products, particularly eggs and chicken meat, have emerged as primary contributors to meeting this demand due to their cost-effectiveness and production efficiency. According to the Food and Agriculture Organization, global production reached approximately 87 million tonnes of eggs and 123 million tonnes of poultry meat in 2017, accounting for nearly 37% of total meat production (Hedman et al., 2020).

The rapid expansion of food-animal production has been accompanied by a parallel increase in antimicrobial use (AMU), raising significant concerns regarding the emergence and spread of AMR. Projections suggest that agricultural intensification will result in a 67% increase in antimicrobial consumption by 2030, largely driven by LMICs. From 2000 to 2010 alone, antibiotic use across 71 countries increased by over 36%, with Brazil, Russia, India, China, and South Africa contributing more than 75% of this rise (Laxminarayan et al., 2016). In several LMICs, AMU levels have already equalled or exceeded those observed in high-income countries, reflecting income growth, intensification of production, and weak regulatory oversight.

Poultry is the fastest-growing livestock sector globally, with an average annual growth rate of approximately 5% over the past five decades- substantially higher than beef, pork, or small ruminants. Chickens (*Gallus gallus domesticus*) account for nearly 90% of global poultry production, with an estimated global population exceeding 23 billion birds (FAO, 2019). The dominance of poultry production is attributed to several biological and economic advantages, including small body size, short production cycles, efficient feed conversion, and adaptability to diverse environments. These characteristics have facilitated the rapid intensification of poultry farming, particularly in LMICs transitioning from subsistence to market-oriented production systems.

The use of antimicrobials in poultry production dates to the mid-20th century, following the discovery that sub-therapeutic antibiotic administration enhanced growth performance. Antimicrobial growth promoters (AGPs) soon became integral to intensive poultry systems, particularly in post-war Europe and North America, and later diffused globally. Regulatory responses to AGP use have diverged across regions. While the European Union banned AGPs in 2006 and several OECD countries subsequently followed suit, AGPs remain widely permitted in many non-OECD countries, including major poultry-producing nations. Evidence suggests that AGP bans have minimal economic impact in high-income settings with robust biosecurity but may pose challenges in LMICs where antimicrobials often substitute for inadequate hygiene and disease prevention measures (Hedman et al., 2020).

Poultry production systems in LMICs and AMR risk

Globally, family-managed and small-scale poultry systems dominate production, particularly in LMICs. These systems range from extensive scavenging operations to semi-intensive and intensive household-level enterprises. While intensification has improved productivity and income generation, it has also increased reliance on routine antimicrobial administration through feed and water. Small-scale intensive poultry operations frequently lack adequate biosecurity, sanitation, personal protective equipment, and safe manure disposal practices. Antimicrobials are often obtained without prescription, and awareness of AMR among farmers remains limited. Close and continuous contact between poultry, other domestic animals, and household members creates high-risk environments for zoonotic

transmission of resistant bacteria and resistance genes.

Transmission pathways of poultry-associated AMR

The AMU in poultry production contributes to AMR dissemination through multiple interconnected pathways, including occupational exposure, foodborne transmission, and environmental contamination. Numerous studies have demonstrated elevated carriage of resistant bacteria among poultry workers and farming households. In resource-limited settings, inadequate slaughter hygiene and informal market chains further amplify foodborne AMR risks. Genomic investigations have provided compelling evidence of shared resistance determinants between poultry and human bacterial populations, highlighting the role of horizontal gene transfer and reinforcing the One Health nature of AMR transmission (Islam et al., 2024).

Poultry production generates substantial quantities of manure and wastewater, which are commonly reused as fertilizer or livestock feed in LMICs. While poultry litter is nutrient-rich, it also serves as a significant reservoir of antimicrobial residues, resistant bacteria, and antimicrobial resistance genes (ARGs). Environmental dissemination occurs via soil, surface water, groundwater, aerosols, insects, and flooding events, particularly in tropical regions. Studies have demonstrated that ARGs can persist in soil for years after poultry waste removal, altering microbial communities and facilitating resistance maintenance (Mu et al., 2015). The environmental resistome thus acts as a long-term reservoir linking animal, human, and ecological health.

Barriers to Antimicrobial Stewardship in poultry systems

Despite global recognition of AMR as a One Health priority, antimicrobial stewardship programs (ASPs) remain weakly implemented in many LMICs. Key barriers include limited surveillance capacity, inadequate political commitment, absence of standardized AMU data, and insufficient laboratory infrastructure. Misconceptions regarding antibiotic use are widespread among small-scale poultry farmers, compounded by over-the-counter availability of antimicrobials and limited access to veterinary services. Studies consistently report prophylactic use of critically important antimicrobials, poor understanding of AMR, and economic pressures driving inappropriate practices.

Effective veterinary services are essential for biosecurity, disease prevention, and responsible antimicrobial use. However, assessments by international agencies indicate that veterinary systems in many LMICs remain under-resourced, fragmented, and poorly regulated. Outdated policies, lack of post-market drug surveillance, and circulation of substandard veterinary pharmaceuticals further undermine stewardship efforts.

Strengthening veterinary education, regulatory frameworks, and international collaboration is critical for addressing AMR at the poultry-human-environment interface.

Implications for One Health- oriented interventions

The convergence of intensified poultry production, widespread AMU, environmental contamination, and weak governance structures underscores AMR as a systemic One Health challenge. Reducing AMR emergence requires coordinated interventions spanning poultry management, waste handling, surveillance, farmer education, and veterinary capacity building. Comprehensive strategies integrating improved biosecurity, alternatives to antimicrobials, environmental risk mitigation, and context-specific stewardship programs are essential to safeguard poultry productivity while protecting public and environmental health (Mu et al., 2015; Nhung et al., 2017).

Antimicrobial alternatives in poultry

In poultry production, the widespread misuse of antimicrobials has accelerated the emergence of multi-drug-resistant *Salmonella* spp., *Escherichia coli*, and *Campylobacter* spp., posing serious threats to meat safety and public health through the food chain and horizontal transfer of resistance determinants. In response to the escalating threat of AMR and the urgent need to reduce antibiotic dependence in animal production systems, contemporary research has converged on two complementary strategies: the development of novel antimicrobial agents and the implementation of effective non-antibiotic alternatives. Heightened concerns related to environmental sustainability, food safety, and public health have further accelerated the transition toward green, non-toxic, and residue-free feed additives, positioning these interventions at the forefront of innovation in modern poultry production (Biswas et al., 2024).

Robust evidence indicates that microbiome-modulating interventions- particularly probiotics, prebiotics, and synbiotic- enhance intestinal homeostasis, improve growth performance, and reduce pathogen colonisation. Organic acids and essential oils, through their antimicrobial and gut-modulatory properties, play a pivotal role in maintaining intestinal integrity and controlling enteric pathogens. Emerging modalities such as postbiotics, comprising bioactive metabolites and microbial cell components, and bacteriophages offer targeted, precision-based pathogen control strategies. In parallel, advances in nanotechnology and antimicrobial peptide design represent promising avenues for improving pathogen specificity and nutrient utilisation (Acharya and Barsila, 2025). Collectively, these approaches align with the Sustainable Development Goals and underscore the need for rigorous research, regulatory validation, and context-specific implementation to support sustainable, resilient, and antibiotic-responsible global poultry production systems.

Conclusion

Emerging antimicrobial alternatives, including phytochemical compounds, EOs, probiotics, postbiotics, bacteriophages, nanotechnology-based interventions, and precision nutrition strategies, demonstrate considerable promise in reducing pathogen burden and limiting resistance selection. When strategically combined and supported by robust surveillance, regulatory oversight, and farmer engagement, these innovations can reshape poultry production into a more resilient and responsible system. However, their successful adoption requires context-specific validation, economic feasibility, and alignment with regulatory and consumer expectations. Ultimately, addressing AMR in poultry demands coordinated action across disciplines and sectors, integrating scientific innovation with policy, education, and environmental stewardship. Embedding One Health principles into poultry production systems not only strengthens control of antimicrobial resistance but also advances broader goals of food security, public health protection, and sustainable development. By rethinking how poultry is produced and protected, the global community can transform a major driver of AMR into a powerful lever for healthier, safer, and more sustainable food systems.

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NANO-INTERVENTION IN POULTRY PATHOPHYSIOLOGY: PRECISION NUTRIENTS FOR HIGHER PRODUCTIVITY

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Modern poultry production is continuously exposed to multiple physiological challenges such as gut dysbiosis, oxidative stress, heat stress, immune suppression, and metabolic imbalance. These pathophysiological factors collectively impair feed efficiency, intestinal integrity, and organ function, resulting in poor productivity and increased antibiotic dependence. Nano-nutrient technologies offer a transformative approach by directly influencing cellular mechanisms and correcting the underlying physiological dysfunctions responsible for performance loss.

Emerging Nano-Betaine provides targeted osmoregulatory support, correcting dehydration and cellular stress pathways by regulating osmotic pressure and enhancing hepatic methylation. Under heat stress, conventional betaine fails to reach deeper tissues, whereas nanostructured betaine improves, protein deposition, and membrane stability through higher bioavailability.

Nanobutyryl addresses the pathology of intestinal inflammation and impaired epithelial tight junctions by delivering sustained butyric acid in distal gut regions. This prevents villus shortening, supports crypt architecture, reduces pathogen translocation, and enhances nutrient absorption—directly improving FCR and growth.

NanoGABA targets stress-related neuroendocrine pathways controlling corticosterone release. Heat and stocking density activate hypothalamic stress circuits that reduce feed intake and immunity. Nano-engineered GABA penetrates neuronal receptors more efficiently, restoring calm state, improving feed intake, and reducing productivity losses.

Nanominerals such as nano-zinc, selenium, copper and manganese improve antioxidant defence, mitochondrial efficiency, and immune cell activation. Their nano-size lowers antagonism and enhances intracellular mineral usage, supporting skeletal health, reproductive performance, and disease resilience at lower doses.

Collectively, nanotechnology modulates underlying pathophysiology rather than simply supplementing nutrients, providing a precision approach for antibiotic-free poultry productivity and healthier commercial flocks.

SYNERGISTIC COMBINATION OF HERBAL EXTRACTS, ESSENTIAL OILS & PROBIOTIC: A NOVEL TOOL AGAINST ANTIMICROBIAL RESISTANCE, STRENGTHENING POULTRY HEALTH, FARMERS ECONOMY AND PUBLIC FOOD SAFETY.

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Antimicrobial resistance, compromised immune, respiratory and gut systems of broiler birds are thought to be one of the most expensive problems in the poultry industry. To evaluate the effectiveness of various combination of commercial preparations containing different herbal extracts (Herbal Care WS®, Detox 365®, Salcoli kill CRD Care®, IBH HPS Care®), with essential oils (Respiraksha®) rich in bioactive compounds, phenolic, and flavonoids—demonstrated Antimicrobial, Antiviral, Anti-coccidial, Anti-inflammatory, Hepatoprotective and Immunomodulatory properties along with supplementation of probiotics and prebiotics (Enzyprolite-P®). The field study was conducted on several broiler flocks subjected to routine environmental and management stressors. Combination of above medicines were delivered via drinking water in a form of Daily scheduled program, under standard commercial rearing conditions. Key performance indicators, including body weight gain, feed conversion ratio (FCR), metabolic, microbial, toxic, nutritional disease incidence, mortality rate, were compared between flocks. Indicated a significant positive and synergistic response, enhancing nutrients absorption, improving gut integrity, and suppressing pathogenic microorganisms, which collectively resulted in better FCR and growth performance. Additionally, respiratory health was supported, with faster recovery during stress period. Treated flocks also showed higher body weight (100-150 gm), improved FCR (Lower than 0.5 to 1 Point), reduced mortality (2-5% less than other flocks), and better flock uniformity compared to other flocks. Activity of the studied herbal complex suggests its use as an alternative approach and presents a promising, natural, and sustainable alternative to conventional reliance on various chemicals & antibiotics. Overall, this novel tool and invention provides a practical solution to transform farmer's economy by strengthening poultry health, welfare and public food safety by addressing the growing concerns surrounding antimicrobial resistance.

POTENTIAL OF CINNAMALDEHYDE AND CITRAL ESSENTIAL OIL AS A POSSIBLE ANTIMICROBIAL AGAINST *E. COLI* INFECTION IN BROILER CHICKENS

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This study investigated in vitro effects of cinnamaldehyde and citral essential oils (EOs) on the inhibition of *E. coli* and their in vivo effects on growth performance and gut health of broiler chickens. A total of 240 one-day-old broiler chicks were divided into six groups and assigned to challenge with *E. coli* and supplementation with EO mixture: A, normal control; B, 1×10^8 CFU/ml of *E. coli* on day seven; C, *E. coli* on day seven and antibiotic lincospectine from day one in feed; D, *E. coli* and EO mixture through drinking water @1:2000 from day one; E, EO mixture from day one @1:2000; F, *E. coli* and EO mixture @1:500 from day 10-21. Birds supplemented with EO mixture showed significantly higher body weight, lower feed intake and better FCR during the experiment as compared to control infection group. FIC index of combination of EO revealed additive effect. Supplementation of EO mixture successfully reduced the caecal load of *E. coli* throughout the experimental period. Lesions in all the segments of intestine were less severe in supplemented groups indicating reduced invasion of *E. coli*. These findings support the potential antimicrobial effects of EO mixture in *E. coli* challenged broilers chickens.

ANTIMICROBIAL EFFICACY OF CINNAMALDEHYDE AND CITRAL ESSENTIAL OIL MIXTURE AGAINST AVIAN PATHOGENIC E. COLI INFECTION IN BROILER CHICKENS.

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The present study is aimed to appraise the impact of supplementation with cinnamaldehyde and citral essential oil (EO) mixture in broiler chickens infected by pathogenic *E. coli*. A total of 240, one-day-old chicks were divided into six groups and assigned to challenge with *E. coli* and supplementation with EO mixture: A, normal control; B, 1×10^8 CFU/ml of *E. coli* on day seven; C, *E. coli* on day seven and antibiotic lincospectine from day one in feed; D, *E. coli* and EO mixture through drinking water @1:2000 from day one; E, EO mixture from day one @1:2000; F, *E. coli* and EO mixture @1:500 from day 10-21. On the 3rd, 7th and 14th day post infection (dpi) with *E. coli*, six birds from each group were randomly sacrificed and observed for gross and histopathological observations. Grossly, liver from most of the birds in group B showed marked congestion, fibrinous perihepatitis, pericarditis and lungs revealed congestion. Histopathologically, birds in group B showed perihepatitis with fibrin meshwork in liver, rosette shaped coli granuloma in the lung, pericarditis, muscular haemorrhages in heart, necrosis of tubular epithelial cells, proteinaceous casts in the tubular lumen, interstitial and glomerular haemorrhages in kidney and mild lymphoid depletion with congestion of red pulp and thickened splenic artery. Severity of lesions was less pronounced in groups supplemented with EO mixture at all the intervals as compared to control infection group. Significant reduction in the lesions in all the supplemented groups supports the potential antimicrobial effects of EO mixture in *E. coli* challenged broilers chickens.

ANTIMICROBIAL EFFECT OF METAL NANOPARTICLES ON *E. COLI* INDUCED PATHOLOGY IN BROILER CHICKENS

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Colibacillosis is a prevalent bacterial disease in the poultry industry throughout the world caused by avian pathogenic *E. coli*. The multidrug resistance in *E. coli* strains makes it a potential reservoir for gene resistance possessing zoonotic health risks. To combat the antibiotic resistance, alternative techniques for diagnosis and treatment of *E. coli* are need at the moment. The present research was outlined to elucidate the therapeutic potential of metal nanoparticle mixture as an alternative for the antibiotic resistance in *E. coli* infection. During the research, the revival of *E. coli* isolates was done and confirmed by PCR. One of the *E. coli* isolates positive for both genes was used for challenging the broilers at the concentration of 1×10^8 CFU/bird by giving 1 ml inoculum through oral route. A notable difference ($P < 0.01$) in the efficacy of Ag nanoparticles followed by the mixed metal nanoparticle mixture was seen in the in vitro assay as compared with other metals viz. Mg, Cu, Fe, Se, Zn, Cr, Sn. In the present experiment, the addition of metal nanoparticle mixture (1–28 days) in birds challenged with *E. coli* infection showed significantly ($P < 0.01$) higher body weight, lower feed intake and comparatively better FCR in respect with other groups. Histopathological lesions in the lungs revealed congestion, haemorrhages, exudates and infiltration of leucocytes. Severe pericarditis and perihepatitis were evident. Peritonitis with distended caeca was observed. Haemorrhages in lamina propria, disruption of villi, sloughing of mucosa along with mononuclear infiltration of cells was found in the intestine. Nonetheless, significant ($P < 0.01$) reduction in intensity of the lesions and bacterial colonization in intestine of birds fed with nanoparticles from the 10th day of age was observed at 21st day of age. The result of present study indicates that metal nanoparticles can be further explored as an alternative to antibiotic growth promoters

Theme 5

*Food Safety and
Foreign Trade Policy*

FOOD SAFETY AND GOVERNMENT POLICIES

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Foods-vegetables, cereals, milk, meat, fish, poultry, and eggs—constitute a major component of India's nutritional security. Food safety is a critical component of public health, nutrition security, and economic development. It refers to the handling, preparation, and storage of food in ways that prevent contamination and ensure that food is safe for consumption. Unsafe food poses serious health risks, contributes to disease burden, undermines consumer confidence, and affects domestic and international trade. Government play a central role in ensuring food safety through policy formulation, legislation, regulation, surveillance, enforcement, and capacity building. In India, food safety governance has evolved significantly over the last two decades, moving from fragmented commodity-based regulations to an integrated, science-based legal framework. India has a complex regulatory ecosystem involving multiple agencies such as the Food Safety and Standards Authority of India (FSSAI), Department of Animal Husbandry & Dairying (DAHD), Bureau of Indian Standards (BIS), and state-level bodies.

Animal-source foods contribute significantly to India's dietary protein intake. However, these foods are prone to contamination by biological, chemical, and physical hazards throughout the value chain—farm, transport, processing, distribution, and retail. In recent years, food-borne illnesses, antimicrobial resistance (AMR), adulteration, and environmental contamination have raised concerns over the safety of animal foods. The Government of India has enacted a robust set of laws and regulatory mechanisms to ensure safety and quality.

Major Hazards in Animal-Derived Foods

i. Biological Hazards

- Food-borne microbes: *E. coli* O157:H7, *Staphylococcus aureus*, *Clostridium* spp. *Salmonella*, *Listeria monocytogenes*, *Campylobacter*, *Brucella*, *Mycobacterium bovis*, etc.
- Viruses and parasites: Hepatitis E, Nipah virus (in pigs), *Toxoplasma*, *Taenia* spp.

ii. Chemical Hazards

- Antibiotic residues
- Pesticide and heavy metal contaminants in feed and water
- Environmental pollutants

iii. Physical Hazards

- Foreign materials from equipment, packaging, or unhygienic handling.

These hazards necessitate stringent laws and monitoring systems across the entire value chain.

Regulatory Framework for Food Safety in India**Importance of Food Safety**

Food safety is essential for:

- Protection of public health by preventing foodborne illnesses
- Ensuring nutritional security, especially for vulnerable populations
- Enhancing consumer confidence in the food system
- Supporting agricultural growth and value addition
- Facilitating domestic and international trade
- Meeting international obligations under Codex Alimentarius and WTO–SPS Agreement

Food Safety Challenges**Key challenges affecting food safety include:**

- Microbial contamination (bacteria, viruses, parasites)
- Chemical hazards (pesticide residues, veterinary drugs, heavy metals)
- Adulteration and food fraud
- Informal and unorganized food sectors
- Inadequate hygiene and sanitation practices
- Antimicrobial resistance linked to food animals
- Climate change impacts on food production and safety

Evolution of Food Safety Regulation in India

Prior to 2006, food regulation in India was governed by multiple laws such as the Prevention of Food Adulteration Act, Fruit Products Order, Meat Food Products Order, and Milk and Milk Products Order. These laws often overlapped and lacked uniformity. To address these limitations, the Government of India enacted the Food Safety and Standards Act (FSS Act), 2006, establishing a unified and science-based approach to food regulation.

i. Food Safety and Standards Act, 2006 (FSS Act)

The FSS Act, 2006 is the umbrella legislation for food safety in India. It created the Food Safety and Standards Authority of India (FSSAI) and consolidated multiple food laws.

Key Features

- Risk-based regulation rather than end-product testing alone
- Responsibility of food safety placed on Food Business Operators (FBOs)
- Emphasis on self-compliance supported by regulatory oversight
- Provision for surveillance, recall, and emergency response

Food Safety and Standards Authority of India (FSSAI)

The FSSAI is the apex body responsible for food safety regulation in India.

Functions of FSSAI

- Framing food safety standards and regulations
- Licensing and registration of food businesses
- Conducting food safety surveillance and monitoring
- Laying down guidelines for food testing laboratories
- Capacity building and training
- Risk communication and consumer awareness

Key Government Policies and Initiatives**Risk-Based Food Safety Regulation**

Government policies promote risk-based inspection and surveillance, focusing regulatory efforts on high-risk foods and processes rather than uniform enforcement.

National Food Safety Surveillance

- Routine and targeted food sampling
- Surveillance of foodborne pathogens and contaminants
- Linkage with public health surveillance systems

Eat Right India Movement**A flagship initiative of FSSAI aimed at:**

- Promoting safe, healthy, and sustainable diets
- Improving food hygiene practices
- Reducing food adulteration and trans fats
- Encouraging responsible food consumption

Hygiene Rating and FoSTaC

- 1 Hygiene Rating Scheme: Encourages transparency and compliance in food establishments
- 1 Food Safety Training and Certification (FoSTaC): Capacity building of food handlers

Food Recall and Crisis Management**Government policies provide for:**

- Mandatory food recall mechanisms
- Rapid response to food safety emergencies
- Coordination between central and state authorities

Key FSSAI Regulations Relevant to Animal Foods**a. Food Safety and Standards (Food Products Standards & Food Additives) Regulations, 2011****Defines compositional standards for:**

- Meat and meat products

- Poultry products
- Fish and fishery products
- Milk and dairy products
- Eggs and egg powder

b. Food Safety and Standards (Licensing and Registration of Food Businesses) Regulations

Mandates licensing for slaughterhouses, meat processing plants, dairy units, fish processing units, and cold storages.

c. Food Safety and Standards (Contaminants, Toxins and Residues) Regulations

Specifies limits for antibiotics, heavy metals, mycotoxins and pesticides

d. Food Safety and Standards (Packaging & Labelling) Regulations

Covers identity, nutrition labelling, date marking, allergen disclosure, and traceability.

