



DAIRY, FISHERIES AND ANIMAL
RESOURCE DEPARTMENT

Training Manual

Advanced Disease Diagnosis in Poultry

(19-23 January, 2026)



**Department of Veterinary Pathology, BVC
Directorate of Extension Education
Bihar Animal Sciences University, Patna-14**



Training Manual

Training Program

"Advanced Disease Diagnosis in Poultry"

(19 to 23 January, 2026)

Sponsored by:



DAIRY, FISHERIES AND ANIMAL
RESOURCE DEPARTMENT

DAIRY, FISHERIES AND ANIMAL RESOURCE DEPARTMENT

Organized by:

**Department of Veterinary Pathology, BVC
Directorate of Extension Education
Bihar Animal Sciences University, Patna-14**

Editor In-Chief

Dr. N. S. Dahiya

Director Extension Education, BASU, Patna

Editors:

Dr. Sanjiv Kumar

Dr. Kausal Kumar

Dr. Imran Ali

Dr. Deepak Kumar

Dr. Saroj Rajak

Dr. Y.S. Jadoun

Dr. Puspendra Kumar Singh

Year of Publication: 2026

Publication No.: 76/2026/DEE/BASU, Patna

Instructions

The information contained in this manual has been obtained from authentic and reliable resources, but the authors/publisher cannot assume responsibility for the validity of all materials or the consequences of their use.

Edition : First

No part of this publication may be reproduced / stored/ retrieved/ transmitted in any form without explicit and prior written permission granted by the publisher. All rights are reserved and vests in publisher.

Note: Due care has been taken while editing printing the manual in the event of any mistake in printing error happens, publisher or editors will not be held responsible.

Publisher : Publication Cell, DEE, BASU, Patna

Coyright © 2026, DEE/BASU-Patna

CORE TEAM MEMBERS OF THE TRAINING

Chief Patron

Dr. Inderjeet Singh
Hon'ble Vice-Chancellor, Basu

Patrons

Dr. N. K. Singh

Director Research, BASU, Patna

Dr. J. K. Prasad

Dean, BVC, BASU, Patna

Dr. P. K. Singh

DRI-cum-Dean, PGS, BASU, Patna

Chairman

Dr. N. S. Dahiya

Director Extension Education, BASU, Patna

Programme Director

Dr. Sanjiv Kumar

Professor & Head
Department of Veterinary Pathology
Bihar Veterinary College
Bihar Animal Sciences University (BASU), Patna.

Programme Co-Director

Dr. Imran Ali

Assistant Professor
Department of Veterinary Pathology
Bihar Veterinary College
Bihar Animal Sciences University (BASU), Patna

Dr. Kausal Kumar

Assistant Professor
Department of Veterinary Pathology
Bihar Veterinary College
Bihar Animal Sciences University (BASU), Patna

Programme Coordinator-cum-Organising Secretary

Dr. Deepak Kumar

Assistant Professor
Department of Veterinary Pathology
Bihar Veterinary College
Bihar Animal Sciences University (BASU), Patna

Convenor

Dr. Saroj Rajak

Associate Professor & Head
Department of Veterinary & Animal Husbandry Extension Education
Bihar Veterinary College
Bihar Animal Sciences University (BASU), Patna.

Dr. Y.S. Jadoun

Associate Professor & Head
Department of Dairy Extension Education
Sanjay Gandhi Institute of Dairy Technology (SGIDT)
Bihar Animal Sciences University (BASU), Patna.

Co-Convenor

Dr. Puspendra Kumar Singh

Assistant Professor & Head
Department of Veterinary & Animal Husbandry Extension Education
Bihar Veterinary College
Bihar Animal Sciences University (BASU), Patna.