Other Relevant Laws and Standards

i. Prevention of Cruelty to Animals Act, 1960

Governs humane handling, transport, and slaughter of animals through rules on animal transport, slaughterhouse conditions, stunning (where applicable), fitness for transport

Bureau of Indian Standards (BIS) Standards

Provides voluntary but widely used standards such as:

- IS 4393: Hygienic conditions for slaughterhouses
- IS 506: Dairy industry quality guidelines
- IS 3026: Methods of microbiological testing

Export Inspection Council (EIC) Standards

Mandatory for export-oriented units, especially seafood, meat, and poultry.

State-Level Municipal and Public Health Acts

Regulate local slaughterhouses, meat retail shops, and food vending.

Institutional Mechanisms for Enforcement

Department of Animal Husbandry & Dairying (DAHD)

- National Livestock Health & Disease Control (LH&DC) Programme
- National AMR Action Plan in livestock
- Regulations on veterinary drugs

Institutes support scientific risk assessment, training, and method development.

APEDA and Export Inspection Council (EIC)

Ensure compliance of export units with international standards including EU, USFDA, Codex.

Role of Other Government Agencies

Food safety governance involves multiple ministries and institutions:

- **Ministry of Health & Family Welfare:** Overall policy direction
- **ICAR:** Research and scientific inputs
- **ICMR:** Human health surveillance and research
- **State Governments:** Enforcement and local implementation

Food Safety, Trade, and International Obligations

India's food safety policies align with:

- Codex Alimentarius Commission standards
- WTO–Sanitary and Phytosanitary (SPS) Agreement
- Importing country requirements for exports

Strong food safety systems enhance India's credibility in global markets and reduce trade rejections.

Emerging Policy Directions

Government food safety policies are increasingly focusing on:

- One Health approach linking human, animal, and environmental health
- Integration of food safety and antimicrobial resistance policies
- Use of digital platforms for traceability and compliance
- Strengthening laboratory networks and genomic surveillance
- Climate-resilient food safety systems

Challenges in Ensuring Animal Food Safety

1. Unorganized slaughter and retail sector
2. Antibiotic misuse in livestock leading to AMR
3. Insufficient cold chain infrastructure
4. Low consumer awareness on food hygiene
5. Feed contamination and environmental pollutants
6. Fragmented regulatory responsibilities
7. Gaps in traceability, especially in poultry and small ruminants

One Health Approach: The Future of Food Safety

India is increasingly adopting a One Health approach to integrate:

- Human disease surveillance, animal health monitoring, environmental protection

Examples include:

- National One Health Initiative
- AMR surveillance across medical, veterinary, and food labs

- Multi-sectoral coordination hubs

Ensuring food safety will increasingly depend on cross-sectoral decision-making.

Conclusions

Food safety is a shared responsibility requiring strong government leadership, effective regulation, scientific support, and stakeholder participation. India's food safety policies have evolved into a comprehensive and integrated framework, but continued strengthening is necessary to address emerging risks, protect public health, and support sustainable food systems and trade. India has made substantial progress in creating a robust regulatory ecosystem for food safety, anchored by the FSS Act, 2006. However, rapid urbanization, rising demand for animal protein, AMR, and environmental pressures require stronger multi-sectoral collaboration. Integrating food safety into the One Health framework, modernizing infrastructure, improving surveillance, and enhancing consumer awareness will be essential for safeguarding public health and achieving the vision of Viksit Bharat 2047.

ROLE OF AVIAN HEALTH PROFESSIONALS IN POULTRY SECTOR

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Abstract

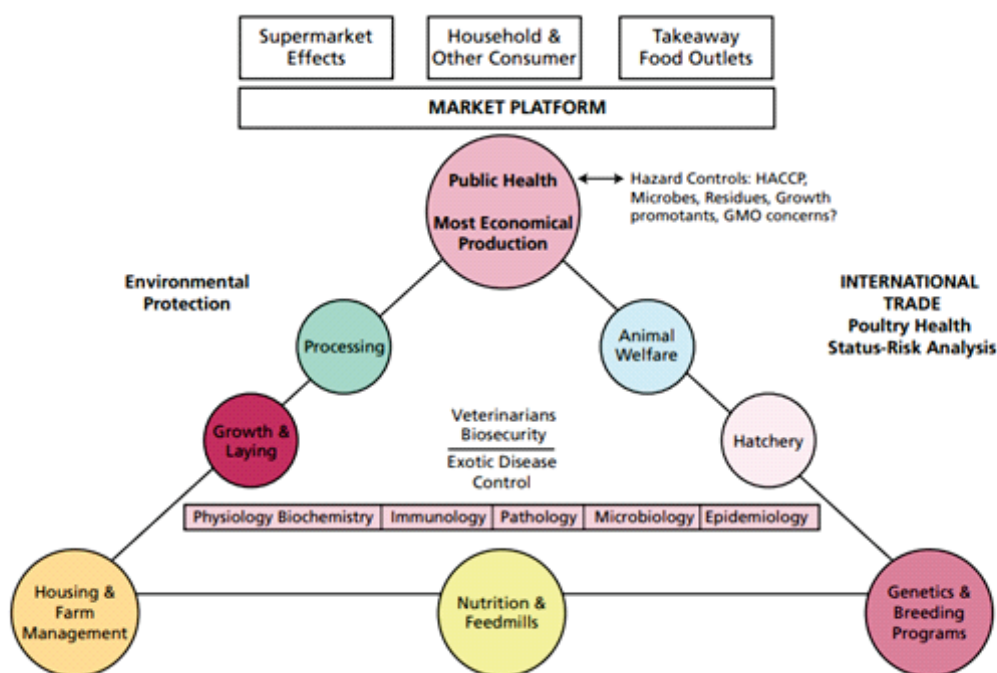
The poultry sector has become one of the fastest-growing segments of the global livestock industry, playing a vital role in ensuring food and nutritional security, creating employment opportunities, and supporting economic development, particularly in developing countries. However, with increasing production, genetic advancements, and the commercialization of poultry production systems, the sector faces complex challenges, including disease emergence, biosecurity threats, antimicrobial resistance, animal welfare concerns, food safety issues, and public health risks. In this evolving scenario, avian health professionals have assumed a pivotal role that extends far beyond conventional disease treatment. Avian health professionals serve as key stakeholders in preventive healthcare, disease surveillance, biosecurity planning, nutritional health management, antimicrobial stewardship, and welfare-oriented production systems. Their expertise is central to early disease detection, outbreak containment, and implementation of science-based health strategies that minimize production losses and ensure sustainable productivity. Furthermore, these professionals act as a critical interface between poultry producers, diagnostic laboratories, regulatory authorities, and consumers, thereby safeguarding both animal and human health under the One Health framework. This lead paper comprehensively ruminates on the multifaceted roles of avian health professionals across the poultry value chain, highlighting their contributions to farm-level health management, food safety assurance, public health protection, and policy formulation. The paper also discusses about the emerging challenges such as climate change, evolving pathogens, and antimicrobial resistance, while emphasizing future opportunities through precision poultry farming, digital health monitoring, and capacity building. Strengthening the role of avian health professionals is essential for achieving sustainable, resilient, and globally competitive poultry production systems.

Keywords: Avian health professionals, Poultry sector, Biosecurity, Disease surveillance, Food safety, One Health, AMR.

Introduction

The poultry sector is strategically significant in global food systems due to its capacity to produce high-quality protein efficiently, contributing substantially to food security and nutrition (Serbessa et al., 2023). The global demand for poultry meat and eggs continues to rise, driven by population growth, urbanization, and consumer preference shifts. However, rapid intensification and commercialization of poultry production have also heightened the incidence and complexity of poultry diseases, including viral (avian influenza), bacterial (salmonellosis), parasitic, and emerging pathogen threats that can

devastate flocks and undermine productivity (Serbessa et al., 2023; Grace et al., 2024). Challenges such as biosecurity lapses, inadequate surveillance, and inconsistent management practices increase disease risks on farms, particularly in developing countries (Islam et al., 2025; FAO Poultry Development Review, 2006). Compounding these risks is the growing concern of antimicrobial resistance (AMR), which emerges from excessive and unregulated antibiotic use in poultry production and poses significant threats to animal and human health alike (Torres et al., 2022). Tackling AMR and optimizing disease control strategies require coordinated, evidence-based interventions at both farm and policy levels.



Source: Bagust (2006); Avian Health Online

Fig.1. A Systems Approach to Poultry Health, Food Safety, and Sustainability

In this complex production environment, avian health professionals play a central and essential role. These professionals possess specialized knowledge in poultry pathology, epidemiology, immunology, clinical care, and health management. They design and implement preventive health programs including vaccination planning, diagnostic surveillance, and biosecurity protocols that are vital for minimizing disease incidence and economic losses (FAO Poultry Development Review, 2006; Serbessa et al., 2023). Their contributions enhance flock health through early detection of health anomalies, strategic vaccination, and rapid response to outbreaks. Beyond disease prevention, avian health professionals also address food safety and public health concerns by supporting hygienic production practices and ensuring poultry products meet national and international safety standards (Lichtensteiger et al., 2021). Moreover, given the zoonotic potential of pathogens like avian influenza, their work supports integrated One Health approaches that involve coordinated actions across animal and human health sectors (Hassan et al., 2023). This lead paper aims to comprehensively elucidate the roles of avian health professionals in modern poultry production systems, emphasizing their contributions to productivity, disease control, biosecurity, welfare, and policy development, while acknowledging emerging challenges and opportunities for innovation and capacity enhancement.

Evolution of Avian Health Profession

The avian health profession has evolved markedly from a traditional, responsive veterinary role focused primarily on clinical treatment of diseases to a highly specialized and preventive discipline aligned with modern intensive poultry production systems. Earlier, veterinarians managed poultry health largely through curative interventions based on clinical signs and post-mortem findings; however, the complexity of contemporary poultry operations has necessitated specialized avian expertise in pathology, nutrition, epidemiology, and production management (Jordan & Pattison, 1996; Swayne, 2017). This evolution has been accompanied by a paradigm shift from curative to preventive and predictive health approaches, emphasizing biosecurity, vaccination, surveillance, and data-driven risk assessment to anticipate and mitigate disease outbreaks before clinical manifestation (Saif et al., 2020; Thrusfield, 2018). Specialized avian pathologists enable accurate disease diagnosis, nutritionists optimize gut health and immune function, and epidemiologists guide population-level disease control strategies, collectively enhancing productivity, sustainability, and public health protection in the poultry sector (Leeson & Summers, 2009; Capua & Alexander, 2009).

Core Roles of Avian Health Professionals



Fig 2. Multi-Disciplinary Avian Health Ecosystem- One Health

a. Genetic Health and Disease Resistance

Poultry geneticists play a crucial role in strengthening avian health by developing genetically resilient birds with enhanced disease resistance, stress tolerance, and production efficiency. Through selective breeding and advanced genomic tools, geneticists contribute to the development of poultry lines with improved resistance to major diseases such as Marek's disease, avian influenza, and Newcastle disease, thereby reducing reliance on therapeutic interventions (Delourme et al, 2011; Dekkers, 2012). Modern genomic selection enables identification of health-related traits associated with immune competence,

gut integrity, and heat tolerance, which are increasingly important under intensive production systems and climate change scenarios (Zhang et al., 2018). By integrating genetic improvement with health management, vaccination strategies, and nutrition, poultry geneticists support sustainable disease control, improved animal welfare, and long-term productivity, reinforcing the One Health objective of reducing disease burden at its biological source (Lamont et al., 2017).

b. Disease Surveillance, Diagnosis, and Epidemiology

Avian health professionals play a critical role in disease surveillance by integrating farm-level observations, laboratory diagnostics, and epidemiological tools to support early warning systems and rapid response mechanisms. Farm-level surveillance involves routine health inspections and mortality analysis, while laboratory diagnostics such as serology, PCR, and histopathology are essential for confirming etiological agents (WOAH, 2019). Epidemiological investigation allows identification of risk factors, transmission pathways, and outbreak sources, thereby enabling data-driven decision making and targeted control strategies (Thrusfield, 2018). Early detection through structured surveillance systems is particularly crucial for transboundary diseases such as avian influenza and Newcastle disease, where delayed response can have severe economic and public health implications (Capua & Alexander, 2009).

c. Disease Prevention and Health Management

Disease prevention constitutes the primary role of poultry veterinarians and forms the backbone of sustainable poultry production systems. They design, implement, and audit farm-specific biosecurity programs, develop scientifically sound vaccination schedules, and continuously monitor flock health to prevent economically important diseases such as avian influenza (AI), Newcastle disease (ND), infectious bronchitis (IB), Mycoplasma infections, and salmonellosis (Swayne, 2017; Saif et al., 2020). Vaccination planning is carried out based on regional disease prevalence, maternal antibody interference, and production type, while flock health monitoring through mortality trends, feed intake, and production parameters enables early disease detection and timely intervention (de Wit, 2018). Well-structured preventive health programs significantly reduce disease incidence, production losses, and dependence on therapeutic interventions (Van Steenwinkel et al., 2011).

d. Antimicrobial Stewardship and AMR Control

Antimicrobial resistance (AMR) has emerged as one of the most critical challenges confronting global poultry production and public health. AMR occurs when microorganisms develop the ability to survive exposure to antimicrobial agents that were previously effective against them. In poultry production systems, the intensive nature of farming, high stocking density, and continuous disease pressure have historically led to frequent antimicrobial use. As a result, poultry has been recognized as an important sector where antimicrobial stewardship is essential to safeguard animal health, food safety, and human health within the One Health framework. The development of AMR in poultry is largely driven by the selective pressure exerted by indiscriminate and prolonged use of antimicrobials.

Avian health professionals play a pivotal role in mitigating AMR through effective antimicrobial stewardship. Veterinarians and poultry scientists are responsible for ensuring rational use of antimicrobials based on clinical necessity, supported wherever possible by laboratory diagnosis and antimicrobial sensitivity testing. Judicious selection of drugs, adherence to correct dosages and duration, and strict observance of withdrawal periods are fundamental responsibilities. By discouraging the use of critically important antimicrobials for human medicine in poultry, professionals directly

reduce the risk of cross-sectoral resistance development. Beyond therapeutic interventions, poultry professionals significantly contribute to AMR control through preventive health strategies. Strengthening biosecurity, implementing effective vaccination programs, improving farm hygiene, and optimizing nutrition and housing conditions help reduce disease incidence and, consequently, the need for antimicrobials. Promotion of antibiotic alternatives such as probiotics, prebiotics, postbiotics, organic acids, phytogenics, enzymes, and immunomodulators has gained prominence, with professionals guiding their scientific application and field-level validation. These measures collectively support sustainable poultry production while minimizing antimicrobial dependence.

Government agencies play a complementary and decisive role in controlling AMR through policy formulation, regulation, and surveillance. Implementation of regulations restricting the use of antibiotics as growth promoters, enforcement of prescription-only antimicrobial sales, and monitoring of drug residues in poultry products are critical regulatory interventions. National AMR action plans aligned with global guidelines of WHO, FAO, and WOAHA provide a coordinated framework to address AMR across sectors. Governments also support integrated AMR surveillance systems, enabling data-driven decision-making and early detection of emerging resistance trends. In addition, government investment in research, infrastructure, and capacity building strengthens national efforts against AMR. Funding research on antibiotic alternatives, supporting diagnostic laboratories, and facilitating training programs for veterinarians, para-veterinary staff, and farmers enhance the overall effectiveness of antimicrobial stewardship. Awareness campaigns and extension activities further empower poultry farmers to adopt responsible practices, thereby reinforcing field-level implementation of AMR control strategies.

The combined efforts of avian health professionals and government authorities directly contribute to the One Health approach by addressing AMR at the animal–human–environment interface. Reduced antimicrobial use and resistance in poultry lower the risk of resistant pathogens entering the human food chain and contaminating the environment. Improved waste management, surveillance, and coordinated action across sectors ensure that poultry production remains safe, sustainable, and socially responsible. Thus, antimicrobial stewardship in poultry is not merely an animal health issue but a critical component of global public health and One Health sustainability.

e. Poultry Nutrition–Health Interface

The interface between nutrition and health is a critical area where avian health professionals particularly nutritionists work to prevent nutritional deficiency disorders and optimize immune competence. Balanced diets support gut integrity, enhance disease resistance, and improve overall productivity, while improper nutrition predisposes birds to metabolic disorders and immunosuppression (Leeson & Summers, 2009). Gut health management through dietary interventions and feed additives plays a key role in disease prevention. Additionally, feed safety and mycotoxin control are essential to prevent subclinical losses and immune dysfunction (Binder, 2007). Stress nutrition strategies, including antioxidant and electrolyte supplementation during heat stress or transport, further improve bird resilience and performance (Surai, 2016).

f. Biosecurity and Farm Management Advisory

Avian health professionals particularly field veterinarians provide expert advisory technical services on biosecurity and farm management to minimize disease risks. This includes guidance on farm layout, traffic flow control, zoning, and controlled access to prevent pathogen introduction and spread (FAO,

2008). They also establish effective cleaning and disinfection protocols and advise on proper litter management, water sanitation, ventilation, and air quality to reduce environmental stress and pathogen load (Van Limbergen et al., 2018). Training of farm personnel is emphasized, as human behaviour is often the most critical factor influencing biosecurity compliance and overall flock health outcomes.

g. Poultry Welfare and Ethical Production

Ensuring poultry welfare is an integral component of avian health management and ethical production systems. Avian health professionals should ensure compliance with welfare standards related to housing, stocking density, handling, transport, and humane culling practices (Broom, 2017). Reducing physical and psychological stress improves immune function, growth performance, egg quality, and survivability (Estevez, 2007). Increasing consumer awareness regarding animal welfare has also made ethical production and welfare certification important determinants of market access, consumer trust, and long-term sustainability of poultry enterprises.

h. Food Safety and Public Health Protection

Avian health professionals play a vital role in protecting public health by controlling zoonotic pathogens such as *Salmonella*, *Campylobacter*, and avian influenza throughout the poultry value chain. Their involvement in hygienic egg and meat production, implementation of Hazard Analysis and Critical Control Point (HACCP) systems, and monitoring of chemical residues and contaminants ensures food safety and regulatory compliance (Newell et al., 2010). By linking flock health with food safety outcomes, avian health professionals operationalize the One Health approach, ensuring that improvements in avian health directly translate into reduced risks to human health (Grace, 2015; FAO & WHO, 2011).

i. Value Chain Management

In poultry slaughter processing, avian health professionals play a critical role in ensuring food safety, quality, and compliance with regulatory standards. They oversee hygienic slaughter, carcass handling, and chilling operations to prevent microbial contamination, while implementing Hazard Analysis and Critical Control Point (HACCP) systems to monitor critical points throughout processing (Newell et al., 2010). Their responsibilities extend to further processing activities such as portioning, ready-to-eat product preparation, and packaging, ensuring adherence to residue limits, chemical safety, and temperature control (FAO & WHO, 2011). Additionally, they facilitate compliance with national and international export standards, certification protocols, and traceability requirements, bridging poultry health management with public health and global trade obligations (Grace, 2015).

j. Crisis Management and Emerging Threats

Poultry consultants are critical in managing crises arising from disease outbreaks and emerging threats. During avian influenza (AI) outbreaks, they implement rapid surveillance, quarantine, and culling measures to limit virus spread, while coordinating with government authorities and international agencies (Capua & Alexander, 2009; WOA, 2019). Climate change exacerbates the risk of vector-borne and temperature-sensitive diseases, demanding proactive adaptation strategies in flock management (Thornton et al., 2014). The emergence of new pathogens and viral variants necessitates continuous genomic monitoring and epidemiological modelling. Emergency preparedness and response frameworks led by avian health professionals—including contingency planning, rapid diagnostics, and communication systems—are essential to mitigate economic losses and protect public health (Swayne, 2017).

k. Capacity Building, Training, and Extension

Avian health professionals play a pivotal role in bridging knowledge between laboratories and farms through capacity building and extension services. They conduct farmer training programs on biosecurity, vaccination, disease recognition, and proper handling of poultry (FAO, 2008). Technical advisory services, skill development workshops, and demonstration of novel technologies facilitate adoption of best practices in health management, nutrition, and welfare (Delourmew et al, 2011). Technology transfer from research institutions to field operations ensures evidence-based interventions reach the farm level, enhancing productivity, animal welfare, and compliance with regulatory standards.

l. Policy, Regulatory, and Institutional Contributions

Poultry veterinarians provide expert inputs for government policy formulation, national disease control programs, and livestock health standards. They contribute to drafting guidelines for vaccination, biosecurity, and emergency response, supporting implementation of national and international regulatory frameworks (FAO & WHO, 2011). Their participation in standard setting, certification, and evaluation of disease surveillance programs informs national livestock policies, ensuring harmonization with global trade and public health objectives (Newell et al., 2010).

Challenges Faced by Avian Health Professionals

Despite their critical role, avian health professionals face multiple challenges. Rapid expansion of the poultry industry increases flock density and pathogen exposure, complicating disease management (Swayne, 2017). Rising antimicrobial resistance (AMR) places pressure to reduce antibiotic use while maintaining productivity (Tang et al., 2017). Resource limitations, including personnel, laboratory infrastructure, and field diagnostic capacity, constrain effective interventions. Additionally, farmer compliance and adoption of biosecurity, welfare, and vaccination practices remain inconsistent; while emerging and re-emerging diseases continually challenge preparedness and response capabilities (Saif et al., 2020).

Future Perspectives and Way Forward

The future of avian health is poised to be shaped by digital and precision technologies. Digital health monitoring and farm-level sensors enable real-time surveillance of flock health and environmental parameters, while AI-based disease prediction models allow proactive interventions (Zhang et al., 2018). Precision poultry farming integrates genomics, nutrition, and environmental control to optimize health and productivity. Strengthening One Health collaborations ensures integrated management of zoonotic risks, environmental sustainability, and public health protection. Expansion of specialized training programs and recruitment of more avian health experts is necessary to meet the growing complexity of modern poultry systems (Lamont et al., 2017).

Conclusion

Avian health professionals occupy a central role in the poultry sector, safeguarding Poultry health, food safety, and public health while supporting productivity and sustainability. Their work spans preventive health, crisis management, nutrition, welfare, policy advisory, and capacity building. Addressing emerging challenges such as AMR, climate change, and novel pathogens requires an interdisciplinary approach integrating genetics, epidemiology, nutrition, and technology. Strengthening training, research, and One Health initiatives is essential for ensuring the long-term resilience of poultry production systems and global food security.

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Theme 6

*GI Tract Health and
Disease Management*

PATHOPHYSIOLOGICAL INSIGHTS AND STRATEGIC MODULATION OF GUT HEALTH FOR ENHANCED PRODUCTIVITY IN CHICKENS

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Abstract

Gastrointestinal tract (GIT) health affects animal productivity. The poultry microbiome has functions that range from protection against pathogens and nutrient production to host immune system maturation. Optimal gut health is vital for chicken performance. The gut is responsible for regulating physiological homeostasis, which provides the host with the ability to withstand infectious and non-infectious stressors. The gut microbiota confers health benefits to the host, including aiding in the digestion and absorption of nutrients, contributing to the construction of the intestinal epithelial barrier, development and function of the host immune system, and competing with pathogenic microbes to prevent their harmful propagation. Modulating gut health in chickens involves manipulating the to improve overall health and productivity. This can be achieved through various strategies, such as dietary adjustments, prebiotics, probiotics, postbiotics, and fecal microbiota transplantation. These methods aim to shift the balance of gut microbes towards a more beneficial composition, enhancing nutrient utilization, boosting immunity, and reducing the risk of diseases.

Keywords: Gut health, intestinal epithelial barrier, prebiotics, probiotics, postbiotics, fecal microbiota transplantation.

Introduction

According to Hippocrates 460-370 BC, "all disease begins in the gut and health is determined by the microbiota in the gut!" Modern broilers are characterized by highly efficient feed conversion and fast growth. Such performance requires massive feed intake, which exerts enormous pressure on the physiology of the gastrointestinal (GI) tract (Svihus, 2014). Poultry health for sustained meat supply is closely linked to its profile and diversity. Microbiome functions include protection against pathogens, nutrient production, and host immune system maturation. The ban on AGP has triggered renewed scientific interest in the intestinal health of animals. While in the past, the focus of gut health research was almost exclusively on the veterinary aspects of pathogenic organisms invading the intestine and/or intestinal tissues (helminths, protozoa, bacteria, and viruses), causing severe damage to the host mucosa and resulting in clinical symptoms of disease, the current focus is on the fundamental aspects of the numerous complex and subtle interactions between the host mucosa, intestinal content, and all organisms residing in the intestinal tract (Vancamelbeke and Vermeire, 2017).

Gut health is defined as "a steady state where the microbiome and the intestinal tract exist in symbiotic equilibrium and where the welfare and performance of the animal are not constrained by intestinal dysfunction. Gut health involves a complex network of interactions, including its structural integrity on a large and microscale. Gut health also involves microbiome balance and impacts the host's immune status. Recently, it has also been shown that there is extensive communication between the brain and the microbiota via the brain-gut-microbiome axis. Through this bidirectional communication, signals from the brain can influence the motor, sensory, and secretory functions of the gut, and visceral messages from the gut can influence brain function (Wiley et al., 2017). Similarly, the gut-kidney axis encompasses the interaction between the intestinal barrier, production of microbial metabolites, and renal physiology.. (Summers and Quimby, 2024).

Intestinal barrier

Intestinal homeostasis is determined by the intestinal epithelium,, and host immune system. The functionality of this unit relies entirely on the integrity of the gut epithelium, which is upheld by junctional proteins, such as adherent junctions, tight junctions (TJs), and desmosomes. These proteins connect neighboring epithelial cells and, along with the lamina propria, form physical barriers. Changes in the expression and functioning of tight junctions cause gut leakage, characterized by body fluid leakage into the intestinal lumen, which may ultimately result in diarrhea. In this context, an organized intestinal barrier prevents uncontrolled microbe-induced immune reactions in the gut. Disruptions in the intestinal barrier lead to significant changes in the delicate balance between luminal antigens and the local immune system. Consequently, a leaky gut permits the translocation of bacteria, other microorganisms, and luminal antigens into the bowel wall, thereby inducing an overwhelming pro-inflammatory mucosal immune response (Wu et al., 2024).

Intestinal health and the microbiome

The microbiome of poultry is a complex community of microorganisms, including viruses, bacteria, fungi, protozoa, and other microbes residing in their digestive tracts, with each region harboring distinct microbial populations. The intestinal microbiota contributes to several physiological functions, protective functions (pathogen displacement, nutrient competition, receptor competition, and production of antimicrobial factors), structural functions (GIT barrier fortifications, induction of IgA, apical tightening of tight junctions, and immune system development), and metabolic functions (fermenting non-digestible dietary residue and endogenous epithelial-derived mucus, synthesizing vitamins, controlling intestinal epithelial cell differentiation and proliferation, and ion absorption) (Marchesi et al., 2016). Several metabolites produced by the microbiota also stimulate neuroendocrine cells in the GIT; therefore, the microbiota plays an important role in the endocrine regulation of gastrointestinal functionality. The diversity and density of the microbiome vary greatly in different regions of the GI tract. Within both the crop and ileum, the Lactobacillaceae family predominates, typically constituting over 90% of the microbial community. The dominant bacterial phyla in poultry are Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria. Lactobacilli are prominent in the upper and middle gastrointestinal tracts (Xiao et al., 2017).

Intestinal inflammation and gut leakage

It is now well established that even in perfectly healthy chickens, there is always a certain level of 'physiologic inflammation' in the GI tract. This allows for an immediate response in the case of an imminent threat, which is important for the intestinal mucosa, as it is continuously exposed to numerous potentially harmful substances and microorganisms that are ingested. For the same reason, the entire mucosa of the GI tract is equipped with sensors that can rapidly alert and activate a powerful defensive inflammatory response through the nuclear factor kappa B (NFκB) cascade. These sensors include Toll-like receptors and a range of other receptors (Ducatelle et al., 2023).

Dysbiosis, a shift in the microbiota towards production of proinflammatory signals

Many proteobacteria are known producers of hydrogen sulfide, which is toxic to epithelial cells at high concentrations and triggers inflammation (Lim et al., 2022). Conversely, depletion of butyrate-producing taxa, especially those belonging to the families Lachnospiraceae and Ruminococcaceae, leads to alterations in epithelial cell metabolism and loss of the anti-inflammatory protection of high butyrate production. Both the expansion of Proteobacteria and the loss of butyrate producers are typical effects of dysbiosis (Rivera-Chavez et al., 2016). Dysbiosis is linked to an increased susceptibility to respiratory diseases and infections.

Beyond the gut

Damage to the gut barrier allows leakage of potentially harmful compounds and microorganisms from the intestinal lumen into the portal circulation and from there directly to the liver. As mentioned above, this causes a change in the metabolism of hepatocytes, known as the acute phase response. As hepatocytes synthesize most plasma proteins, this will also lead to a change in the composition of plasma in the general circulation, potentially impacting all organ systems in the body. However, the vascular route is not the only route by which intestinal health affects different organ systems (O'Reilly and Eckersall, 2014).

The gut microbiome promotes and calibrates multiple aspects of immune system maturation and function via the release and production of various molecular motifs and metabolites, respectively. These molecules not only modulate local (gut) immunity but also exert remote functions on peripheral organs, including the lungs, a phenomenon referred to as the gut-lung axis (Dang et al., 2019).

As for the immune system, the gut microbiota has an important impact on the growth and function of myeloid cells in many organs and at different stages of cellular development. Myeloid cell growth in the bone marrow is diminished in the absence of gut microbiota, resulting in a delay in the clearance of systemic bacterial infections. The complexity of the gut microbiota also influences the level of myelopoiesis, which is modified according to the presence of GM-derived Toll-like receptor (TLRs) ligands in the blood. Short-chain fatty acids (SCFA), the main metabolites produced by bacterial fermentation of dietary fiber in the gastrointestinal tract, may also promote myelopoiesis in the bone marrow.

AIV-induced dysbiosis in early life (due to direct, local, and/or type-I IFN-mediated remote effects) could impair the proper maturation of the immune system of young chickens. In contrast, dysbiosis due to an unbalanced GM composition and altered immune system development and functioning could favor respiratory and/or intestinal AIV infection with increased severity and negative consequences for the poultry production sector. LPAIV infection retards cecal microbiota diversification in chickens. Yitbarek and colleagues already suggested that shifts in the composition of the GM may result in changes in cell- and antibody-mediated immune responses to vaccination against avian influenza viruses. Therefore, enhancing immunity along the gut-lung axis via increased GM diversity in early life is among the best strategies to foster resilience to economically important poultry viral pathogens, such as AIV. Therefore, the relevance of supporting robust immune system development along the gut-lung axis in early life is still one of the best strategies to promote health and welfare and limit economic losses due to viral infections in poultry flocks (Yitbarek et al., 2019).

Modulation of gut health

Modulation of gut health can play a key role in reducing the dependence on antimicrobials to protect animals from diseases and maintain production. Modulating barrier function may be a promising approach for treating a wide range of intestinal and extraintestinal diseases. There are several methods for modifying gut health, including dietary modifications and the use of prebiotics, probiotics, synbiotics, and postbiotics. Currently, numerous novel therapeutic concepts are being explored to directly or indirectly enhance the barrier function. Recently, our group has developed metal nanoparticle conjugated with phytomolecules to maintain the gut health of chickens.

Metagenomics for the identification of gut microbiome composition

However, many members of the microbiome are non-culturable and require more sensitive methods for their identification and enumeration. Nonetheless, given the advancements in molecular biology and the emergence of culture-independent high-throughput molecular sequencing technologies, new and interesting scientific data continue to emerge and shed light on microbiome interactions in their hosts.

Methods for Testing Gut Permeability and Other Markers of Intestinal Barrier Disruption

One of the issues with determining gut barrier dysfunction is the lack of specific biomarkers. A variety of parameters can be evaluated when testing intestinal permeability. Moreover, the fact that permeability varies along the GI tract must be considered, with the small intestine being more permeable than the large intestine (Mateer et al., 2016). Briefly, methods for testing gut permeability *in vivo* involve the administration of a tracer molecule by oral gavage or intestinal instillation. Commonly used tracers include non-digestible sugars such as lactulose or mannitol, PEG, fluorescently labeled dextrans, and Cr-EDTA, which can be quantified in urine or blood. The size of a tracer can indicate the probable permeability route. To obtain comprehensive information regarding epithelial leakiness, it is recommended that *in vivo* and *ex vivo/in vitro* tests of permeability be used in combination with the detection of permeability-associated biomarkers (Galipeau and Verdu).

Conclusion

Several complex mechanisms are involved in the GIT functionality and health of poultry. Gut microbial comparison and analysis have the potential to improve our understanding of the pathogenesis of various gut-linked diseases in chickens and the development of corresponding strategies to decrease the collateral damage. It is crucial to deepen our understanding of these interactions so that strategies for the modulation of GIT functionality and health can be developed in the context of improved chicken performance.

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GUT MICROBIOTA MODULATION THROUGH ESSENTIAL OILS: IMPACT IN POULTRY HEALTH

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Abstract

The irrational use of antibiotics in poultry production contributes to the global challenge of antimicrobial resistance (AMR). The need has arisen to explore for alternative of non-antibiotic growth promoters for poultry which could help revamp the gut microbiota of poultry circumventing the adverse effects of anti-microbials. Essential oils (EO) produced from aromatic plants have become increasingly interesting owing to their potential effects as antioxidants, antimicrobials, antifungals, and stimulants of digestive enzymes. The EOs help enhance the digestion of nutrients, modulates gut microbiota, reduce oxidative stress, control free radicals, strengthen the immune system and improves the productive performance of broiler chickens. Healthy gut microbiota is essential for optimum growth of chicken and unhealthy microbiota invigorate intestinal pathogens leading to reduced growth and increased mortality rates. EOs have antibacterial activity and help modulate gut microbiota by supporting microbial balance and enhancing gut resilience. It influences poultry gut health through cellular, microbial and molecular mechanisms. The widespread adoption of EOs in modulation of gut microbiota remains limited due to several practical and scientific challenges, although having potential benefits ranging from enhanced gut health and immunity to improved growth performance. EOs with their roles in pathogen control, immune modulation and gut health are well suited for the global shift toward antibiotic free poultry production.

Keywords: Essential oils, Gut health, microbiota, poultry

Introduction

In developing countries, poultry is the fastest rising sub-sector of food industry. The demand for chicken meat has been steadily increasing (<https://data.oecd.org/agroutput/meat-consumption.html>). Poultry products such as chicken and eggs serve as rich sources of high-quality protein, essential vitamins, and minerals. There is an increasing trend of feeding antimicrobial agents to poultry as growth promoters. The antibiotic selection pressure and the inappropriate usage of antibiotics are not only responsible for the resistance of pathogenic bacteria but also of the endogenous flora of exposed population. In addition to this, many synthetic drugs and growth promoters are also supplemented to broilers to effect rapid growth. However its use has shown adverse side effects and has raised serious concerns due to drug residues in poultry meat and eggs. The irrational use of antibiotics in poultry production contributes to the global challenge of antimicrobial resistance (AMR).



The need has arisen to explore for alternative of non-antibiotic growth promoters for poultry which could help revamp the gut microbiota of poultry circumventing the adverse effects of anti-microbials (Salinas-Chavira et al., 2024). Since ages, plants have been used for food preservation and treatment of diseases as well (Sakkas and Papadopoulou, 2017). Plants have pivotal role in influencing the microbiota of chickens (Qamar et al., 2021). Essential oils (EO) produced from aromatic plants have become increasingly interesting owing to their potential effects as antioxidants, antimicrobials, antifungals and stimulants of digestive enzymes (Khwaza and Aderibigbe, 2025). EOs contain secondary plant molecules as active ingredients with enormous uses in both food industry and medicine. The EOs help enhance the digestion of nutrients, modulates gut microbiota, reduce oxidative stress, control free radicals, strengthen the immune system and improves the productive performance of broiler chickens (Ulukanli et al., 2014; Ding et al., 2020). Supplementation of poultry rations with EOs could be a safer and more promising alternative to antibiotics (Abd El-Hack et al., 2022).

Role of gut microbiota in poultry health

The gut microbiota (GM) includes wide assortment of microorganisms inhabiting the gastrointestinal tract and has a central position in poultry nutrition, health, physiology and immunity. Microbiota controls normal host physiology, such as nutritional status, energy metabolism, immunity, infection, behavior and stress response, explaining its tolerance by the host organism. A well-balanced gut microbiota is essential to ensure homeostasis at the intestinal mucosa and beyond (Sekirov et al., 2010). Any exogenous and endogenous changes leads to change in microbiota and the change of its pattern further impact on the host health status (Lazar et al., 2022). The gut microbiota of chicken is a diverse population of microbes which is classified into pathogenic and commensal populations (Ding et al., 2017). Induction of infection, intestinal degeneration and toxin production are mediated by pathogenic population whereas vitamin synthesis, stimulus for immune system and avoiding establishment of pathogenic mirco-organisms are mediated by commensal populations (Jeurissen et al., 2002; Smith et al., 2014). The gut microbiota acts as a first line of defence against infections which in aid affects nutritional, physiological and health conditions on chickens. On the basis of effects on the host, gut microbiota has been divided into pathogenic (*E. coli*, *Compylobacter* and *Salmonella*) and beneficial (*Lactobacilli* and *Bifidobacteria*). The gut microbiota inhibits pathogens by competing for resources, generating harmful substances such as volatile fatty acids, low pH, competing for binding sites on the gut epithelium and stimulating immune system (Oni and Oke, 2025). Environmental aspects like accomodation, litter material and nutrition may have influence on gut microbiota (Boroluzzi et al., 2019). Active ingestion and effective absorption of feed nutrients are the key roles of healthy gut (Kairie et al., 2013). Healthy gut microbiota is essential for optimum growth of chicken and unhealthy microbiota invigorate intestinal pathogens leading to reduced growth and increased mortality rates. Various molecular factors impacts on the GM and can be clustered into various categories. Host-derived factors include specific microRNAs and nonspecific molecules, such as antimicrobial peptides, mucus, immunoglobulin A and hormones (Hasan and yang, 2019). Microbial factors include lipopolysaccharides, metabolites, short chain fatty acids and quorum sensing signaling molecules (Wen

and Duffy, 2017). Dietary molecules like fats, sugars, proteins and medications (mainly antibiotics) impacts GM. However, any drugs including natural products used in traditional medicine, prophylaxis or immune stimulation could impact the gut microbiota.

Essential oils in shaping microbiota

Various molecules and particles ingested in various situations, represent the most significant shaping agents for GM. Among these, essential oils (EOs) and plant extracts are frequently reaching microbiota following ingestion. EOs have antibacterial activity and help modulate gut microbiota by supporting microbial balance and enhancing gut resilience (Mafe et al., 2025). It influences poultry gut health through cellular, microbial and molecular mechanisms. Compounds like thymol, carvacrol and cinnamaldehyde reduced the growth of harmful bacteria like *Clostridium perfringens* and *E. coli* by disrupting bacterial cell membrane, changing membrane permeability and inhibiting quorum sensing (Yang et al., 2015). EOs and their derivatives have effect on cytoplasm and cell walls which results in radical alteration of the cells's shape (Yang et al., 2015). The antibacterial properties in thymol, carvacrol and eugenol are due to phenolic compounds found in thyme, oregano and clove EOs. Combination of carvacrol and thymol was effective in combating *C. perfringens* in broiler gut in cases of necrotic enteritis (Koscova et al., 2006). A combination of carvacrol and thymol or 1% oil supplementation decreased *Compylobacter* (Arsi et al., 2014). The widespread adoption of EOs in modulation of gut microbiota remains limited due to several practical and scientific challenges, although having potential benefits ranging from enhanced gut health and immunity to improved growth performance. EOs with their roles in pathogen control, immune modulation and gut health are well suited for the global shift toward antibiotic free poultry production.

The use of EOs in poultry production is promising alternative to antibiotic growth promoters, though several factors hinder their optimal use. Studies on synergistic or antagonistic interactions between EOs or other phytochemicals or feed additives like probiotics, prebiotics, enzymes and organic acids is of prime concern towards use of EOs in poultry production. The stability, bioavailability and site specific release of EOs in gut can be enhanced by advanced technologies such as microencapsulation, nanoemulsions and pH sensitive coating.

Conclusion

Essential oils represent a natural, effective and sustainable alternative to antibiotics in poultry nutrition. Their antimicrobial, antioxidant, and growth-promoting properties make them invaluable in enhancing poultry health and productivity. Various bioactive components in EOs help to lessen the need of antibiotics by strengthening immune responses, improving nutritional absorption and improving balance of gut microbiota. With further research to standardize dosages and formulations, EOs can redefine the future of poultry feed additives, catering to both industry and consumer needs.

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**“EFFECT OF POMEGRANATE (*PUNICA GRANATUM*) PEEL POWDER
AND EUCALYPTUS (*EUCALYPTUS GLOBULUS*)
LEAVES POWDER ON GUT MICROBIAL STUDY AND
COMPARATIVE ECONOMICS OF BROILERS”**

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Feed additives, which are used to promote disease resistance, growth rate and feed conversion ratio, are one of the key instruments in poultry nutrition. Pomegranate peels and Eucalyptus leaves are used in poultry as natural alternatives to antibiotics offering benefits like anti-inflammatory, antimicrobial, anti-diabetic, heart-protective properties, plus high fiber for digestion, boosting immunity, better gut/respiratory health. In an effort to investigate the possibilities of using of pomegranate peel powder and eucalyptus leaves powder in broiler ration, a feeding trial of six weeks was conducted using 120 broiler chicks (Vencobb-430Y) in completely randomized block design. The four dietary treatments were designated as control group (Basal diet), T1 supplemented with pomegranate peel powder @ 0.5% level, T2 supplemented with eucalyptus leaves powder @ 0.5% level and T3 supplemented with pomegranate peel powder @ 0.25% and eucalyptus leaves powder @ 0.25% (1:1) level in the diet. Highly significant ($P < 0.01$) effect was found on decreased total bacterial count and *E. coli* count in treatment groups as compared to control group. Highest net return per bird was observed in T2 group and lowest was observed in control group C. Looking at the results of the current study, it could be assumed that supplementation of eucalyptus leaves powder @ 0.5% reduce gut microbial count of broilers and increase the net profit without any deleterious impact.

EVALUATING THE EFFECT OF DIETARY SUPPLEMENTATION OF TULSI LEAF AND GINGER POWDER AS FEED ADDITIVES ON CARCASS AND GUT MICROBES OF BROILERS

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The use of antibiotics in birds for growth may result in antibiotic residues in foods such as eggs and meat, and may cause various side effects, such as the transfer of antibiotic resistant bacteria to humans. This study evaluated the effect of tulsi and ginger as feed additives on broiler carcass and gut microbial parameters of broilers. A total of 210 day-old chicks, divided into 7 groups in a completely randomized design, with 30 chicks in each group and reared for 42 d. Control group was fed on basal diet and groups T1 and T2 were supplemented with 0.5 and 1% of tulsi and G1 and G2 fed with 0.5 and 1% of ginger powder in the ration, respectively. T1G1 and T2G2 treatment groups were supplemented with 0.5% and 1% of both herbs in combination, respectively. Highest dressing percentage was observed in the group supplemented by 0.5 per cent of tulsi leaf and ginger powder. Abdominal fat percentage was significantly ($P<0.01$) reduced in all treatment groups. However highest reduction ($P<0.01$) in *E. coli* and total microbial count were observed in the T2G2 group as compared to the control. Supplementing tulsi leaf and ginger powder @ 0.5% each resulted in improved dressing percentage and reduced *E. coli* and total microbial count of gut of the broiler chicks.