डॉ० इन्द्रजीत सिंह
कुलपति

Dr. Inderjeet Singh
Vice Chancellor

बिहार पशु विज्ञान विश्वविद्यालय
बिहार पशु चिकित्सा महाविद्यालय प्रांगण, पटना-800014
BIHAR ANIMAL SCIENCES UNIVERSITY
BIHAR VETERINARY COLLEGE CAMPUS, PATNA-800014

☎ 0612-2222221
☎ +91-94728-64654

Email : vc-basu-bih@gov.in
vicechancellorbasu@gmail.com

Message

I am pleased to learn that the **Department of Veterinary Pathology** is organizing a specialized training programme on “**Advanced Disease Diagnosis in Poultry**” from **19-23 January, 2026** for the **Veterinary Officers of the Government of Bihar**. The poultry sector is witnessing rapid intensification and the emergence of complex disease conditions necessitates a strong foundation in advanced diagnostic methodologies for effective prevention and control.

Accurate disease diagnosis forms the backbone of efficient poultry health management. The integration of clinical examination with gross pathological, histopathological, cytological and laboratory diagnostic tools has become indispensable in modern veterinary practice. This training programme reflects the institution’s commitment to capacity building and professional excellence by equipping Veterinary Officers with updated scientific knowledge and practical skills relevant to field conditions.

I am confident that this initiative of the department of Veterinary Pathology will empower the participating Veterinary Officers to adopt a systematic, scientific and evidence-based approach to poultry disease diagnosis, thereby reducing economic losses and improving flock health. I extend my sincere appreciation to the organizing department and resource persons and wish the programme great success.

Dr. Inderjeet Singh
Vice Chancellor, BASU, Patna



Dr. Nirmal Singh Dahiya
Director Extension Education

BIHAR ANIMAL SCIENCES UNIVERSITY
BIHAR VETERINARY COLLEGE CAMPUS, PATNA- 800014
Directorate of Extension Education

Ph. No. : 0612-2227261

Email: deebasupatna@gmail.com

Message

It is a matter of great satisfaction to be associated with the training programme on “**Advanced Disease Diagnosis in Poultry**”, being conducted from **19–23 January 2026**, organized by the **Department of Veterinary Pathology** for the **Veterinary Officers of the Government of Bihar**. Strengthening diagnostic capabilities of field veterinarians is crucial for effective disease surveillance, timely intervention and sustainable poultry production.

This training programme has been thoughtfully designed to bridge the gap between academic knowledge and field application by emphasizing hands-on training in poultry disease diagnosis, including post-mortem examination, sample collection, pathological interpretation and laboratory confirmation. Veterinary Pathology forms the cornerstone of disease diagnosis and such focussed programmes are essential for enhancing diagnostic precision at the grassroots level.

I appreciate the dedicated efforts of the Department of Veterinary Pathology and the organizing team in conceptualizing and implementing this need-based training programme. I am confident that the knowledge and skills acquired by the participating Veterinary Officers will translate into improved diagnostic services and better poultry health management across the state. I wish the programme every success and the participants a productive and rewarding learning experience.

Dr. Nirmal Singh Dahiya
DEE, BASU, Patna

INDEX

Sl. No.	Topic	Author's Name	Page no.
01	Post-Mortem Based Diagnosis of Common Poultry Diseases	Deepak Kumar, Sanjiv Kumar, Kaushal Kumar, Imran Ali and Vishal Kumar Sinha	1-6
02	Serology and Molecular Diagnostics for Poultry Diseases	Pradeep Kumar Ray	7-17
03	Poultry Necropsy Techniques: Identification of Gross Lesions & Sample Collection	Deepak Kr., Sanjiv Kr., Imran Ali, Kaushal Kr. and Neha Kumari	18-21
04	Poultry sector Overview and Health Challenges in Bihar	Pawan Kumar and Sanjiv Kumar	22-25
05	PPE Demonstration, Biosecurity Checklist Preparation and Identification of Farm-Level Gaps in Poultry Farms	Kaushalendra Kumar, R. R. K. Sinha and Ratnesh Kumar Choudhary	26-32
06	Integrated Disease Management in Poultry and Vaccination Protocol	Kaushal Kumar, Deepak Kr., Imran Ali and Sanjiv Kumar	33-37
07	Viral Diseases of Poultry : Field Diagnosis and Sampling	Imran Ali, Sanjiv Kumar, Kausal Kumar and Deepak Kumar	38-43
08	Collection and Handling: Blood, Swags and Tissue Samples of Poultry	Imran Ali, Sanjiv Kumar, Kausal Kumar and Deepak Kumar	44-49
09	Poultry Farm and Hatchery Biosecurity, Disinfection and Sanitation	Anjay, Purushottam Kaushik, Seuli Saha Roy, Bhoomika and	50-57
10	Transportation Mortality in Heavy weight Birds in Bihar Scenario	Sanjiv Kumar, Imran Ali, Deepak Kumar and Kaushal Kumar	58-61
11	Outbreak Investigation in Poultry	Sumant Kumar and Sanjiv Kumar	62-66
12	Parasitic Diseases of Poultry: Prevention & Control	Pankaj Kumar, Sanjiv Kumar and Deepak Kumar	67-72