COMPARATIVE EFFICACY AND PERFORMANCE OF ANTIMICROBIAL GUT HEALTH ADDITIVES FED TO BROILER CHICKEN

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A six-week biological experiment was conducted to evaluate the comparative efficacy and performance of different antimicrobial gut health additives in commercial broiler chickens. A total of 640, day-old male commercial broiler chicks of the Cobb-430Y strain were randomly divided into four groups (A to D), containing 160 chicks per group with eight replicates of 20 chicks in each. Group A was the control without any antimicrobial gut health additive. Broilers from groups B, C, and D were fed with Enramycin 8% @ 100g/ton, Virginiamycin 50% @ 40 g/ton, and Phytogenic Feed Additive (PFA) @100g/ton, as per manufacturers' recommendations, respectively. The broilers were fed with iso-caloric and iso-nitrogenous pre-starter, starter, and finisher diets from 0-10, 11-21, and 22-42 days, respectively. The different antimicrobial feed additives significantly ($P<0.05$) improved the finisher phase BW, cumulative BWG, ADG, and overall FCR in broilers, achieving the best with PFA @ 100 g/ton among antimicrobial feed additives. The addition of PFA and other antimicrobial feed additives significantly decreased *Clostridium* spp. count of caecal digesta and Total Bacterial Enteritis Scoring (TBE) on the 35th day, leading to better gut health. The PFA significantly decreased serum BUN and ammonia levels compared to other antimicrobial feed additives and the control group on the 35th day of age, indicating a positive effect on protein metabolism by decreasing the protein catabolism rate. The PFA significantly decreased oxidative stress-induced serum lipid peroxidation levels as well as serum total nitrite levels compared to other antimicrobial feed additives and the control group on the 35th day of age, while the values of SOD antioxidant enzyme levels were highest in the PFA group. It was concluded that the PFA was found to be promising for the replacement of antibiotic growth promoters in commercial broilers for improving gut health and growth performance.

STUDIES OF CARBON DOT ENCAPSULATED ESSENTIAL OILS ON PERFORMANCE AND GUT HEALTH OF BROILER CHICKEN

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Encapsulation of essential oils has emerged as a practical strategy to enhance their stability, control release, minimize volatility, and improve bioavailability in GIT while reducing the cost of production. Carbon-dot conjugation, in particular, allows for the efficient delivery of active compounds such as carvacrol and cinnamaldehyde at lower dosages, maintaining efficacy and reducing production costs. A total of 240 day-old Vencobb 430 chicks were randomly assigned to four treatment groups T0, T1, T2 and T3 with three replicates of 15 birds each. The T0 group was fed as per BIS (2024), while T1 received Avilamycin (100 mg/kg). Groups T2 and T3 were supplemented with carbon-dot conjugated carvacrol and cinnamaldehyde essential oils, respectively, at 200 mg/kg feed. The T3 group (cinnamaldehyde EO) achieved the highest ($p \leq 0.01$) body weights and cumulative weight gains by the end of the fifth week, followed by T2 and T1. The feed conversion ratio (FCR) was better in essential oil group. Significant improvements were observed in nutrient digestibility (DM and CP), intestinal morphology (greater villus height and villus height-to-crypt depth ratio) and caecal microbial counts, with reduced levels of *E. coli* and total viable count (TVC), particularly in group supplemented with carbon-dot conjugated carvacrol and cinnamaldehyde essential oils. The net profit per kg live weight was highest in T3 group (Rs. 16.82). It is concluded that the supplementation with carbon-dot conjugated essential oils especially cinnamaldehyde presents a safe, effective, and economical alternative to antibiotic growth promoters in broiler chicken.

STUDIES OF DIETARY INCLUSION OF RICE DDGS THROUGH DIFFERENT GROWTH PHASES ON PERFORMANCE AND GUT HEALTH OF BROILER CHICKEN

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Four hundred twenty day old straight run commercial broiler chicks were divided into seven groups of three replications each and fed as T0 (Control) received standard basal diet as per BIS (2007) specification, T1 with 20 percent rice DDGS from 1st to 42nd day, T2 with 20 percent rice DDGS from 1st to 42nd day supplemented with protease enzyme @ 300g/ton of feed, T3 with 20 percent rice DDGS during starter and finisher stage, T4 with 20 percent rice DDGS during starter and finisher stage supplemented with protease enzyme @ 300g/ton of feed, T5 with 20 percent rice DDGS during finisher stage and T6 with 20 percent rice DDGS during finisher stage supplemented with protease enzyme @ 300g/ton of feed. The final body weights were significantly higher for group fed 20 percent rice DDGS during finisher stage supplemented with protease enzyme. The overall cumulative FCR reflected on 6th week indicated non significant difference amongst the group. The metabolizability of nutrients revealed non significant differences for various nutrients except CP metabolizability, where it was significantly higher for group with 20 percent rice DDGS supplemented with protease enzyme. The total viable count (TVC) and E-coli count revealed non significant differences and no significant difference was observed in crypt depth, villus width and villus height (VH) between different groups. It is concluded that inclusion of rice DDGS in broiler diet at 20 percent level during starter and finisher phase supplemented with protease enzyme @ 300 g/ton of feed proved to be more economical.

GUT PATHOLOGY AND MOLECULAR CHARACTERIZATION OF VIRULENT C. PERFRINGENS FROM NECROTIC ENTERITIS IN COCKRELS

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Clostridium perfringens is a normal inhabitant of the intestinal tract of chickens as well as a potential pathogen causing necrotic enteritis. *C. perfringens* is a major etiological agent due to its toxinogenic nature and ubiquitous presence in the poultry environment. In present research, intestine and cloacal swabs were collected from diseased cockrels. The present study aimed at pathology and molecular detection of *C. perfringens* from suspected cases of necrotic enteritis using culture and PCR-based techniques. All samples were processed for anaerobic bacterial isolation and molecular characterization. Samples were inoculated in RCM broth, incubated anaerobically using a gas pack system at 37°C for 24 hrs. Further the broth was streaked on *Perfringens* agar base (PBA) and colonies showing characteristic lecithinase and black colour were subjected to Gram staining, revealing Gram-positive rod-shaped bacteria. All these culturally positive isolates were further subjected to molecular characterization by using species specific PCR followed by toxinotyping of *C. perfringens* for *cpa*, *cpb* and *cpb2* toxin gene. Gram staining revealed Gram positive rod shaped organisms. Species specific PCR showed the amplicon size of 481 bp indicating the isolates being *Clostridium perfringens*. Further, toxins typing indicated the amplification of a specific 324 bp fragment confirming the presence of Toxin type A of *C. perfringens*. Toxinotyping of the isolates revealed that the necrotic enteritis among the cockrels was mainly caused by *C. perfringens* type A.

EFFECT OF DIETARY B-MANNANASE ON GUT HEALTH OF BROILERS FED WITH LOW ENERGY

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Six-week experiment was undertaken to evaluate β -mannanase supplementation in low-energy broiler diets. A total 300, day-old Cobb-430Y broiler chicks were assigned to five groups, containing 60 chicks with three replicates of 20 in each. The group PC was the positive control and contained 3000, 3125, 3250 kcal ME/kg feed in the pre-starter, starter, and finisher phases, respectively. Negative controls (NC1 and NC2) had 75 and 100 kcal less ME/kg diet, respectively, than PC. The 100g/ton β -mannanase (Natupulse® TS) was supplemented in these negative controls forming groups NC1 + BM and NC2 + BM. Jejunal histomorphology, Lactobacillus and E.coli count was performed 35th day. The β -mannanase supplementation in low-energy diet significantly ($P < .05$) lowered crypt depth, increased villus height : crypt depth ratio and lowered goblet cell count in the Jejunum compared to PC and negative controls (NC1 and NC2), indicating that β -mannanase helps to improve gut health. The β -mannanase significantly ($P < 0.01$) improved Lactobacillus count and decreased the E coli count of jejunal content. Supplementation of β -mannanase in low-energy diets alleviates negative effects of dietary mannans and significantly improved broiler gut health.

STUDIES OF PHYTOSOME CONJUGATED ESSENTIAL OILS ON PERFORMANCE AND GUT HEALTH OF BROILER CHICKEN

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Phytosome enable the utilization of lower concentrations of phyto constituents such as carvacrol and cinnamaldehyde in phyto conjugated products compared to their pure forms, translating into cost savings, better performance, nutrient metabolizability, gut health in broiler production. The 300-day-old Vencobb 430 straight run commercial broiler chicks were randomly assigned to five dietary regimens. There were four replicates of 15 birds in each treatment group. The group T0 served as the control and received the standard diet as per BIS 2007. Groups T1, T2, T3, and T4 received the standard broiler chicken diet with BMD antibiotic, phytosome-conjugated carvacrol EO, phytosome-conjugated cinnamaldehyde EO, and a combination of both (1:1), respectively. The essential oils were supplemented @ 100 mg/kg feed in T2, T3, and T4 groups. By the end of the 6th week, broiler birds in groups T3 and T1 exhibited significantly higher ($p \leq 0.01$) weekly body weight gains compared to the control group (T0), while other treatments showed non-significant differences. Overall, groups supplemented with phytosome-conjugated essential oils and antibiotic (T1) had lower FCR than the control. Phytosome-conjugated carvacrol and cinnamaldehyde improved DM and crude protein utilization. The groups supplemented with essential oils and antibiotic had significantly lower *E. coli* and TVC counts in caecal content, suggesting antimicrobial effects. Gut villi morphology did not show significant differences across treatment groups. Broiler birds supplemented with phytosome-conjugated essential oils (T3, T4, and T2) generated higher net profits compared to the control group. It is concluded that supplementing broiler chicken diets with phytosome-conjugated carvacrol EO (T2) and phytosome-conjugated cinnamaldehyde EO (T3) alone or in combination (T4) at 100mg/kg feed may serve as a safe, effective and economical natural growth promoter.

EFFECT OF DIFFERENT COMBINATIONS OF DIFFERENT FEED ADDITIVES ON PATHOLOGY OF EIMERIA INFECTION IN BROILERS

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Avian coccidiosis caused by *Eimeria* species remains a major challenge in commercial broiler production due to its adverse effects on intestinal health and performance. The present study was planned to evaluate the effect of different combinations of different feed additives on colonization of *Eimeria* spp., intestinal health, production parameters, gross and histopathological lesion induced by *Eimeria* spp. in experimental broiler chickens. A total of 240 day-old broiler chicks were randomly assigned to eight experimental groups, including a positive control, coccidiostat control and treatment groups receiving essential oil and herbal based feed additives. Birds were challenged via floor contamination with sporulated *Eimeria* oocysts ($4.0 \times 10^5/\text{m}^2$). Production performance parameters showed numerically improved body weight gain and FCR in supplemented birds. Grossly, severe lesions in intestine characterized by ballooning and caecal hemorrhages were observed in the positive control group. Histopathological examination revealed extensive villous disruption, crypt necrosis, inflammatory cell infiltration, and abundant endogenous developmental stages of *Eimeria*. In contrast, treated groups exhibited markedly reduced lesion severity, improved intestinal architecture, and lower parasitic load. Oocyst shedding was significantly reduced in all supplemented groups compared to the control, with herbal formulations showing the greatest reduction. A significant decrease in caecal *E. coli* counts was also recorded in treatment groups.

Theme 7

*Metabolic, Toxic, Nutritional
Diseases and their Management*

TOXICITY AND INCLUSION BODY HEPATITIS (IBH) IN POULTRY: AN OVERLOOKED LINK IN FIELD OUTBREAKS

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Introduction

Fowl adenovirus (FAdV) is recognized as the primary etiological agent of Inclusion Body Hepatitis (IBH), which has re-emerged as a significant disease in commercial poultry production in several countries, including India. IBH is classically characterized by sudden onset mortality, pale and friable liver, and the presence of basophilic intranuclear inclusion bodies in hepatocytes. To prevent and control IBH, inactivated IBH vaccines have been widely used in breeder flocks as well as in day-old commercial broilers. However, frequent field cases presenting with hepatitis and swollen kidneys continue to be reported. In many affected flocks, mortality patterns were inconsistent, responses to IBH vaccination were variable, and concurrent toxicity were commonly observed. Other immunosuppressive diseases such as Infectious Bursal Disease (IBD), Chicken Anemia Virus (CAV), and Marek's Disease (MD) play a critical role in weakening the immune foundation of poultry flocks and significantly influence the outcome of multifactorial diseases, including Inclusion Body Hepatitis. These infections are frequently subclinical, causing lymphoid depletion, poor vaccine response, uneven growth, and increased susceptibility to secondary viral and bacterial infections without obvious mortality. The situation is further complicated by the global emergence of variant and very virulent IBDV strains, diverse CAV genotypes, and evolving MDV variants, which challenge vaccine efficacy and immune protection.

In India, widespread circulation of IBDV, CAV, MDV, along with fowl adenoviruses and avian reoviruses, often occurs in combination with non-infectious immunosuppressive factors such as mycotoxins, nutritional deficiencies, heat stress, and poor water quality. Chronic exposure to aflatoxins and fumonisins, common under tropical conditions, suppresses lymphoid organ function and vaccine responsiveness, while deficiencies of vitamin E, selenium, and essential fatty acids further impair immune competence. These overlapping infectious and non-infectious stressors erode the "solid immune base" required for effective disease resistance, allowing pathogens like FAdV to cause severe clinical disease under field conditions. Therefore, maintaining immune competence through strong breeder immunity, optimal maternal antibody transfer, precision vaccination scheduling, mycotoxin control, stress reduction, and advanced diagnostics such as real-time PCR and molecular surveillance is essential for sustainable poultry health and performance.

Toxicity-IBH Interaction – Field Observations

Field evidence increasingly indicates that Inclusion Body Hepatitis (IBH) in poultry is strongly influenced by concurrent toxic stress, particularly mycotoxicosis, which acts as a critical trigger and disease amplifier rather than a coincidental finding. Diagnostic investigations conducted at Poultry

Diagnostic and Research Centre (PDRC), Pune, have shown that a substantial proportion of IBH-suspected cases exhibit histopathological lesions consistent with toxicity, including hepatocellular degeneration, fatty changes, bile duct hyperplasia, and renal tubular necrosis. In some flocks, Fowl Adenovirus (FAdV)-positive cases were simultaneously associated with detectable levels of aflatoxin B1 and fumonisins in feed, especially during monsoon and post-monsoon periods. Even when individual mycotoxin levels were within permissible limits, the presence of multiple mycotoxins likely exerted synergistic immunosuppressive effects, impairing liver detoxification capacity and reducing antiviral immune responses. This toxic stress compromises the hepatic and immune defenses, allowing enhanced viral replication and progression of latent or low-grade FAdV infections into clinically severe IBH outbreaks. The overlapping gross and microscopic lesions of IBH and mycotoxicosis further complicate field diagnosis and often lead to underestimation of the toxic component. These observations clearly support the concept that IBH under field conditions is a multifactorial disease, wherein toxicity plays a decisive role in determining disease severity, mortality, and outbreak dynamics, necessitating integrated control strategies addressing both viral infection and toxic exposure.

Understanding Toxicity in Poultry Production

Toxicity in poultry refers to adverse effects caused by ingestion or exposure to harmful substances present in feed, water, or the environment. In commercial poultry systems, toxicity is most commonly chronic and subclinical, making diagnosis difficult.

Common Sources of Toxicity

In commercial poultry production, toxicity arises from multiple sources. These include mycotoxins such as aflatoxin, T-2 toxin, ochratoxin, fumonisins and deoxynivalenol etc present in contaminated feed ingredients; residues of pesticides and fungicides used during crop production or storage; heavy metals like lead and cadmium; poor-quality drinking water containing excess salts or nitrates; and accidental drug or chemical overdosing. Among these, mycotoxins are the most important contributors due to tropical climate, poor storage conditions, and variable raw material quality.

Toxic stress also induces oxidative damage, further worsening liver injury, and interferes with vaccine response, reducing protective immunity. As a result, even low-grade or latent FAdV infection can progress to severe IBH outbreaks in the presence of toxic stress.

In such outbreaks, correction of feed quality and reduction of toxin load through the use of toxin binders and management interventions frequently lead to a marked reduction in mortality, even without specific antiviral treatment. This clearly supports the role of toxicity as a significant severity of IBH disease mortality.

Diagnostic Approach:

A virus-only diagnostic approach may lead to incomplete conclusions and ineffective control measures. A comprehensive investigation of IBH outbreaks should include detailed clinical history with special emphasis on recent feed changes, careful evaluation of gross liver and kidney lesions, and histopathological examination (liver, Kidney, Bursa, Thymus, Spleen and Bone marrow) to identify both inclusion bodies in liver, pancreas and lesions suggestive of toxicity. Molecular confirmation of FAdV by PCR is essential, but it should be complemented by mycotoxin analysis of feed and raw materials and assessment of concurrent immunosuppressive diseases such as IBD and CAV. An integrated diagnostic approach allows more accurate interpretation and formulation of effective control strategies.

Surveillance for FAdV infection:

In PDRC poultry disease surveillance was carried out regularly and last 10 years, several FAdVs were isolated from IBH/HPS/GE cases in different zones from commercial birds. Molecular typing was carried out using zone-wise representative isolates every year. Multiple serotypes were detected which are circulating in the field. Its pathogenicity study was carried using different serotypes of FAdV isolates in SPF birds. In vivo pathogenicity studies showed that the FAdV serotype 4, 8 and 11 are pathogenic and other FAdV serotype 1,2,6 and 9 were detected but disease was not produced experimentally in SPF chicks. In the year 2024-25, more than 2000 feed/feed ingredient samples were tested for Mycotoxins from chicken farms of multiple locations by using ELISA. Aflatoxin B1 was present in 78% samples below 20 ppb while 22% samples were higher than the permissible limit of 20 ppb. Ochratoxin A was present in 0.05% samples above the permissible limit and the remaining samples were below the permissible limit of 40 ppb. T-2 was detected in almost all samples below the permissible limit of 200 ppb. Fumonisin was detected in 16% samples above the permissible limit of 1500 ppb, which is higher than last year tested samples 2023-24. The samples of dead birds from corresponding feed samples having high feed toxicity in histopathology showed toxicity changes mainly in liver, kidney and gizzard with substantial fatty changes in hepatocytes and necrosis of tubules and haemorrhages in the kidney.

Control and Prevention Strategies

Effective prevention of IBH requires simultaneous control of viral infection and toxic exposure. This includes the use of good quality raw materials, proper feed storage and routine monitoring of feed for mycotoxin contamination. Inclusion of broad-spectrum toxin binders in feed, avoidance of indiscriminate drug use, and strengthening breeder vaccination programs are essential components of prevention. Control of immunosuppressive diseases and maintenance of good biosecurity further reduce the risk of IBH outbreaks. During active outbreaks, liver support supplements and antioxidants may help reduce severity, but they should be viewed only as supportive measures rather than substitutes for preventive strategies. An inactivated FAdV vaccine was shown to protect chicks against IBH by maternal antibody (MAb) transfer from vaccinated breeders. Monovalent vaccines do not cross-protect against heterologous FAdV species. For the effective prevention of IBH, multivalent vaccines are needed. Multivalent vaccines have been shown to provide broad-spectrum protection against avian viruses. In India inactivated oil emulsion vaccines are used to prevent the disease.

Conclusion

Toxicity alone, particularly mycotoxicosis, is capable of inducing severe hepatic and renal lesions in poultry that closely resemble those observed in Inclusion Body Hepatitis. Mycotoxin-induced hepatitis and nephritis commonly present with pale, enlarged, friable livers, hemorrhages, fatty degeneration, and swollen kidneys, leading to frequent misinterpretation as IBH based solely on gross pathology. Such lesion overlap can result in incorrect field diagnosis and inappropriate control measures when laboratory confirmation is not pursued. In the absence of Fowl Adenovirus infection, toxic injury may independently cause significant mortality and performance loss, emphasizing that gross lesions alone are insufficient for definitive diagnosis. Accurate differentiation between IBH and toxicity-related disease therefore requires an integrated diagnostic approach incorporating histopathology, molecular detection of FAdV, and comprehensive feed toxin analysis. Recognizing toxicity as both a disease mimic and a disease modifier is essential for effective field diagnosis and rational disease management.

PHYTOCHEMICAL PROFILING AND ANTI-GOUT EFFICACY OF *ZINGIBER OFFICINALE* AND *PIPER BETLE* IN BROILER CHICKENS

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The current study assessed the therapeutic efficacy of aqueous extract of *Zingiber officinale* roots and *Piper betle* leaves in experimentally induced gout in broiler chickens. GC-MS analysis revealed presence [6] - gingerol, gingerol, zingiberone, and paradol in *Z. officinale* and hydroxychavicol and eugenol in *P. betle*. Day old broiler chicks (N = 90) were randomly divided into 6 equal groups. T1 served as control whereas group T2, T3, T4, T5, and T6 were given sodium bicarbonate @15 g/L in drinking water from day 11-14 for induction of gout. Group T3, T4, T5, and T6 were treated with allopurinol (@20mg/L, twice daily), aqueous extract of *Z. officinale* (10%), *P. betle* (10%) and aqueous extract of *Z. officinale* (10%) + *P. betle* (10%) in drinking water, respectively from day 15 to 21. There was significant reduction in body weight, weight gain, and increased FCR in group gout positive control group, while treatment groups showed significant improvement. On day 14, gout induced groups showed significant increase in Hb, PCV, TEC, TLC, lymphocyte, total protein, albumin, AST, ALT, creatinine, BUN, and uric acid, while on day 21 there was significant reduction and on day 42 values were comparable with control group. Oxidative stress markers (MDA, SOD) exhibited beneficial nephroprotective effects. Gross observations of dead birds during 3rd week showed deposition of crystals of urates on heart, liver, spleen and kidney and histopathological observation revealed gout related lesions. On day 42, sacrificed birds of group T2 showed mild degenerative lesions in liver, heart, kidney, whereas treatment groups had no significant lesions. Thus, findings suggested that aqueous extract of *P. betle* (10%) alone or in combination with *Z. officinale* (10%) showed therapeutic beneficial effect against sodium bicarbonate induced gout in broiler chickens, presenting a potential alternative to allopurinol.

SAFETY STUDY OF NOVEL QUERCETIN PHYTOSOMES IN BROILER CHICKEN BY IMPLEMENTING OECD GUIDELINE

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Quercetin is a plant derived pigment, classified as a polyphenolic flavonoid group of phytochemicals. It has an excellent antioxidant property and other pharmacological activities. However, with less water solubility and little bioavailability reduces therapeutic applicability of quercetin in humans and veterinary medicine. Hence, present study was planned to fabricate, characterize and then investigate the safety of quercetin phytosomes, in broiler chickens. Quercetin was complexed with phytylphosphotidylcholine to form phytosomes by thin film hydration method and characterized for its size, shape and stability. Afterwards safety study of quercetin phytosomes was performed orally in 12 healthy broiler chicken (Vencob 400); birds were divided in control and treatment groups (n=6) as per OECD guideline 223. Birds from the treatment group was orally administered with quercetin phytosomes at a selected limit dose of 2000 mg/Kg body weight as a single dose and birds from control group received sham dose. Results of present study showed that, the particle size of quercetin phytosomes were in the range of 150 - 250 nm with PDI < 0.4, with zeta potential was higher than -22 mV and phytosomes were stable for 30 days at room ambient room temperature. SEM analysis showed 150-250 nm size of quercetin phytosomes. At the end of safety study, all the chicken were scarified humanely. Postmortem procedure was executed in treatment and control birds to note any gross changes in visceral organs and tissues from these organs were collected for histopathological studies. So, this study testified the safety of quercetin phytosomes for broiler chicken without any mortality or other clinical symptoms of toxicity at dose of 2000 mg/Kg body weight in broiler chicken.

OCCURRENCE OF VISCERAL GOUT IN A BROILER POULTRY FARM

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In November 2025, an outbreak of visceral gout occurred in a commercial broiler poultry farm in Nadia district of West Bengal. Affected birds showed clinical signs including hyporexia, lethargy, ruffled feather and progressive emaciation followed by mortality of few percentage of birds. The necropsy was subsequently performed, which revealed the presence of chalky white deposits on the surfaces of the liver, pericardium, and epicardium, along with marked thickening of the pericardium. The kidneys were enlarged, greyish in colour, and mildly congested. Histopathological examination of the heart showed multifocal tophi formation in the pericardium, surrounded by inflammatory cell infiltration, along with pericardial haemorrhages and congestion. The kidneys exhibited degeneration and necrosis of tubular epithelial cells, dilatation of renal tubules, and tophi formation in both proximal and distal convoluted tubules, accompanied by tubular mineralization. The liver showed fatty degeneration with tophi formation in the hepatic capsule. The lungs revealed congestion with focal tophi formation. Based on the characteristic clinical signs and gross and histopathological findings, the condition was diagnosed as visceral gout.

**PROTECTIVE EFFECT OF *ALLIUM SATIVUM*
SUPPLEMENTATION AGAINST EXPERIMENTALLY INDUCED
ESCHERICHIA COLI INFECTION IN BROILER CHICKENS**

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The present investigation was conducted over a period of 29 days on 150 day-old VenCobb broiler chicks which were procured and randomly allocated into six experimental groups (T1–T6). Group T1 served as the control. Birds in Group T2 were orally inoculated with *E. coli* broth at a dose of 0.5 ml (1×10^9 CFU/ml) on the 8th day of age. Group T3 received garlic powder at 1.5 g/kg feed from the 8th to the 15th day of age. Group T4 birds were administered *E. coli* broth along with garlic powder at the same dose and duration. Group T5 birds were challenged with *E. coli* broth and treated with levofloxacin at 1 g/10 kg body weight for seven days. Group T6 received *E. coli* broth, garlic powder (1.5 g/kg feed from the 8th to the 15th day), and levofloxacin at 1 g/10 kg body weight for seven days. Haemato-biochemical parameters were recorded on the 0th, 7th, 14th, and 28th days post-infection, *E. coli*-infected birds showed marked alterations in haemato-biochemical parameters compared to the control group. Gross pathological examination of Group T2 revealed congestion of the lungs, trachea, kidneys, and spleen, along with fibrinous pericarditis and perihepatitis. Histopathological examination showed treatment- and duration-dependent minimal to moderate lesions in the lungs, trachea, heart, spleen, liver, kidneys, and intestine. Garlic supplementation through feed exhibited a partial protective effect against experimentally induced *E. coli* infection. Levofloxacin treatment proved effective in mitigating disease effects, while the combined administration of garlic and levofloxacin demonstrated superior efficacy, as evidenced by improved growth performance, haemato-biochemical parameters, and reduced pathological lesions.

EFFECT OF MICROENCAPSULATED ESSENTIAL OIL AND ORGANIC ACID IN DIETS ON PERFORMANCE OF *ESCHERICHIA COLI* CHALLENGED BROILERS

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A study was conducted to evaluate the effects of microencapsulated essential oil i.e., cinnamon oil and microencapsulated organic acid i.e., butyric acid at a level of 0.02 and 0.12 g/kg in 180 broilers. There were four treatment groups with three replicates of 15 birds in each treatment group, the chicks in group (A) served as control. The birds in B group were inoculated at 8th day with avian pathogenic field strain of E. Coli. The birds in C group were received diet as in A + 0.02 g/kg cinnamon oil and 0.12 g/kg butyric acid in micro encapsulated form (MEOA). The birds in D group were also inoculated with E. Coli. Broiler growth performance, carcass traits, gut health was assessed and also production cost was calculated to know economic benefits. Broilers receiving feed having MEOA recorded improved ($p < 0.05$) body weights. The birds inoculated with E. Coli showed decrease in body weights and gain in weights with or without MEOA. Increase in feed intake was evident in group C getting MEOA in diet. The E. Coli challenged birds recorded lower feed intake compared to control. MEOA yielded better FCR however, E. Coli challenge has negatively influenced the FCR. Mortality was within standard range though it was comparatively higher in E. Coli challenged birds. The MEOA in feed has favourably reduced the total viable and coliform count in treated broilers. There was improvement in dressing and eviscerated yield in birds offered MEOA diet. The cost-economics revealed lesser production cost expressed on live weight basis in MEOA supplemented group C, however, it was higher in challenged groups. These findings are suggestive of beneficial effects of microencapsulated cinnamon oil at 0.02 g/kg and butyric acid 0.12 g/kg in diet on performance, carcass traits, gut health and margin of profit in broiler production.

ASSESSMENT OF NOVEL PROBIOTIC SUPPLEMENTATION ON GROWTH PERFORMANCE AND ECONOMICS OF BROILER PRODUCTION IN CHALLENGED DIETARY CONDITION

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The experiment was conducted on 960 Vencobb-430Y strain straight-run broiler chicks up to 5 weeks. The chicks were randomly distributed into eight groups viz., A, B, C, D, E, F, G and H having six replicates, each comprising of 20 birds. The control group A received basal diet without probiotics, group B supplemented with Enramycin@10ppm/T in basal diet. Groups C and D birds supplemented with Basal diet + Enramycin@10ppm/T + CO@200g/T of feed and Basal diet + Enramycin@10ppm/T + KN @ 200g/T of feed, respectively. Whereas, groups E and F diet supplemented with KN (KURACO™) @ 200g/T and ET (ENTEROSURE™) @ 200 g/T of feed in basal diet, respectively. However, groups G and H diet supplemented with MSF @ 350 g/T and XC @ 300g/T of feed in basal diet, respectively. At the end of 5th week, live body weights and cumulative weight gains were significantly ($P<0.01$) higher in treatment groups C, D, E, F, G and H supplemented with novel probiotics as compared to control group A. At 5th week, the cumulative FCR was significantly ($P<0.01$) better in treatment groups C to H as compared to control group A. However, significantly ($P<0.01$) better cumulative FCR was found in group F birds fed diet with ENTEROSURE™ @ 200 g/ton of feed as compared to groups A, B, C and H. The higher survivability was observed in treatment group C and followed by groups E, F, G, H, D, B and A. The significantly ($P<0.01$) better European Production Efficacy Factor (EPEF) was recorded in birds supplemented probiotics in basal diet for treatment groups C, D, E, F, G and H as compared to group control group A. The cost of production per kg live weight for the treatment groups A to H were Rs. 73.19, 71.65, 71.16, 70.40, 69.04, 68.55, 69.44 and 71.17, respectively. The highest net profit per kg live body weight was observed in treatment group F followed by groups E. Hence, it could be concluded that supplementation of novel probiotics KURACO™ @ 200g/T, ENTEROSURE™ @ 200 g/T and MSF @ 350 g/T of feed in challenged dietary condition improved growth performance and found to be more economically beneficial in broiler production.

AMELIORATION OF INDUCED FATTY LIVER SYNDROME IN BROILERS BY SUPPLEMENTATION OF FORTIMAS WITH OR WITHOUT HERBS

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Fatty liver syndrome (FLS) is a significant metabolic disorder in broiler chickens, characterized by excessive fat accumulation in hepatocytes, leading to impaired liver function, poor growth performance, reduced feed efficiency, immunosuppression, and increased mortality, thereby causing substantial economic losses to the poultry industry. The present study was planned to evaluate the efficacy of Fortimas with or without herbs on the pathology of induced Fatty liver syndrome in commercial broilers. A total of 270 one-day-old Cobb 430 broiler chicks were randomly distributed into six experimental groups and reared under uniform management conditions for 42 days. Fatty liver syndrome was induced through dietary modifications during the early growth period. Fortimas alone or in combination with herbs was administered either as a preventive or therapeutic supplement. Growth performance parameters, liver-body weight ratio, liver fat content, serum biochemical indicators of hepatic function, immune response against Newcastle disease virus, and liver histopathology were evaluated. Broilers supplemented with Fortimas or Fortimas with herbs showed improved body weight gain, better feed conversion ratio, reduced mortality, and significantly lower liver fat accumulation compared to the fatty liver induced control group. Improved serum biochemical profiles and enhanced immune response were also observed in supplemented birds. Histopathological findings revealed reduced hepatocellular vacuolation and milder fatty changes, particularly in preventive supplementation groups. The study emphasizes the importance of nutritional liver protectants in the prevention and management of fatty liver syndrome in broiler chickens.

IMPACT OF DIETARY INCLUSION OF ORGANIC ZINC AND CHROMIUM ON PHYSIOLOGICAL RESPONSE OF BROILER CHICKENS EXPOSED TO COLD STRESS

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The study was planned on 120 one-day-old broiler chickens, randomly distributed into 5 dietary treatments with 3 replicates having eight chickens each. Control birds (CON) were reared under thermoneutral conditions and offered a basal diet to meet their nutrient requirements. Treatment CS was offered the basal diet under cold stress. While, treatments ZNC100, CR2, and ZN+CR were offered basal diet under cold stress, supplemented with organic Zinc, organic Chromium or their combinations at 100, 2, or 100 + 2 mg/kg DM, respectively. Birds under CS treatment had higher feed intake compared to other treatments. Body weight gain and feed conversion ratio were not significantly affected by treatments. Digestibility of dry matter, crude protein, and nitrogen-free extra of birds under CON treatment were greater than CS, ZNC100, and CR2 treatments. The concentration of blood urea nitrogen and albumin to globulin ratio in CS treatment was higher compared to CON treatment. The antibody response of 21-day-old broiler chickens in the CS group was lower than the birds of the ZN+CR group. The lowest activities of glutathione peroxidase and superoxide dismutase were found in the blood serum of the CS group. The percentage of dressing carcass in both CON and CS treatments were lower than those of birds under ZN+CR treatment. Moreover, the yield of breast and drumstick of the ZN+CR treatment was higher ($P < 0.05$) than the CON treatment. There was better production performance along with improved immune status in broiler birds fed a diet supplemented with organic Zinc and Chromium alone or their combination under cold stress.

ENHANCING HEAT STRESS RESILIENCE IN BROILER CHICKENS USING *WITHANIA SOMNIFERA* MEDIATED ZINC OXIDE NANOPARTICLES

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Effect of supplementation of *Withania somnifera* mediated zinc oxide nanoparticles under heat stress in broiler chicken was studied. Characterization of *Withania somnifera* mediated zinc oxide nanoparticles confirmed synthesis of ZnONPs with size ranging from 40 nm to 200 nm. XRD analysis revealed hexagonal wurtzite structure while FESEM analysis showed virtually flakes like irregular and disc shape of ZnONPs. EDX analysis confirmed purity of ZnONPs with zinc as major element comprising of more than 50% of total constituent along with oxygen. The experiment was undertaken during summer months with apparent heat stress. The daily minimum temperature and daily maximum temperature during the period was above 24°C and 38°C respectively. Total 60, day old broiler chicks were equally divided into four groups. Group T1, served heat stress control group supplemented with basal diet, group T2 was supplemented with *Withania somnifera* root powder @1% of feed. Group T3 was supplemented with zinc oxide powder @ 80mg/kg of feed, and group T4 was supplemented with *Withania somnifera* mediated ZnONPs @ 50mg/kg of feed for a period of 42 days. Group T1 showed significant decrease in the average weekly body weight and weight gain with decrease in pooled mean feed consumption and increased FCR. Serum total protein was significantly decreased while serum AST, ALT, creatinine, cholesterol, blood glucose, MDA and corticosterone were significantly increased in control group, while, T4 group showed restoration of biochemical values followed by group T2 and T3. Microscopically, liver, heart, kidney, spleen, thymus, brain, bursa of Fabricius, duodenum, and ileum showed marked degenerative changes in T1 group, while T4 group followed by group T2 and T3 showed mild degenerative changes. Thus, *Withania somnifera* mediated ZnONPs showed some protective effects during heat stress in broiler birds at given dose.

BENEFICIAL ROLE OF INDIAN BAY LEAF POWDER (IBP) SUPPLEMENTATION ON ENVIRONMENTAL THERMAL STRESS-INDUCED DELETERIOUS IN WHITE PEKIN DUCKS

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The present experiment was performed to assess the effect of supplementing Indian Bay leaf powder (IBP) in feed on environmental thermal stress-induced deleterious changes on body weight, feed conversion ratio (FCR), haemato-biochemical, and antioxidant parameters in White Pekin ducks. Ninety-six (96) Day old White Pekin ducks were randomly allocated into 4 groups with 24 birds in each group; Group 1: No IBP supplementation, Group 2: IBP supplanted at the dose of 400 mg/kg b.wt., Group 3: IBP @800mg/kg b.wt., and Group 4: IBP supplemented @1200 mg/kg b.wt., those were maintained for 0 to 9 weeks. Supplementation of IBP was done over a period of 42 days (from 3 weeks old to 9 weeks old ducklings. The blood collection was done from randomly selected 6 ducks from each group, totalling to 24 birds in one occasion, on day 0 (3week old duckling) and subsequently on 21st and 42nd day till 9th week. The exposure to increasing THI (Temperature-Humidity Index) or thermal stress over a period of 42days inflicted changes in the ducklings, which include reduced body weight gain, feed intake and increased water intake, along with open mouth breathing, dullness, ruffled feathers. These signs were also noticed in IBP (400mg/kg b.wt. in feed) treated birds, but in very milder form. In the experiment there was persistent increasing THI from Day 0 to Day 42, revealing increasing level of stress to ducklings from 3-week-old to 9-week-old. There was significant reduction in body weight gain from Day 0 to Day 42 of experiment in G1, which was significantly alleviated by feed supplementation of Indian Bay leaf powder @ 800 and 1200 mg/kg b.wt. Thermal stress impacted mean values of Hb, PCV, Heterophil %, Lymphocyte %, H/L ratio with increasing level of THI (Thermal stress) and significant reversal of these effects was noticed with supplementation of IBP @ 1200mg/kg b.wt. in feed. Significant increase in mean levels of AST, ALT, ALP, LDH, glucose, cholesterol, total globulin, BUN, creatinine, uric acid and corticosterone, indicating liver and kidney dysfunction with increasing thermal stress in body. These levels were significantly decreased with supplementation of IBP (1200 mg/kg b.wt.) in feed. The supplementation of IBP @ 800 mg/kg b.wt. in feed helped in reducing mean values of AST, creatinine, glucose and corticosterone significantly. Supplementation of IBP (400mg/kg b.wt.) in feed has only been proven significant to reduce mean values of creatinine, glucose and corticosterone. Significant decrease in mean levels of TP, ALB, Globulin, Ca and P has been noticed indicating hypoproteinaemia along with mineral deficiency due to impaired absorption with thermal stress. It is concluded that prophylactic inclusion of IBP to feed can help alleviate the deleterious effect of environmental thermal stress, such as growth disturbances, haemato-biochemical alterations, and antioxidant level variations in White Pekin duckling.

SURVEILLANCE AND RISK ASSESSMENT OF MYCOTOXIN CONTAMINATION IN POULTRY FEED SAMPLES DURING 2024-2025

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Mycotoxins are known to decrease health and performance in poultry production. This study investigates the scale of mycotoxicity in the poultry feed during 2024-2025. In the year 2024-25, more than 2000 feed/feed ingredient samples were tested for Mycotoxins from chicken farms of multiple locations and subjected to the ELISA Assay. Tissue samples from morbid or dead birds were also collected for histopathology and microbial identification. Aflatoxin B1 was present in 78% samples below 20 ppb while 22% samples were higher than the permissible limit of 20 ppb. Ochratoxin A was present in 0.05% samples above the permissible limit and the remaining samples were below the permissible limit of 40 ppb. T-2 was detected in almost all samples below the permissible limit of 200 ppb. Fumonisin was detected in 16% samples above the permissible limit of 1500 ppb, which is higher than last year tested samples 2023-24. Histopathology findings revealed toxicity changes mainly in liver, kidney, gizzard and ovaries. Substantial fatty changes in hepatocytes and necrosis of tubules and haemorrhages in the kidney. Immunosuppression due to toxicity also leads to secondary complications in commercial broilers. Mycotoxicity cases are also observed in Commercial Layers and desi birds. Samples received in June to December showed the highest positivity. Mycotoxin Prevalent in India AfB1 > Fum > OTA > T2 > ZEN > DON. Multiple mycotoxins causes synergistic effects. These findings highlight significant regional and temporal variations in mycotoxin contamination, particularly Aflatoxin B1, which poses a continuous risk to feed quality and bird health. The data emphasize the importance of routine feed monitoring, stringent raw material screening, and detoxification strategies at the field level to ensure feed safety and minimize the impact of mycotoxicosis on poultry productivity. Mycotoxicosis is the most important predisposing factor for many bacterial and viral infections. Histopathology examination is an important tool for differential diagnosis of mycotoxicosis. Environmental conditions and storage conditions are major factors for development of mycotoxicosis. Use of good quality toxin binder with the appropriate dose is advisable to reduce impact of mycotoxicosis.

PATHOLOGY OF EXPERIMENTALLY INDUCED SUBACUTE AND SUB-CHRONIC ZIRAM TOXICITY IN KADAKNATH CHICKENS

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To Study the pathomorphological and haemato-biochemical alterations in ziram-induced subacute and sub-chronic toxicity in Kadaknath chickens. Dithiocarbamate fungicides are widely used across the globe for various crops, primarily because of their effectiveness in managing plant fungal diseases and their relatively low acute toxicity to mammals. Ziram, a specific dithiocarbamate, is utilized to combat various fungal diseases affecting potatoes, nuts, certain fruits and grains. The current study was conducted from May to October 2024 at the college poultry farm unit and the Department of Veterinary Pathology, COVSc. , MHOW, aimed to study the pathomorphological and haemato-biochemical alterations in the ziram-induced subacute and sub-chronic toxicities in Kadaknath chickens. The clinical signs of toxicity included lethargy, loss of appetite, huddling, ruffled plumage, muscle weakness, uncoordinated gait and oedema in the crop. Weight gain and overall live weight were significantly lower in the experimental groups compared to the control group. Haematological changes included decreased haemoglobin, TEC, PCV and lymphocytes, with increased leucocyte count and heterophils. Biochemical analysis showed increased AST, ALT, creatinine and globulin levels, while total protein and globulin decreased. Zinc levels were significantly elevated, while copper showed no significant change. Gross pathology revealed liver congestion with necrotic foci, dark red spleen, hyperaemic intestines and atrophied bursa of Fabricius and thyroid. Histopathological findings included liver oedema, necrosis, spleen haemorrhages, intestinal necrosis and kidney degeneration. The study highlighted the toxic effects of ziram on various organs in Kadaknath chickens.

A CASE STUDY ON HEAT STRESS IN POULTRY

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Birds are 'heat stressed' if they have difficulty achieving a balance between body heat production and body heat loss. This can occur at all ages and in all types of poultry. Around 53 birds were brought to the department of veterinary pathology, College of veterinary and animal sciences, Arrabari Kishanganj, BASU, Patna for post-mortem examination in the period of 15 days. The time duration lies in the month of May-June, when the temperature and humidity both were on peak at the place of study. Cardiorespiratory failure due to overheating was observed as the possible cause of death. Scalp cyanosis, skeletal muscle anemia that looked like cooked meat or par-boiled appearance, petechiae, and internal organ hyperaemia were reported as typical macroscopic findings. Heat stress indeed has a detrimental effect on poultry production causing farmers facing economic crises.

THE POTENTIAL RISKS OF HIGH-DOSE PROBIOTIC ADMINISTRATION: INVESTIGATING BACTERIAL TRANSLOCATION AND MORTALITY IN NEONATAL CHICKS

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Probiotics, particularly *Lactobacillus* species, are generally regarded as beneficial and widely promoted as safe alternatives to antibiotics in poultry production. However, limited information is available on their safety at high doses during early life stages. This study investigated the effects probiotic gel supplementation containing specific *Lactobacillus* strains, on the survival and systemic health of young chicks. Day-old chicks were accidentally administered a probiotic gel containing three distinct strains of *Lactobacillus* species. These strains were administered both individually and as a multi-strain consortium at a concentration of 10^8 CFU/ml. Following probiotic administration, a significant mortality rate was observed in the treated groups within the first week of life. To investigate the cause, post-mortem examination was conducted on succumbed chicks (5-day-old), tissue samples including liver, spleen, yolk sac and cloacal swabs were collected from succumbed chicks for microbiological analysis to assess bacterial presence and translocation. Microbiological evaluation of the succumbed chicks revealed presence of administered *Lactobacillus* strains not only in cloacal swabs but also systemically within the liver, spleen, and yolk sac. This indicates a clear occurrence of bacterial translocation from the gastrointestinal tract to vital organs. The systemic presence of these strains suggests that at high dosage, these typically commensal bacteria may overwhelm the immature gut barrier and immune system in young chicks, leading to septicemia-like conditions and death. These findings raise concerns regarding the safety of administering high-dose probiotics during early developmental stages and highlight the importance of careful dose optimization before field application.

A POST-MORTEM STUDY ON TESTICULAR PATHOLOGY OF POULTRY

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Avian reproductive pathology research predominantly focuses on females due to the economic implications of egg production; however, male reproductive integrity is a fundamental determinant of flock fertility and genetic progress. This study aimed to characterize the prevalence and morphological nature of gross and histopathological lesions in the male reproductive tract across various avian species and age groups. A systematic post-mortem investigation was conducted on 132 male birds, comprising chickens (n=118), ducks (n=13), and a turkey (n=1), spanning chick, grower, and adult life stages. Following detailed gross examination, testicular and epididymal tissues were fixed in 10% neutral buffered formalin, processed routinely, and sectioned at 4 micron thickness. Slides were stained with Haematoxylin and Eosin (H&E) for light microscopic evaluation. Gross pathological alterations were identified in 10.6% (14/132) of the birds. The primary gross observations included vascular congestion of the tunica albuginea (8.87 %), unilateral and bilateral testicular atrophy (5.65%), sporadic instances of epididymal lithiasis and a testicular neoplasia. Histopathological analysis revealed a significantly higher incidence of subclinical pathology, with lesions evident in 61.3% of the subjects. Predominant microscopic findings included interstitial vascular congestion, congestion of the tunica albuginea, and multifocal degeneration of the seminiferous tubules. Notably, a rare case of testicular teratoma was identified in an adult rooster, exhibiting multi-lineage differentiation including glandular epithelium, cartilage, and keratinized epithelial pearls. The high prevalence of microscopic lesions, even in the absence of gross abnormalities and within younger age groups (chicks), underscores the susceptibility of the avian male reproductive system to early-onset pathological insults.