POST-MORTEM BASED DIAGNOSIS OF COMMON POULTRY DISEASES

**Deepak Kumar, Sanjiv Kumar, Kaushal Kumar, Imran Ali and Vishal
Kumar Sinha**

Department of Veterinary Pathology, Bihar Veterinary College, Patna

Introduction

Post-mortem examination is a vital diagnostic approach in poultry disease investigation and remains one of the most practical tools for identifying causes of mortality in poultry flocks. Gross pathological findings when interpreted in conjunction with flock history, age, vaccination status and mortality patterns frequently allow a presumptive diagnosis prior to laboratory confirmation. In many field situations, postmortem-based diagnosis guides immediate disease control and preventive strategies.

Principles of Poultry Postmortem Examination

Necropsy should be conducted on freshly dead or humanely euthanized birds to minimize autolytic changes that may obscure lesions. A systematic examination of the external body surface, coelomic cavity and visceral organs is essential for accurate lesion interpretation. Representative tissue samples should be collected aseptically for histopathology, bacteriology, virology and molecular diagnostics to confirm tentative diagnoses.

Postmortem Lesions of Common Poultry Diseases

Newcastle Disease (ND)

Newcastle disease is a highly contagious viral disease affecting birds of all ages. On postmortem examination, petechial to ecchymotic haemorrhages are commonly observed at the tips of proventricular glands along with congestion and haemorrhages of the trachea. Necrotic and haemorrhagic lesions in the intestine particularly in the caecal tonsils are characteristic of velogenic strains. In layers, ovarian haemorrhages and follicular regression may also be evident.

Avian Influenza (AI)

Highly pathogenic avian influenza produces severe systemic disease with high

mortality. Gross lesions include severe congestion and haemorrhages in the respiratory tract, cyanosis of comb and wattles, oedema of the head and neck and petechial haemorrhages on serosal surfaces (OIE, 2023). Multifocal haemorrhages in internal organs reflect viraemia and endothelial damage associated with highly pathogenic strains.

Infectious Bursal Disease (IBD)

Infectious bursal disease primarily affects chickens between 3 and 6 weeks of age. The bursa of Fabricius is characteristically enlarged, oedematous and may contain gelatinous or haemorrhagic exudate during the acute phase. In chronic cases, bursal atrophy, haemorrhages in thigh and breast muscles and nephrosis with urate deposition are commonly observed.

Marek's Disease (MD)

Marek's disease is a lymphoproliferative condition caused by Gallid herpesvirus-2. Postmortem findings include enlargement and loss of striations of peripheral nerves such as the sciatic nerve, along with greyish-white tumorous infiltrates in visceral organs including the liver, spleen, kidneys, heart and gonads. Ocular lesions such as irregular pupils and iris depigmentation may also be present.

Fowl Pox

Fowl pox occurs in cutaneous and diphtheritic forms. The cutaneous form is characterized by proliferative nodular lesions and scab formation on the comb, wattles, eyelids and other unfeathered areas. In the diphtheritic form, yellowish-white necrotic membranes are observed in the oral cavity, pharynx, larynx and trachea often leading to respiratory distress.