HAEMATOBIOCHEMICAL CHANGES IN EXPERIMENTALLY INDUCED HEAT STRESS IN BROILER BIRDS

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In this study, a total of 150 broiler chickens were divided into six groups, each consisting of 25 birds. The study was carried out for a period of 35 days. Group T1 served as the normal control, while group T2 acted as the heat stress control (HS). Group T3 received dried tamarind pulp powder (DTPP) at a concentration of 2.5 g/kg of fodder, group T4 was supplemented with Vitamin C powder at 250 mg/kg of fodder, group T5 consisted of heat-stressed (HS) birds receiving 2.5 g/kg of DTPP, and group T6 included heat-stressed birds supplemented with Vitamin C powder at 250 mg/kg of fodder. Hematological analysis revealed a decrease in hemoglobin (Hb), packed cell volume (PCV), and total erythrocyte count (TEC) in heat-stressed birds. However, parameters such as total leukocyte count (TLC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and clotting time remained within normal ranges. Notably, heat stress induced a significant reduction in lymphocyte count, an increase in heterophil count, and a higher heterophil-to-lymphocyte (H/L) ratio. Biochemical analysis demonstrated a decline in total protein, albumin, globulin, and the albumin/globulin (A/G) ratio in the heat-stressed group. Additionally, heat stress led to increase in the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum cholesterol, uric acid, and creatinine. Serum sodium and potassium levels were also elevated in heat-stressed birds. Oxidative stress markers revealed increased lipid peroxidation in red blood cells (RBCs) of heat-stressed broilers. Furthermore, serum corticosterone levels were significantly higher in the heat stress group, indicating a stress response. The estimated values shown improvement in treated birds against heat stress. These findings suggest that heat stress adversely affects the hematological and biochemical profiles of broilers, and supplementation with DTPP as well as Vitamin C had shown mitigating effect on experimentally induced heat stress.

GROSS AND HISTOPATHOLOGICAL STUDIES ON OVARIAN ADENOCARCINOMA IN A 7-YEAR-OLD TURKEY BIRD: A CASE REPORT

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Ovarian neoplasms are infrequently reported in avian species, particularly in turkey (*Meleagris gallopavo domesticus*), and available published literature remains scarce. The ovarian adenocarcinoma in turkeys were thought to be primarily arisen from the germinal epithelium and could be significantly influenced by environmental factors, especially photoperiod (light exposure), with longer summer days promoting neoplastic growth. The present case report describes ovarian adenocarcinoma in a seven year-old turkey bird. The necropsy of an adult bird with the history of chronic illness, including progressive abdominal distension, reduced feed intake and reduced physical activity was conducted. Prominent necropsy finding revealed presence of a large quantity of amber-colored fluid within the peritoneal cavity, indicative of ascites likely associated with neoplastic involvement of ovarian tissue. The liver appeared pale, yellowish, and glistening, suggestive of degenerative changes possibly secondary to chronic systemic stress and impaired metabolism. The ovary was markedly enlarged occupying major space in abdominal cavity and appeared multilobulated, firm to hard, raised whitish nodules with a characteristic gritty consistency. On cut section, these nodules exhibited diffuse white necrotic areas, consistent with malignant epithelial tumor growth. Additionally, small multifocal whitish nodules were observed on the peritoneal surfaces, particularly in the region of the reproductive tract, indicating transcoelomic spread of the tumor. The presence of peritoneal implantation nodules along with ascites supports the aggressive and invasive nature of the neoplasm. The gross pathological findings were characteristically suggestive of a malignant ovarian tumor. The representative histopathological examination the ovarian nodular growth showed glandular morphology with cuboidal neoplastic cells having basal round nuclei and prominent nucleoli arranged in a tubulo-acinar pattern. Focally, sheets of proliferating cells separated by strands of fibrous connective-tissue were noted. Based on the characteristic gross and microscopic lesions, including ovarian nodular masses, necrosis, ascites, and peritoneal dissemination, the condition was diagnosed as ovarian adenocarcinoma. Ovarian adenocarcinoma is a common malignant ovarian tumor in birds and is known for its rapid progression and tendency for peritoneal spread. This case contributes valuable information to the limited data on ovarian tumors in turkey birds and underscores the importance of systematic post-mortem examination for accurate diagnosis of avian reproductive neoplasms.

A RARE CASE OF ADRENAL TERATOMA IN CHICKEN

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Teratomas are uncommon tumors of domestic fowl. These are the rare germ cell tumors composed of tissue derived from two or more embryonic germ layers. In teratomas, adrenal teratomas have been found rarely in chicken. Adrenal gland plays important role in metabolism, tissue differentiation, resistance to infection, stress, low temperature. Present report describe rare case of adrenal teratoma of weight 250 gm in four weeks old chicken identified during routine post mortem examination. Grossly a well demarcated, firm, lobulated mass was observed in abdominal cavity with heterogenous cut surface. Histopathological examination revealed that the tumour included structures derived from all three germ cell layers: ectoderm, mesoderm and endoderm. This report documents a rare case of adrenal teratoma in a chicken diagnosed during post-mortem examination and emphasizes the diagnostic value of histopathology.

COMPARATIVE EVALUATION OF NOVEL PROBIOTICS IN BROILER DIETS ON INTESTINAL MICROMETRY, ISI SCORE, CARCASS TRAITS AND MEAT ORGANOLEPTIC PROPERTIES UNDER DIETARY CHALLENGE CONDITIONS

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The study was conducted on 960 Vencobb-430Y strain straight-run broiler chicks up to 5 weeks. The chicks were randomly distributed into eight groups viz., A, B, C, D, E, F, G and H having six replicates, each comprising of 20 birds. The control group A received basal diet without probiotics, group B supplemented with Enramycin@10ppm/T in basal diet. Groups C and D birds supplemented with Basal diet + Enramycin@10ppm/T + CO@200g/T of feed and Basal diet + Enramycin@10ppm/T + KN @ 200g/T of feed, respectively. Whereas, groups E and F diet supplemented with KN (KURACO™) @ 200g/T and ET (ENTEROSURE™) @ 200 g/T of feed in basal diet, respectively. However, groups G and H diet supplemented with MSF @ 350 g/T and XC @ 300g/T of feed in basal diet, respectively. The Vilus height: crypt depth ratio of duodenum at 28th day was significantly ($P<0.01$) increased in groups E, F, G and H supplemented with various probiotics as compared to groups A and B. Supplementation of ENTEROSURE™ (F) was recorded significantly ($P<0.01$) higher Vilus height: Crypt depth ratio than all the treatment groups except groups E and G. The mean values of ISI score of jejunum at 28th day were significantly ($P<0.01$) reduced in groups F and G as compared groups A and B. The mean values of eviscerated weight, gilet and abdominal fat pad percent relative to live body weight were non-significant in all treatment groups at 35th day. At 35th day, the mean values of water holding capacity (WHC) were significantly ($P<0.01$) increased in groups F and G as compared groups A, B and C. However, the values of shear force of breast meat of broilers were significantly ($P<0.01$) reduced and Myofibril Fragmentation Index (MFI) was significantly ($P<0.01$) increased in treatment groups E, F, G and H as compared to groups A, B and C. Hence, it could be concluded that supplementation of novel probiotics ENTEROSURE™ @ 200 g/T, KURACO™ @ 200g/T and MSF @ 350 g/T of feed in challenged dietary condition improved micrometry of duodenum, ISI score of jejunum, carcass traits and organoleptic properties of breast meat in broilers.

EFFECT OF DIETARY SUPPLEMENTATION OF PREBIOTIC AND ACIDIFIER POWDER ON COMPARATIVE ECONOMICS OF BROILERS

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The present study evaluated the effect of dietary supplementation of prebiotics and acidifiers on the comparative economics of broiler production. A total of 120 one-day-old commercial broiler chicks (Vencobb-430Y) were utilized in a completely randomized block design and reared for 42 days. The birds were randomly distributed into four dietary treatment groups with 30 chicks per group. The control group (C) was fed a standard broiler mash, while treatment T1 received prebiotics in the form of fructo-oligosaccharides (0.5% during 0-3 weeks and 0.25% during 4-6 weeks). Treatment T2 was supplemented with acidifiers at the same inclusion levels, and T3 received a 1:1 combination of prebiotics and acidifiers. Economic evaluation was carried out by analyzing feed cost, total cost of production, cost per kilogram live weight, gross return, net return and benefit-cost ratio. Although the supplementation of prebiotics and acidifiers slightly increased the feed cost due to the inclusion of additives, the overall cost of production per kilogram live weight was reduced in the supplemented groups. Improved feed efficiency and better utilization of nutrients resulted in enhanced economic performance compared to the control group. Among the dietary treatments, birds receiving the combined supplementation of prebiotics and acidifiers (T3) showed the highest economic advantage, followed by those supplemented individually with prebiotics (T1) and acidifiers (T2). The improved profitability in the supplemented groups was mainly attributed to better feed conversion efficiency and reduced feed requirement per unit of weight gain. Consequently, higher net returns and improved benefit-cost ratios were observed in these groups compared to the control. In conclusion, dietary inclusion of fructo-oligosaccharides and acidifiers, particularly in combination, proved to be economically beneficial in broiler production. The findings suggest that these non-antibiotic feed additives can be effectively used to enhance profitability and sustainability in commercial broiler farming systems.

GROWTH PERFORMANCE AND NUTRIENT UTILIZATION IN BROILER CHICKENS SUPPLEMENTED WITH ZINC AND *SACCHAROMYCES CEREVISIAE*

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The present study was conducted to evaluate the effect of dietary supplementation of organic and inorganic zinc, with or without probiotic (*Saccharomyces cerevisiae*), on growth performance and nutrient utilization in broiler chickens. A total of [insert number] birds were reared under standard managemental practices up to 35 days of age. Experimental groups were designed to assess the impact of zinc sources and probiotic inclusion on parameters such as body weight gain (BWG), feed intake, feed conversion ratio (FCR), and nutrient digestibility (crude protein, ether extract, and metabolizable energy). Results demonstrated significant ($p < 0.01$) improvements in growth traits in zinc + *S. cerevisiae* supplemented groups. The T6 group (organic zinc + 1% *S. cerevisiae*) recorded the highest BWG (1852.33 g), lowest FCR (1.65), and maximum crude protein digestibility (78.42%). Similarly, ether extract digestibility (79.11%) and metabolizable energy utilization (3100.22 kcal/kg) were superior in T6 compared to control. The T3 group (inorganic zinc + 1% *S. cerevisiae*) also showed notable enhancement (BWG: 1765.22 g; FCR: 1.72; CP digestibility: 75.11%), while the control group exhibited comparatively lower performance (BWG: 1620.45 g; FCR: 1.85; CP digestibility: 71.02%). Feed intake remained consistent across treatments, but efficiency of nutrient utilization was markedly improved in supplemented groups. Overall, supplementation of zinc, particularly in organic form with *S. cerevisiae*, enhanced nutrient absorption and supported superior growth performance in broiler chickens, indicating its potential for improving productivity in commercial poultry farming.

IMPACT OF SUPPLEMENTATION OF PEPPERMINT AND EUCALYPTUS ESSENTIAL OILS ON THE GROWTH PERFORMANCE OF JAPANESE QUAILS

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Traditionally antibiotics were being used as feed supplement in the ration of farm animals causing increase in the incidence of anti-microbial resistance (AMR) in livestock and humans. In order to overcome this issue, global organizations are trying to find out potential replacements for antibiotics. Essential oils are the volatile aromatic compounds having antimicrobial, anti-inflammatory, and antioxidant properties. Apart from their medicinal properties their aroma makes them useful in cosmetic industry and also as flavoring agents. This study was undertaken to investigate the impact of dietary inclusion of peppermint (PEO) and eucalyptus essential oils (EEO) on the growth performance of the Japanese quails. For this, 180 Japanese quail chicks of six day old were randomly grouped into 5 treatments which were further divided into 3 replicates having 12 chicks each. The experimental treatments were T0 (Basal diet), T1 (Basal diet+0.2% of PEO), T2 (Basal diet+0.2% of EEO), T3 (Basal diet+0.1% each of PEO+EEO) and T4 (Basal diet+0.05% each of PEO+EEO). The trial was carried out for 35 days and after this one-week metabolic trial was undertaken. The study revealed that the Japanese quails supplemented with 0.1% of peppermint and eucalyptus essential oils in combination reduced the overall feed intake and enhanced the average body weight gain resulting in better FCR. This group also showed least feed cost per kg body weight gain compared to all the other groups. Thus, it can be concluded that the supplementation of 0.1% of PEO and EEO in combination in the Japanese quails improved the growth performance, feed efficiency, and lowered the production cost which is beneficial for the farmers. Therefore, 0.1% of peppermint and eucalyptus essential oils in combination can be a promising supplement in feed of Japanese quails which can help reduce the application of antibiotics in animal feed industry.

HISTOMORPHOLOGICAL CHANGES IN INTESTINE OF BROILER IN NORMAL AND *SACCHAROMYCES CEREVISIAE* FERMENTED FED PRODUCT

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The current study is focused on the morphological differences in the intestinal segments of broiler chickens fed with *Saccharomyces cerevisiae* and normal diet. The birds from each group were slaughtered on 21st and 42nd day of age and tissue samples from the duodenum jejunum, ileum and cecum were collected. The small intestine of both the groups was found to have three layers: the tunica mucosa, tunica muscularis, and tunica serosa, from inner to outer. The submucosa was inappreciable. The tunica mucosa presented different villi that varied in its shape, size and appearance. The tunica muscularis had 3 distinct layers the inner and outer longitudinal and middle circular layers. The tunica mucosa had numerous blood vessels and adipose tissue. Feeding of *Saccharomyces cerevisiae* had shown beneficial impact on the broiler performance in terms of carcass weight. This may be due to alteration in bird's metabolism which is due to the structural difference that occurred due to feeding of *Saccharomyces cerevisiae*. It increased the height and density of villus which is responsible for better rate of nutrient absorption. It also showed increase in thickness of tunica muscularis, goblet size and diameter of lymphatic nodule that helped in inhibiting the growth of pathogenic microbes.

Theme 8

*Immunosuppression
and Immunomodulation*

IMMUNOSUPPRESSION AND IMMUNOMODULATION IN POULTRY

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Immunosuppression in Poultry

Immunosuppression in poultry refers to an impaired condition of bird's immune system (innate and adaptive immune responses) making them more susceptible to infections. This results in stunted growth, poor vaccine response and high mortality. It is considered as one of the major hidden causes responsible for economic losses in poultry sector.

Immunosuppression in poultry is multifactorial. Its major causes include viral diseases i.e. Infectious Bursal Disease (IBD), Chicken Infectious Anaemia (CIA) and Marek's Disease (MD); Avian Leukosis (AL), Reticuloendotheliosis (RE), few Avian Adeno and Reo virus infections; secondary infections caused by certain bacteria, fungi and parasites; mycotoxins; nutritional imbalance; genetic, managemental and environmental stress factors, and improper use of chemicals and pharmaceuticals.

Viral causes of immunosuppression

Infectious Bursal Disease

IBD in chickens is caused by infectious bursal disease virus (IBDV) of the Birnaviridae family. IBDV targets B cells in bursa of Fabricius, causes depletion of B-cells and results in long-lasting humoral deficits. Immunosuppressive effects caused by IBDV infections were first of all reported by Allan et al. (1972) and Faragher et al. (1974). IBDV primarily infects bursa of Fabricius, causes severe apoptosis and lysis of B lymphocytes and their precursors. It leads to severe depletion of lymphocytes in bursa of Fabricius, thus crippling the humoral immune response. In addition, IBDV also cause indirect immunosuppression by dysfunction of T-cells. The IBDV exposure results in activation of host macrophages and produces anti-proliferative substances, such as nitric oxide which inhibits proliferation of T-cells. Besides, IBDV proteins interfere with antiviral responses produced by the host and cellular signalling pathways. It affects functions of helper T-cell and causes cytokine interference by disturbing cytokine balance. Other organs such as spleen, gut-associated lymphoid tissue and Harderian glands are also affected by IBDV infection (Zhang et al., 2024).

Chicken Infectious Anaemia

CIA is caused by chicken infectious anaemia virus (CIAV), a member of the genus Gyrovirus, family Anelloviridae. The immunosuppression by CIAV is primarily caused by destruction of T-lymphocyte

precursors present in the cortex of thymus. This is mediated by apoptosis through non-structural viral protein 3 (VP3) of the virus, also known as "apoptin". The lymphocytic destruction and depletion leads to atrophy of thymus. It causes significant reduction in the population of mature T-helper (CD4+) and cytotoxic (CD8+) lymphocytes throughout the body of host mainly in blood and spleen (Fatoba and Adeleke, 2019). The significant reduction in number of T-helper cells hampers overall immune signalling network. It causes substantial reduction in release of cytokines such as interleukin-2 and interferons which are essentially required for normal proliferation of T-cells and their functions as well as antibody production by B-cells. It also causes significant reduction in the production of antigen-specific cytotoxic T lymphocytes, which are critical for fighting against other viral infections such as MD.

Marek's Disease

Marek's Disease Virus (MDV) also causes significant immunosuppression in poultry. It is mediated by depletion of lymphocytes, dysregulation of T-cells, molecular interference via cytokine imbalance and activation of cyclooxygenase-2 (COX-2)/prostaglandin E2 (PGE2) pathways. MDV encodes several proteins viz. Meq oncogene, vTR (viral telomerase RNA), vIL-8, and pp38 which interfere with the survival and proliferation of lymphocytes, cytokine signaling, and immune cell trafficking resulting in marked immunosuppression, facilitating viral persistence and tumourigenesis in infected chickens. Depletion of B lymphocytes occurs during early cytolytic phase of MDV infection. It is mediated by apoptosis, inhibition of cell proliferation and atrophy of bursa of Fabricius and thymus. Apoptosis targets uninfected bystander cells near the MDV infected cells likely due to paracrine signaling or contact with infected cells. However, in thymus; MDV infection triggers the process of apoptosis directly in infected cells. MDV infections strongly inhibits the proliferation of B-cells by arresting the G0/G1 phase of cell-cycle and down regulation of cell-cycle key regulators, preventing the division of B cells from dividing and repopulating the lymphoid organs. Within few days of infection with MDV, severe atrophy of bursa of Fabricius and thymus occurs due to massive cell death and lack of new cell growth (Berthault et al., 2018). Thus, MDV infection causes severe immunosuppression by significant B-cell lymphopenia. MDV infection also causes dysregulation of T-cells by preferential targeting and transforming CD4+T lymphocytes. Dysregulation causes functional alterations such as impaired proliferation, altered cytokine production, and resistance to apoptosis. It also facilitates clonal expansion and development of lymphoma (Nair, 2018). Virulent MDV strains increase population of regulatory T-cells that expresses immunosuppressive cytokines such as transforming growth factor-beta (TGF- β). It suppresses effector T-cell responses and results in early immunosuppression (Gurung et al., 2017). Activation of T-cells is hampered by upregulation of COX-2 and PGE2 pathways, resulting in reduced production of IL-2 and diminished T-cells' proliferation (Kamble et al., 2021). In addition, MDV infection also results in exhaustion of T-cells and impaired antiviral responses by increased expression of programmed death-1 and cytotoxic T-lymphocyte antigen-4 immune check points (Sato et al., 2025). Altogether, it leads to disruption of T-cell homeostasis, resulting in compromised cell-mediated immunity, increased viral persistence and tumour production in infected chickens.

Avian Leukosis

ALV mainly subgroup J (ALV-J) causes immunosuppression in the chickens involving several mechanisms that affect both innate and adaptive immune responses. ALV-J infection in chickens causes atrophy of bursa of Fabricius and thymus that impairs the development of B and T lymphocytes and reduced immune competence. Infected chickens show significant depletion of lymphocytes and altered

distribution of T-cell subsets with marked reduction in the population of CD4+ and CD8+ T cells resulting in compromised cell-mediated immunity (Nair, 2020). Cytokine dysregulation induced by ALV infection in chickens is characterized by enhanced expression of immunosuppressive cytokines (IL-10) and abnormal production of pro-inflammatory cytokine (IL-6) which adversely affects the normal immune signalling and antiviral responses (Dai et al., 2016; Gao et al., 2016). ALV-J infection blocks the NF- κ B signalling pathway. It suppresses innate antiviral immunity by inhibition of type I interferon responses. ALV-J infection leads to upregulation of I κ B α resulting into reduced production of IFN- β and thus causing weakened early antiviral defence mechanisms (Lin et al., 2018). ALV infection results in exaggerated immunosuppression in cases with IBD and results in severe immune dysfunction (Chen et al., 2022).

Reticuloendotheliosis

Reticuloendotheliosis virus (REV) infection affects primary and secondary lymphoid organs of chickens and results in persistent immunosuppression. This causes marked atrophy of bursa of Fabricius as well as thymus resulting in depletion of B lymphocytes and CD4+ T helper cells. It leads to immunosuppression by impairment of both humoral and cell-mediated immunity (Zavala and Nair, 2020).

Miscellaneous Infectious Causes

Avian adenoviruses are not primarily responsible for producing immunosuppression in poultry. However, few serotypes of fowl adenoviruses lead to reduced immune competence due to atrophy of lymphoid organs and apoptosis of lymphocytes (Schonewille et al., 2008). A few strains of avian reoviruses also cause immunosuppression by reduced proliferation of T and B cells. Recent genomic studies and transcriptome analyses suggested that emerging variants of avian reoviruses modulate expression of host immune-related genes, particularly interferon and inflammatory pathways that results in immune dysfunction (Wang et al., 2023; Nour and Mohanty, 2024). Secondary infections caused by certain bacteria (*Escherichia coli*, *Salmonella* spp., *Clostridium perfringens* and *Mycoplasma gallisepticum*), fungi (*Aspergillus fumigatus* and *Candida albicans*) and parasites (*Eimeria* species, *Cryptosporidium baileyi* and *Histomonas meleagridis*) may cause immunosuppression.

Non-infectious Causes

Mycotoxins

Mycotoxins are toxic secondary metabolites produced by various fungi viz. *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. in contaminated poultry feed. Aflatoxins, Ochratoxins, and Trichothecenes are the primary mycotoxins which cause significant immunosuppression in poultry. Besides, fumonisins also disrupts the immune system of poultry. The immunity of the chickens is compromised due to chronic or even subclinical exposure to mycotoxins. The affected chickens develop decreased resistance, much susceptibility to infections and cause impaired response to vaccines (Gomez-Osorio et al., 2024; Olariu et al., 2025). Aflatoxin B1, ochratoxin A, and trichothecenes induce impaired humoral and cell-mediated immunity due to depletion of lymphocytes and atrophy of bursal follicles. Thus, the reduced output of T and B cells from thymus and bursa of Fabricius, respectively leads to development of long-lasting immune incompetence. There is interference in synthesis of DNA and RNA in immune cells by aflatoxicosis in poultry and it leads to suppression of clonal expansion of antigen-specific lymphocytes. Trichothecenes produce immunosuppression by initiating apoptosis of T and B lymphocytes, inhibiting ribosomal protein synthesis, and suppression of the mitogen induced lymphocyte proliferation.

Trichothecenes (e.g. Deoxynivalenol) and fumonisins impair cell-mediated immune response by reducing the population of CD4+ helper T cells and CD8+ cytotoxic T cells, altering CD4+/CD8+ ratios, and increasing the number of regulatory T cells (Shanmugasundaram et al., 2025).

Nutritional Factors

Nutritional imbalance is considered as one of the most important factors responsible for producing immunosuppressive effects in poultry. Dietary deficiency of proteins and energy impairs the antibody synthesis, proliferation of lymphocytes, cytokine production and atrophy of major lymphoid organs viz. bursa of Fabricius and thymus. Deficiency of vitamins A, D3 and E disrupts mucosal integrity, immune regulation and antioxidant defence mechanisms. It leads to the impairment of humoral and cell-mediated immunity (Kidd, 2004). Deficiency of selenium and zinc produces oxidative stress and causes immunosuppression in poultry.

Miscellaneous Non-infectious Causes

The intensive genetic selection particularly for high feed efficiency and rapid growth in commercial broiler chickens leads to reduced immune response and more prone to diseases (Hartcher and Lum, 2020). Environmental and managerial stress factors also contribute to immunosuppression in poultry. Heat stress result in reduced feed intake, induces oxidative damage, disrupts intestinal integrity, and suppresses both humoral as well as cellular immune responses (Lara and Rostagno, 2013; Nanto-Hara et al., 2020). Elevated levels of ammonia cause increased secretion of corticosterone (Edens, 2015), atrophy of lymphoid organs and reduced antibody production (Rahman et al., 2024). Immunosuppression is also aggravated by improper use of chemicals and pharmaceuticals. Gut microbiota is disrupted by prolonged and indiscriminate use of antibiotics resulting in the impairment of gut-associated lymphoid tissue development and mucosal immunity (Zhan et al., 2025). Excessive exposure to heavy metals, disinfectants and pesticides results in oxidative stress and causes apoptosis in immune cells. However, activities of macrophages, production of cytokines and antibodies are directly suppressed by use of exogenous corticosteroids and results in immunosuppression (Aljohani, 2023; Tachibana et al., 2023).

Immunomodulation in Poultry

Immunomodulation is a strategic alteration of the immune function by using nutritional, phytogetic, microbial or biological agents to the improve disease resistance capacity, vaccine responses and overall health in poultry. To combat with rising trend of disease challenges in intensive system of poultry rearing and restrictions on the use of antibiotic growth promoters; immunomodulation is playing a pivotal role in sustainable poultry production (Idowu et al., 2025; Ahmed et al., 2025). The most common immunomodulators for poultry health include nutritional, phytogetic and herbal compounds, probiotics, prebiotics, microbial metabolites, vaccines and biological immunomodulators.

Vitamins and Minerals

Vitamins such as A, E, C, and D₃ are responsible for the antioxidant defence, regulation and proliferation of cytokines and lymphocytes, respectively. Due to their deficiencies, there is impairment of humoral as well as cell mediated immunity (Kidd, 2004; Surai et al., 2017). In broiler and layers, omega-3 fatty acids play a key role in improving the immunological resilience and regulation of inflammatory pathways (Alagawany et al., 2019). Trace minerals such as selenium, copper and zinc act as cofactors for various antioxidant enzymes and immune signalling molecules. So, these are very much helpful for enhancing

the activities of macrophages and antibody production (Richards et al., 2010).

Phytobiotics

Phytobiotics derived from various herbs, spices, and plant extracts viz. aloe vera, garlic, turmeric and neem exhibit antioxidant, anti-inflammatory and immunomodulatory properties. Essential oils and polyphenols modulate innate and adaptive immunity through improvement in gut health and stimuli for development of immune organs. They improve the immune cell population of gut i.e., CD4+ and CD8+T-cells (Obianwuna et al., 2024; Wang et al., 2024). Astragalus and Atractylodes polysaccharides enhance the immunoglobulin levels and helps in protection of lymphoid organs (Zhao et al., 2023). Experimental investigations carried out at Department of Veterinary Pathology, Lala Lajpat Rai University of Veterinary and Animal Sciences have shown that the dietary inclusion of *Azadirachta indica* (Neem) leaf extract at 10% and *Ocimum sanctum* (Tulsi) leaf powder at 5 g/kg feed confers significant protective benefits and modulate immune responses in broilers chickens experimentally exposed to *Salmonella Gallinarum* and hydropericardium syndrome. *Withania somnifera* (Ashwagandha) root extract and aloe vera leaf extract, each provided at 20 ml per litre of drinking water, was found to alleviate disease severity and accelerated recovery in broiler chickens suffering from colibacillosis (Kumari et al., 2020). Supplementation with eugenol at 0.5% in diet has also been demonstrated to enhance antioxidant capacity while exerting a protective effect on liver in broiler chicks (Nehra et al., 2024).

Probiotics, Prebiotics, and Synbiotics

The probiotics are known to strengthen mucosal immunity by increasing production of immunoglobulins i.e., IgA, IgM, and IgG and regulating cytokine responses. *Lactobacillus*, *Bifidobacterium* and *Enterococcus* species; and yeast-based preparations e.g. *Saccharomyces cerevisiae* are some of the most commonly used probiotics in broilers (Halder et al., 2024; Chen et al., 2025). They stimulate activities of phagocytic cells such as macrophages and natural killer cells. Prebiotics promote the beneficial gut bacteria and reduce colonization of pathogens. Mannan and fructo-oligosaccharides enhance the production of short chain fatty acids such as butyrate and propionic acid, which strengthen the integrity of intestinal barrier and gut derived inflammation (Kamal et al., 2025). However, synbiotic combine both effects and improve antibody titres and vaccine response (Talebi et al., 2015; Ahmed et al., 2025).

Vaccines and Biological Immunomodulators

The vaccination plays an important role in immunomodulation in a controlled way by stimulating both humoral as well as cell-mediated immune response. Live attenuated vaccines used for most important viral diseases of poultry such as Newcastle disease, and IBD activate pathways of innate immune response and induce a long-lasting adaptive immunity, including memory T and B cells (Abdelaziz et al., 2024). The inactivated and subunit vaccines mainly enhance antibody-mediated immune response, however, these vaccines can also be optimized for stimulating the cell mediated immune response by use of adjuvants (He et al., 2024). The modern vaccination approaches such as in ovo vaccination help in early immune priming by stimulating the development of immune organs and further increasing the post-hatch disease resistance without affecting their performance (Astill et al., 2018; Abd El-Ghany, 2025). However, vaccine efficacy may be reduced with the improper timing, stress, or any prevailing immunosuppressive conditions.

Conclusion

The immunosuppression in chickens arising from complex interactions among infectious agents, mycotoxins, nutritional imbalances, genetic causes, environmental stress factors and managemental practices act as pivotal constraints for the sustainable poultry production. The immune homeostasis, enhanced disease resistance capability and remarkable improvement in production can be achieved by implementing strategic immunomodulation through balanced nutrition, effective vaccination and biosecurity programmes, judicious use of various types of immunomodulators i.e. phytobiotics, microbial metabolites, probiotics, prebiotics, synbiotics, and biological immunomodulators. Therefore, the specific immunomodulatory strategies are essential for reducing immunosuppression associated risks and enhancing immune resilience in chickens to promote healthy, highly efficient and sustainable poultry systems.

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MONITORING OF CHICKEN INFECTIOUS ANEMIA IN COMMERCIAL POULTRY FLOCKS BY PCR, SEROLOGY AND HISTOPATHOLOGY IN 2025

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Chicken Infectious Anemia (CIA) is an economically important immunosuppressive disease of poultry that adversely affects productivity and flock health worldwide. The disease is caused by Chicken Anemia Virus (CAV), a small, non-enveloped virus with a single-stranded DNA genome. In commercial poultry under field conditions, CAV infection frequently remains subclinical but results in severe immunosuppression, leading to poor vaccine response, increased susceptibility to secondary infections, and economic losses. The present study was conducted to evaluate the current status of CAV infection in commercial poultry flocks in India during 2025 using molecular, serological, and histopathological tools. More than 2,000 samples were collected from commercial poultry flocks across different geographical regions of India as part of routine disease monitoring for immunosuppressive conditions. Presumptive diagnosis was based on clinical signs and gross pathological lesions. Confirmatory diagnosis was performed using PCR for detection of the CAV genome and serological assays for antibody detection. Histopathological examination was carried out on tissues collected from deceased birds, and lesion scoring was used to assess correlation with laboratory findings. Overall, 23.23% of the samples tested positive for CAV by PCR. Histopathological findings included moderate lymphoid depletion in the bone marrow, thymus, bursa of Fabricius, and spleen; increased bone marrow adiposity; follicular atrophy and fibrosis in the bursa; loss of corticomedullary demarcation in the thymus; gizzard mucosal erosion; hepatic degeneration with hemorrhages; and renal tubular necrosis. Microscopic lesions showed strong correlation with PCR positivity. Serological analysis revealed seroconversion patterns consistent with molecular and histopathological results. Subclinical infections were predominantly detected in broiler birds below three weeks of age, while grower birds (3–10 weeks) showed higher PCR and serological positivity. Most positive cases were observed between June and October. The study confirms widespread circulation of CAV in commercial poultry flocks in India and highlights its significant role in immunosuppression. Continuous surveillance and effective breeder flock vaccination programs are essential to minimize the impact of CIA on poultry production.

PREVALENCE STUDIES ON ENDOGENOUS AND EXOGENOUS AVIAN LEUKOSIS VIRUS CIRCULATING IN KADAKNATH BIRDS OF FARM

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Avian Leukosis Virus (ALV) is a member of Retroviridae family responsible for causing a variety of tumor in domestic fowl economically impacting the poultry industry. The virus, based on host range, amino acid sequence of envelope protein, viral interference and type of cell infected, is classified into 11 distinct subgroups. Subgroup A, B, C, D, E, J and K mainly infects the domestic fowl. The present study was conducted to detect the prevalence of endogenous and exogenous ALV in 315 Kadaknath birds by group specific (p27) antigen ELISA. The ELISA positive females and males were mated and fertile eggs were collected individually from each bird and chicken embryo fibroblast (CEF) was prepared from 9 day old embryonated eggs of each bird separately. The DNA was extracted from CEF of each bird and screened for exogenous ALV subgroup A, B, J and K using specific primers. The screening was also done for four endogenous retroviral families viz., EAV, ART-Ch, ev loci and ev- J as well as for six different genotypes of ev loci i.e., E1, E3, E6, E9, E12 and E21 using specific set of primers. The group specific antigen ELISA test revealed overall prevalence of ALV's is 20.32% (64/315) including 23.2% (46/198) females and 15.38% (18/117) males. Among 64 positive Kadaknath birds, 31 were selected for mating and further screening of exogenous and endogenous ALV's. Out of 31 tested for exogenous viruses, 9.6% (3/31) tested positive for ALV-J. The overall prevalence of endogenous retroviral families was 100%, 70.96%, 67.7% and 54.8% for EAV, ev-J, ART-Ch and ev-loci, respectively. The prevalence of ev-loci was found to be 29.03% (9/31) for E1 and E3, 93.5% (29/31) for E6 and 9.6% (3/31) for E21 while none of the birds were found positive for E9 and E21. Only one bird out of 9 was found homozygously positive for E1 genotype while rest 8 were heterozygously positive. However, none were homozygously positive for E3 and E21. In conclusion, both the exogenous and endogenous ALV's were found circulating in the Kadaknath birds. The presence of endogenous viral families poses a great risk by providing sequences for recombination and emergence of new ALV variants, as has been seen with ALV J. The endogenous viruses are integrated in the bird genome which are inherited in a Mendelian fashion, as a result the bird becomes immunotolerant to ALV and are unable to mount antibody responses against ALV infection. These immunotolerant birds acts as a continuous source of infection in the farm which makes it necessary for screening of both endogenous and exogenous viruses.

EFFECT OF DIETARY YEAST-DERIVED MANNAN OLIGOSACCHARIDE AND BETA-GLUCANS (1-3 1-6) ON PERFORMANCE, INTESTINAL MORPHOLOGY, REDOX STATUS, IMMUNE RESPONSE, AND GUT MICROBIOTA IN BROILERS

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The present study was performed to explore the impact of dietary supplementation with ImmunoWall®, a yeast-derived feed additive containing mannan oligosaccharides (MOS) and β -glucans (1-3 1-6) on growth performance, immune responses, antioxidant status, gut health, and microbiota in broiler chickens. Two hundred Cobb 430 broilers were divided into control (basal diet) and treatment (basal diet + 500 g/MT ImmunoWall®) groups. The results showed that body weight and feed intake remained unaffected, whereas the feed conversion ratio (FCR) was significantly ($P < 0.05$) improved in the supplemented group. ImmunoWall® significantly enhanced antibody titers against Newcastle Disease Virus (NDV) and Infectious Bursal Disease Virus (IBDV), immune organ weight at day 28, and lowered pro-inflammatory cytokines (IL-1 β , IL-6, and IL-8). ImmunoWall® supplementation increased glutathione peroxidase (GPx) activity and reduced malondialdehyde levels, indicating improved oxidative stress management. Histological analyses revealed improved gut morphology, with a significantly ($P < 0.05$) increased duodenal villus height and villus height-to-crypt depth (VH/CD) ratio. Cecal microbial analysis revealed higher Lactobacillus counts and reduced Clostridium spp., reflecting a shift toward healthier intestinal microbiota. These improvements collectively support enhanced nutrient absorption and immune modulation in the host. These findings suggest that MOS and β -glucan (1,3 and 1,6) supplementation via ImmunoWall® improves feed efficiency, immune competence, antioxidant defense, and gut health in broilers, offering a promising supplement for sustainable poultry production and health.

ANALYSIS OF BONE MARROW IN CHICKEN INFECTIOUS ANEMIA AFFECTED COMMERCIAL CHICKEN

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Chicken Infectious Anemia (CIA) is a highly contagious, emerging, immunosuppressive disease caused by “Chicken Infectious Anemia Virus.” In the present study, CIA was suspected in 135 commercial chicken in coastal districts of Andhra Pradesh as they exhibited severe anemia, generalized weakness and depression. At necropsy, small shrunken thymus and pale pink bone marrow of femur with jelly to watery consistency was noticed in all affected birds. Cytologically, bone marrows revealed a marked decrease in cellularity along with presence of vacuolar spaces due to replacement of hematopoietic tissue by adipose tissue. Histologically, congestion of capillaries and aplasia of all cell types including granulocytic, agranulocytic and hemopoietic precursors along with complete replacement of bone marrow by adipose tissue was noticed along with eosinophilic, intranuclear inclusions in few hemocytoblasts. Bone marrow samples on TEM revealed severe depletion of all precursor cells and large adipose cells replacing the bone marrow and few electron dense viral aggregates in the nucleus of hemocytoblast. The DNA samples from bonemarrow were used for amplification of VP1 gene of CIAV using specific primers and yielded the desired product of 1390 bp confirming CIA.

IMMUNOMODULATORY EFFECT OF ZINC NANOPARTICLE CONJUGATED WITH *TINOSPORA CORDIFOLIA* IN INFECTIOUS BURSAL DISEASE VIRUS INDUCED INFECTION IN BROILERS

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The present research work was carried out to evaluate immunomodulatory effect of zinc nanoparticle conjugated with *Tinospora cordifolia* (cZnONPs) on infectious bursal disease virus induced infection in broilers. During experiment, chicks were challenged with IBDV on 28th day of age (B, D and E @ 102EID50/0.1 ml/bird by intraocular route) and supplemented with cZnONPs @15ppm in drinking water from 1-42 day of age as prophylactic group (D) and from 28-42 day as therapeutic group (E). Phytochemical study of *T. cordifolia* extract by qualitative and GC-MS analysis revealed presence of various bio-active compounds. Conjugated zinc nanoparticles were characterized by UV-visible spectroscopy, NTA, zeta potential and FTIR. UV-visible absorption peak was found at 548 nm and average size was 66 nm by NTA. FTIR showed stretching frequency at 3333, 1634, 1437, 1133cm⁻¹. Isolation and confirmation of IBDV specific VP2 gene was carried out by RT-PCR. Broilers from group D supplemented with cZnONPs showed significantly higher body weight and lower FCR at 35th and 42nd day of age indicating beneficial effect of cZnONPs as preventive dose. Histopathologically various pathological lesions typical of IBDV were noticed in control infection group however lesions observed in cZnONPs supplemented group as preventive dose were less pronounced indicating immunomodulatory effect of cZnONPs on IBDV induced pathology in broilers. Levels of pro-inflammatory cytokines were higher in group B as compared to supplemented groups indicating regulation of cytokine expression. *T. cordifolia* conjugated with zinc nanoparticles could modulate cytokine expression possibly due to anti-inflammatory, immunomodulatory and antiviral properties.

COMPARATIVE EVALUATION OF PLAIN AND PHYTOSOME-CONJUGATED CINNAMALDEHYDE ESSENTIAL OILS ON IMMUNITY AND GUT HEALTH OF BROILERS

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Natural feed additives play a vital role in poultry production due to their safety and multifunctional properties, including antioxidant, antimicrobial, and growth-promoting effects. The present study evaluated the influence of dietary supplementation of essential oils on immunity and gut health in straight-run broiler chickens up to six weeks of age. A total of 240 one-day-old broiler chicks (Vencobb 430) were randomly allocated into four groups (60 chicks/group) with four replicates. The control group received a basal diet, while the remaining groups were supplemented with an antibiotic growth promoter (500g/ton), plain cinnamaldehyde essential oil (200 g/ton), and phytosome-conjugated cinnamaldehyde essential oil (100g/ton) for 42 days. Broilers supplemented with phytosome-conjugated cinnamaldehyde essential oil (100 g/ton) showed significantly higher ($P<0.001$) live body weight and improved feed conversion ratio compared to other groups. Haemagglutination inhibition titres against Newcastle disease virus did not differ significantly among groups at 14, 28, 35, and 42 days of age. Significant differences in villus height and villus height-to-crypt depth ratio were observed in the antibiotic-supplemented group. On day 42, villus width and crypt depth remained unchanged; however, infiltration of inflammatory cells and epithelial cell sloughing were noted in the antibiotic group. Essential oil supplementation significantly ($P<0.001$) altered caecal microbial counts throughout the experimental period. Overall, phytosome-conjugated cinnamaldehyde essential oil enhanced growth performance, immunity, and gut health in broilers. In conclusion, both plain and phytosome-conjugated cinnamaldehyde essential oils can serve as effective alternatives to antibiotic growth promoters (500 g/ton), with phytosome-conjugated cinnamaldehyde showing superior overall performance.

PREVALENCE OF MAREK'S DISEASE AND MOLECULAR CHARACTERIZATION OF MEQ GENE OF MDV CIRCULATING IN INDIA

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Marek's Disease (MD) is caused by Gallid alphaherpesvirus 2 (GaHV-2), belongs to genus Mardivirus within the subfamily Alphaherpesvirinae and is characterized by development of lymphoid tumors, immunosuppression and paralysis. In recent years, the incidence of Marek's Disease (MD) in India has increased despite extensive vaccination strategies. The objective of this study was to determine the prevalence of MD and molecular characterization of MEQ gene of Marek's Disease Virus (MDV). Tumor samples were collected from a total of 175 birds showing lesions suggesting of MD and screened for MD by PCR using specific primers. The meq gene was amplified from MD positive samples and PCR product (CEDA-116 Feather follicle) was subjected to sequencing and phylogenetic analysis. The results revealed that 58.8% of samples (103/175) were found positive for MD. The Meq gene nucleotide sequence analysis revealed that the sequence of this study was 98.5-100% sequence homology with that of reference strains. The consecutive five proline rich repeats (PPPP) of the Meq transactivation domain, the proline content (21.53 %) and the presence of amino acid (aa) substitutions were determined. Further, phylogenetic analysis revealed that CEDA strain is clustered with Indian virulent (v)MDV strains. In conclusion, the results revealed that MDV is prevalent and virulent strains are circulating in Indian poultry industry in spite of stringent vaccination strategies. Further investigations are required to assess the pathotyping of MDV strains circulating in India.

VISCERAL LYMPHOPROLIFERATIVE LESIONS ASSOCIATED WITH MAREK'S DISEASE IN LAYERS

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Marek's disease is a highly contagious lymphoproliferative viral disease of poultry and remains a significant threat to commercial poultry production due to increasing virulence of field strains. The present report describes pathological, histopathological, and molecular findings associated with Marek's disease in a layer flock. In a flock of 7400 layer birds, progressive mortality of 110 birds was reported in a span of 1 month duration of birds aged between 16 to 20 weeks. In the detailed necropsy of 20 birds, total 06 layer birds of 20 week-old were suspected for presence of Marek's disease with gross lesions of nodular masses in vital organs. Gross examination of birds revealed focal to generalized, multifocal whitish raised to flat nodules on visceral organs viz. heart, liver and spleen, accompanied with marked organomegaly. The proventricular wall was markedly thickened in one bird. Whitish raised focal nodules were also observed on the heart, while the intestines showed wall thickening with multiple whitish nodular lesions. One bird from the same flock showed nodular lesions with involvement of the lungs and thymus. The lungs were enlarged, diffusely greyish, and contained round nodular lesions with consolidation. Impression smears from the spleen and liver demonstrated marked pleomorphic lymphoid cellular populations with dark basophilic nucleus, a characteristic feature of Marek's disease. The H&E stained histopathological sections revealed infiltrative aggregates of pleomorphic lymphoid cells in the hepatic parenchyma, splenic white pulp, as well as in the myocardium and lung alveolar parenchyma. The molecular confirmation of Marek's Disease virus was achieved through real time polymerase chain reaction (PCR) assay targeting the ICP4 gene using 04 tissue samples of spleen and liver from affected birds. These findings collectively confirmed the diagnosis of Marek's disease in pullet and layer birds and highlight its continued occurrence and pathological significance in vaccinated commercial layer flocks.

PATHOLOGY AND MOLECULAR CHARACTERISATION OF RE-EMERGING IMMUNOSUPPRESSIVE VIRAL PATHOGENS IN CHICKENS

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Immunosuppressive viral diseases threaten the poultry industry by causing heavy mortality and huge economic loss. Due to their severe immunosuppressive nature, they lead to secondary bacterial, viral or parasitic infections and cause vaccination failure. The present study was undertaken to know the prevalence, pathology and molecular characterisation of immunosuppressive viral pathogens in chickens. The viruses chosen in our study are Chicken Infectious Anemia, Infectious Bursal Disease, Marek's Disease, Avian Leucosis / Lymphoid Leucosis and Avian Reovirus infection. A total of 260 samples from 50 different flocks suspected of immunosuppressive diseases were collected, screened, and evaluated. Out of 50 flocks, co-infections with secondary bacterial, viral and parasitic diseases were observed in 35 flocks and mono-infection in 15 flocks. The prevalence of CIA was found to be 74%, IBD 18%, MD 8%, AL 6% and ARV infection 4% on the basis of PCR/RT-PCR assay. Gross lesions observed in CIA were pale comb and wattle, atrophied thymus and pale fatty bone marrow. Microscopically, depletion of lymphocytes from thymus cortex and atrophy of stem cells in bone marrow were consistent. In IBD, enlarged bursa with presence of caseous exudates and atrophied bursa was noticed. Microscopically, there was severe depletion of lymphocytes in bursal follicles. In MD and AL infection, enlargement and presence of tumorous growths in liver, spleen, proventriculus and kidney was noticed. Microscopically, MD showed proliferation of pleomorphic lymphoid cells whereas ALV showed proliferation of monomorphic lymphoid cells. In ARV infection, enlargement of gastrocnemius tendon, roundening of proventriculus and enteritis was noticed. Microscopically, infiltration of lymphoid cells in proventriculus and intestine was observed. Molecular characterisation of whole genome of CIA and capsid protein (VP1) revealed high homology with Namakkal isolate and circulation of Genogroup IIIB in Nagpur region. IBD revealed high homology with India, Iraq, Japan and China. MDV showed 99% homology with Kerala isolate. ALV revealed circulation of serotype E in Nagpur region. ARV isolates revealed high similarity with Saudi Arabia isolate.

PATHOLOGY AND MOLECULAR CHARACTERIZATION OF CHICKEN INFECTIOUS ANEMIA VIRUS IN NAGPUR REGION, INDIA

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Immunosuppressive viral diseases are causing heavy mortality and huge economic loss to poultry industry. One of the important immunosuppressive viral disease affecting poultry birds is Chicken Infectious Anemia. The present study was undertaken to study the pathology and characterize Chicken Anemia virus from Nagpur region, India during February 2023 to February 2024. Gross lesions observed in CAV were pale comb & wattle, atrophied thymus and pale fatty bone marrow. Microscopically, depletion of lymphocytes from thymus cortex and atrophy of stem cells in bone marrow. Molecular diagnosis was carried out by the amplification of VP1, VP2 and VP3 gene. All the 3 gene sequences were combined and trimmed using BioEdit software to obtain a complete genome of approximately 2.3 kb size. Upon BLAST analysis, our obtained sequence exhibited highest homology of 99% with Indian isolate. Molecular characterisation of whole genome of CAV and capsid protein (VP1) revealed high homology with Namakkal isolate and circulation of Genogroup IIb in Nagpur region.

PATHOLOGY OF CHICKEN INFECTIOUS ANEMIA WITH CONCURRENT INFECTIONS IN CHICKENS OF CHHATTISGARH

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Poultry industry is constantly exposed to various immunosuppressive agents such as viruses, mycotoxins and environmental stress. Chicken Infectious Anemia is one of the important immunosuppressive viral agents caused by circular single stranded Circovirus which leads to increased susceptibility to concurrent infection and heavy economic losses in terms of mortality and reduced growth performance. During present study, mortality of 10 to 18% due to Chicken infectious anemia in thirteen, 5-12 weeks old commercial layer and cockerel farms located at Balod, Durg, Rajnandgaon and Raipur district of Chhattisgarh was noticed. Increased mortality of 15 to 24% was noticed in the chickens with concurrent infections. Characteristic clinical signs of anaemia, typical pale bone marrow and thymic atrophy were suggestive of CIA. Generalized lymphoid atrophy was the most constant and characteristic lesion found in CIA affected birds. Concurrent infections with one or more infections due to Gangrenous dermatitis, Coccidiosis, Colibacillosis and Salmonellosis were noticed in nine flocks. Clinical samples of thymus collected from thirteen flocks were confirmed as CIA by PCR amplification of 419bp of VP2 gene of virus. The clinical signs, gross and histopathological findings along with PCR amplification of VP2 gene confirmed the outbreak of CIA in commercial layers and cockerels.

Theme 9

*Biosecurity and
Biorisk Management*

BIOSECURITY MEASURES FOR DISEASE PREVENTION IN AVIAN HOLDING FACILITIES

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Biosecurity in wild animal holding facilities is crucial for keeping animals, staff, and the surrounding environment safe from infectious diseases. Effective containment depends on simple but well-planned practices that reduce the chances of disease entering or spreading within the facility. These include controlling who enters the facility, keeping newly arrived animals under quarantine, and regularly monitoring animal health. Cleanliness plays a major role, with routine cleaning, disinfection, and proper waste disposal forming the backbone of daily operations. Staff safety is ensured through the use of protective clothing and clear guidelines for handling animals and biological materials. Managing pests, restricting unnecessary movement of people and equipment, and having clear plans to respond quickly to disease outbreaks further strengthen containment. Together, these measures create a safe and secure environment that protects wildlife, people, and the ecosystem beyond the facility.

Theme 10

Young Scientist Masters

EFFICACY OF GREEN SYNTHESIZED NANO SILVER WITH ENTRAPPED CINNAMALDEHYDE ON CHICKEN INFECTIOUS ANEMIA VIRUS INDUCED PATHOLOGY IN COCKERELS

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Chicken infectious anemia virus (CIAV) is the economically significant and immunosuppressive disease of poultry. The current research work was carried out to investigate the efficacy of green synthesized nano silver with entrapped cinnamaldehyde on chicken infectious anemia virus induced pathology in cockerels. The silver nanopartilces (AgNPs) were green synthesized from *Lactobacillus acidophillus* and entrapped with cinnamaldehyde essential oil and characterised. The CIA virus was isolated from thymus and liver samples collected from cockerels. Detection and molecular characterization were done by targeting VP1 gene of CIAV. Total 180 cockerel birds were equally divided into 6 groups (A-F). The birds from group B, C, D, E and F were challenged on 14th day of age with CIA virus (@10 4.5 EID₅₀ /ml per bird by intramuscular route. Silver entrapped cinnamaldehyde (AgC) was given @ 12.5 ppm in drinking water from day 1-35 in group C as prophylactic and AgC @ 50 ppm in drinking water from day 21- 35 in group D as therapeutic. The group E received plain AgNPs @ 12.5 ppm in drinking water from day 1-35 as a prophylactic group and group F received plain AgNPs @ 50 ppm in drinking water from day 21- 35. Cockerels from group C supplemented with AgC showed the significantly higher body weight and low FCR at 35th day. AgC at prophylactic dose significantly improved the immune organ indices and CAV-specific antibody titers of infected chickens. Grossly and histopathologically group B showed the severe lesions in group B. The severity of lesions was lowest in group C then group D, E and F. The relative expression of anti-inflammatory cytokine, IL-10 and IFN- γ in thymus was higher in AgC prophylactic group C at 7th, 14th and 21st dpi. The birds supplemented with silver nanoparticles entrapped with cinnamaldehyde essential oil at prophylactic dose of effectively reverted the pathological changes induced by chicken infectious anemia virus in cockerels and induced robust immune response.

ASSESSMENT OF EMBRYONATED CHICKEN EGGS AS A MODEL IN UNRAVELING INFECTIOUS BRONCHITIS VIRUS PATHOGENESIS AND HOST IMMUNE RESPONSE

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Infectious bronchitis (IB) is an acute, highly contagious respiratory disease of chickens caused by infectious bronchitis virus (IBV), a Gammacoronavirus of the family Coronaviridae. This study evaluated embryonated chicken eggs (ECEs) as a model to investigate IBV pathogenesis and innate immune responses using three field isolates with varied tissue tropism (RP, NP, MT) and a commercial vaccine strain. The isolates IBV/Chicken/India/IVRI/Rajasthan/01/2023 (RP), IBV/Chicken/India/IBVS1/2024 (NP), and IBV/Chicken/India/UP/07/2022 (MT) were propagated in 11-day-old ECEs, and the 7th passage allantoic fluid was titrated to determine EID₅₀. A dose of 10⁵ EID₅₀/0.2 mL was inoculated in 11-day-old SPF ECEs via the allantoic route, and embryos were harvested at embryonic days 17 and 20. Trachea, lungs, kidneys, caecal tonsils, proventriculus, liver, and spleen were examined for histopathology, immunohistochemistry (IHC), viral load, and innate immune gene expression. Mild tracheal lesions occurred in all infected groups, prominent in RP and MT. Lung lesions ranged from mild to moderate, particularly in MT, RP and vaccine groups, while NP induced the most severe renal lesions. The MT isolate additionally showed distinct microscopic lesions in the proventriculus at both time points. No significant lesions were noted in liver and spleen across all groups, suggesting limited tropism for these organs in the embryonic model. IHC revealed strong renal immunoreactivity in NP and MT and mild-to-moderate lung immunoreactivity in RP, vaccine, and MT groups and Vaccine group showed only mild tracheal immunoreactivity. Viral load peaked at ED17 in trachea and lungs and at ED20 in kidneys and caecal tonsils, with MT, RP, and NP showing organ-specific dominance. PRRs (TLR-3, TLR-7, MDA5) and pro-inflammatory cytokines (IL-1 β , IL-6) were significantly upregulated at ED17 in all organs and at ED20 in kidneys and caecal tonsils, while IFN- β and IFN- γ showed non-significant elevation. In conclusion, ECEs effectively differentiated tissue tropism and virulence among IBV strains. MT exhibited the broader tissue involvement, including lungs, kidneys, and proventriculus, whereas NP and RP largely remained nephro- and respiratory-tropic, respectively. Overall, ECEs represent a valuable experimental platform for comparative IBV pathotyping and studying host-virus interactions at the embryonic stage. Further validation in post-hatch birds is recommended to extend these observations to field-relevant conditions.

OCCURRENCE, PATHOLOGY AND MOLECULAR CHARACTERIZATION OF SALMONELLA SPP IN BACKYARD AND FARMED CHICKENS

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The present study described occurrence, pathology, virulence gene profile and antibiotic resistance in cases of salmonellosis in chicken. A total of 212 dead birds, comprising of broilers, layers and backyard birds in and around Akola, Maharashtra, India were considered for the mortality investigation. Bacterial cultural examination of liver and intestinal content samples revealed salmonellosis in 28 birds which were further confirmed by PCR assay and revealed *Salmonella* spp. in 21 birds indicating occurrence of 11.32% (24/212). Moreover, *S. Gallinarum* was identified in 75% (18/24) samples by using the PCR targeting the fragment of *ratA* gene. Multiplex PCR analysis revealed 58.33 % (14/24) samples positive for *S. Typhimurium* (Flic-C) and 8.33% (2/24) samples positive for *S. Enteritidis* (IE-1). Virulence gene profile using PCR indicated presence of *invA*, *iroB* and *stn* in all *Salmonella* isolates (100%), while none of the isolates contained the *spvC* and *spvR* genes. Antimicrobial susceptibility testing (AST) showed higher degree of sensitivity to amikacin and piperacillin-tazobactam while trimethoprim and ampicillin showed resistance. Additionally, distribution of ESBL genes showed that all the PCR positive samples were positive for the *blaTEM* gene (100%), followed by *blaSHV* (37.5%), *blaCTX-M* (25%) and all samples were negative for *blaOXA*. Gross pathological examination revealed hepatomegaly, splenomegaly, necrotic liver foci, hemorrhages and congestion in multiple organs. Microscopic findings included liver necrosis, renal tubular epithelium desquamation, bronchiolar wall necrosis and myocardial degeneration, among others. Spleen exhibited lymphoid depletion, intestine displayed inflammatory cell infiltration and gland atrophy. This study has been focused on giving a clear pattern of the current situation of *Salmonella* spp. infection in broiler, native and layer chickens including occurrence, serotyping and an antimicrobial resistance.

"PATHOLOGICAL STUDIES OF INDUCED SUBACUTE OCHRATOXICOSIS IN KADAKNATH CHICKENS AND AMELIORATION WITH SACCHAROMYCES BOULARDII"

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Objectives:

To study the effect of Ochratoxin A on haemato-biochemical parameters, gross and histopathological alteration in liver, kidney, spleen, bursa of fabricus and thymus in Kadaknath chickens and to evaluate the efficacy of drug containing *Saccharomyces boulardii* for combating ochratoxicosis.

Materials and Methods:

Day old Kadaknath chicks (40 in number) procured from college poultry farm were divided into four groups: Group A (control), Group B (OTA-exposed), Group C (*S. boulardii*) and Group D (OTA + *S. boulardii*). Ochratoxin A was procured from SIGMA-ALDRICH CHEMICALS, Bangalore. Parameters assessed included productive performance (body weight, weight gain, feed consumption, feed conversion ratio), haematobiochemical markers (Hb, TLC, ALT, AST, Cr and BUN) and pathological alterations in the liver, kidney, spleen, bursa of Fabricius, thymus and small intestine.

Results revealed that OTA exposure significantly reduced growth performance, impaired feed efficiency and caused severe biochemical and pathological changes including elevated ALT, AST, Cr and BUN levels. Gross and histopathological examination showed hepatic and renal damage, lymphoid depletion in the spleen, thymus and bursa of Fabricius and congestion in small intestine.

Conclusion:

Birds in the probiotic-only group (C) exhibited enhanced growth performance and stable haematobiochemical parameters, indicating its growth-promoting and health-supporting roles. In the OTA-exposed group (D) supplemented with *S. boulardii*, partial mitigation of ochratoxin-induced damage was observed, as reflected in improved weight gain, feed efficiency, and reduced severity of organ pathology.

Theme 11

Young Scientist Doctoral



EVALUATING THE EFFECT OF HERBAL CHOLINE FORTIFIED WITH MILK THISTLE (*SILYBUM MARIANUM*) ON GROWTH, LIVER FUNCTION AND GUT HEALTH IN BROILERS

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The present study evaluated the effects of herbal choline fortified with milk thistle on growth performance, liver health, gut morphology and intestinal microbial load in commercial broiler chickens. The experiment was conducted over five weeks at the Department of Poultry Science, Mumbai Veterinary College, using 240 straight-run Vencobb 430Y broiler chicks. Birds were randomly allocated to four dietary treatment groups with six replicates of ten birds each. Group A received herbal choline with milk thistle @ 10 g/T (4 %) used @ 250 g/T and served as the control, while Groups B, C and D received the same product at inclusion levels of 125, 500 and 750 g/T, respectively. Growth performance parameters including body weight gain, feed intake and feed conversion ratio showed no significant differences among treatments ($P \geq 0.05$). However, birds fed higher inclusion levels of 500 and 750 g/T recorded numerically higher final body weights, cumulative weight gain and improved feed efficiency, indicating a dose-dependent response. Mortality remained low and within acceptable limits in all groups, confirming the safety of supplementation. Liver histomorphology revealed significantly lower hepatocellular vacuolization scores in birds fed lower inclusion levels of 125 and 250 g/T, suggesting improved hepatic integrity and fat metabolism. Intestinal microbial analysis showed marginal reductions in total viable and coliform counts with increasing supplementation levels, reflecting mild antimicrobial activity. Intestinal morphometric evaluation demonstrated dose-specific responses, wherein supplementation at 250 g/T favoured villi length, villi architecture and villi-to-crypt balance, while higher inclusion levels of 500 to 750 g/T enhanced crypt depth, indicating increased epithelial cell proliferation and regeneration. Overall, supplementation of herbal choline with milk thistle produced dose-dependent improvements in growth trends, liver health, intestinal morphology and microbial balance. Lower inclusion levels primarily supported liver integrity and absorptive surface development, whereas higher levels promoted growth performance and gut epithelial renewal.

Theme 12

Case Study

CASE REPORT OF EIMERIA BRUNETTI IN COMMERCIAL LAYERS

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Samples were collected from a commercial layer farm (Chandrabhaga Poultry Farm, Taluka Haveli, Pune), located in the vicinity of Pune city, where birds exhibited typical clinical signs of coccidiosis, including diarrhoea, reduced body weight, and mortality. Intestinal samples were collected from affected birds and transferred to the Poultry Disease Research Centre (PDRC) for further processing. Coccidiosis caused by *Eimeria brunetti* was confirmed based on clinical signs, gross pathological lesions, and oocyst morphology. Sporulated oocysts were separated using the standard flotation technique and purified by repeated centrifugation with phosphate-buffered saline (PBS) for three to four cycles. The purified samples were passed through a 96-well plate, and oocysts were randomly selected according to standard size and shape criteria. These isolated oocysts were used for experimental infection and subsequent propagation. One-day-old specific pathogen-free (SPF) chicks were orally inoculated with an adequate dose of oocysts and reared in cages for 14 days to prevent cross-contamination. Faecal samples were collected daily from day 3 to day 14 post-infection to assess oocyst shedding. Selected birds were euthanized between days 4 and 8 post-infection for examination of the intestinal tract for species-specific lesions. Oocyst output per gram of faeces was determined using the McMaster technique, and final oocyst counts were performed using a Neubauer haemocytometer. The sporulated oocysts measured approximately $24.6 \times 18.8 \mu\text{m}$. Gross pathological examination revealed coagulative necrosis, mucoid and bloody enteritis in the lower intestine. The lesions were predominantly observed in the lower mid-intestine, rectum, and caeca, with thickening of the intestinal wall, greyish discoloration, and sloughing of salmon-coloured material. These findings confirm the occurrence of *Eimeria brunetti* infection in commercial layer birds, highlighting its pathological significance, particularly in long-living poultry, and emphasizing the need for effective surveillance and targeted control strategies.

FIELD TRIAL OF PHYTOGENIC EXTRACTS OF TRIBULUS TERRESTRIS (GOKHRU) TO REDUCE ASCITES AND IMPROVE RENAL HEALTH IN BROILERS

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Background:

Indian poultry farms frequently experience cases of Ascites in broilers, usually after 21 days of age. The condition is often linked not only to diseases but also to nutritional and managemental factors. No specific curative or supportive treatment is currently available, and thus affected birds often succumb. Post-mortem lesions typically include enlarged liver, swollen kidneys, and accumulation of clear or gel like fluid in the abdomen.

Materials and Methods:

Broiler farms (capacity 9,000 birds) around Saswad, Pune, known for recurring Ascites and increased mortality upto 2%, were selected. Kidney samples from affected birds were collected for histopathology. Treatment involved administering phytogetic extracts from *Tribulus terrestris* (Gokhru) in drinking water for 10 days as 10 ml/100 birds, along with Neomycin and Doxycycline for 5 days as 1g /5 lit of water for 5 days to prevent secondary infections. Before and after treatment kidney samples were sent to Nagpur Veterinary College for laboratory analysis.

Histopathology:

Before treatment, kidneys showed nephritis with heterophilic and lymphocytic infiltration, tubular disruption, and glomerular hypercellularity. After treatment, kidney sections revealed normal tubular epithelium, intact glomeruli, and minimal inflammation, indicating marked improvement in renal integrity.

Results:

Ascites incidence declined markedly due to the diuretic and kidney-protective effects of Gokhru. Mortality dropped from 2% to 0.15% within five days, with noticeable improvement in appetite, activity, and overall bird performance.

Conclusion:

Phytogetic extracts of *Tribulus terrestris* (Gokhru), combined with antibiotics, effectively controlled Ascites, enhanced kidney health and reduces mortality. Its diuretic and restorative properties make Gokhru a useful natural aid in Ascites management in broilers.

HANDLING OF RESPIRATORY INFECTIONS IN BROILER BREEDERS

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Respiratory infections are the commonest diseases in poultry. CRD, Colibacillosis, Infectious coryza, Infectious bronchitis, LPAI and HPAI are some of the most common respiratory diseases of poultry. Mycoplasma, colibacillosis and infectious coryza are diseases of bacterial origin and can be controlled with bacterins and antibiotics. Others are of viral origin and could be controlled with vaccines only. Among bacterial diseases, CRD is the most important one because it is a vertically transmitted disease. It adversely impacts the hatchability and the chick quality. Tiamulin, the most commonly used drug for CRD, is now showing reduced efficacy due to prolonged usage. Fortunately, newer antibiotics and vaccines have made the CRD manageable. Colibacillosis is a water-borne disease. So water sanitation or usage of filters have made it a lesser known disease now. Infectious coryza can be easily managed with the vaccines and antibiotics. Infectious bronchitis is a very old disease in India. Fortunately, it's mainly targeting the reproductive and not the respiratory organs. AI hit the industry in 2006 and became the catalyst for the rapid spread of respiratory infections. AI damages the trachea extensively. The resultant damage becomes the entry point for the pathogens. Stress is one of the major factors responsible for respiratory issues. The stress resulting from poor density and poor ventilation induces stress on the birds. Stress elevates the cortisol levels in the blood. Cortisol is known to induce catabolism. Catabolism causes break down of proteins leading to immunosuppression which invites respiratory issues. Ammonia damages the tracheal cilia inviting the respiratory pathogens. The heat stress leads to panting. Panting means the air bypasses the tracheal cilia. So anything non-sense in the surrounding air directly settles down deep in the respiratory system. Environmentally controlled houses are needed of the hour. They help in avoiding heat stress and provide a comfortable atmosphere which helps in optimising the immunity and productivity. Optimum biosecurity and the availability of HPAI vaccines like the LPAI will help the poultry industry to minimize the respiratory challenges.

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