Colibacillosis

Colibacillosis is caused by pathogenic strains of *Escherichia coli* and commonly occurs as a secondary infection. Postmortem lesions include fibrinous pericarditis, fibrinous perihepatitis and airsacculitis with caseous exudate producing the typical picture of polyserositis. Septicaemic congestion of visceral organs may be observed in acute cases.

Fowl Cholera

Fowl cholera is an acute to chronic contagious disease of poultry caused by

Pasteurella multocida often associated with high morbidity and mortality particularly in adult birds. In acute cases, postmortem examination reveals septicaemic changes characterized by generalized congestion and haemorrhages of visceral organs with petechial haemorrhages on the heart and abdominal fat. Chronic cases show localized lesions such as caseous exudate in the wattles, joints and sinuses along with fibrinous pericarditis and perihepatitis. Multiple small necrotic foci in the liver are considered a characteristic lesion of fowl cholera.

Salmonellosis

Pullorum disease and fowl typhoid are caused by *Salmonella Pullorum* and *Salmonella Gallinarum* respectively. Postmortem lesions include hepatomegaly with bronze discoloration and focal necrosis, splenomegaly and caseous material in the caeca. In chicks, unabsorbed yolk sac with yolk sac infection and peritonitis is a consistent finding.

Coccidiosis

Coccidiosis is caused by different species of *Eimeria* producing species-specific intestinal lesions. Haemorrhages, intestinal thickening and necrosis are common findings. Ballooning of caeca filled with blood clots is typical of *Eimeria tenella* while white transverse ladder-like lesions in the duodenum are characteristic of *Eimeria acervulina*.

Ascites Syndrome

Ascites syndrome is a metabolic disorder predominantly seen in fast-growing broilers. Postmortem findings include accumulation of clear or straw-coloured fluid in the abdominal cavity, right ventricular hypertrophy, cardiac dilation and hepatic congestion. These changes are associated with pulmonary hypertension and right-sided heart failure.

Role of Ancillary Diagnostic Techniques

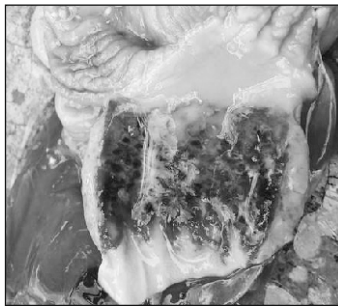
Although gross postmortem examination provides a strong presumptive diagnosis, confirmatory diagnosis requires laboratory investigations such as histopathology, bacteriological culture, serology and molecular techniques including PCR and RT-PCR.

Conclusion

Postmortem-based diagnosis remains an indispensable component of poultry disease investigation. Accurate recognition of characteristic lesions, supported by epidemiological data and laboratory confirmation, ensures effective disease control and prevention strategies in poultry production systems (Calnek *et al.*, 2013; Saif, 2008).



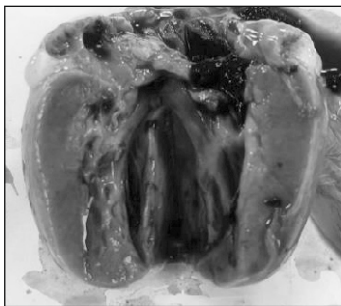
Severe congestion on the comb of bird affected from New Castle Disease.



Petechial to ecchymotic haemorrhages are observed at the tips of proventricular glands of bird suffering from New Castle Disease.



Trachea showing severe congestion of bird suffering from avian influenza.



Haemorrhages on the surface of heart of bird suffering from avian influenza.



Bird showing haemorrhages on the shanks and feet, a characteristic gross lesion suggestive of avian influenza, associated with severe vascular damage and systemic infection.



Haemorrhages in thigh of bird suffering from Infectious Bursal Diseases.



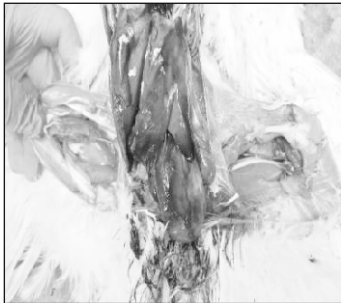
Inflamed and haemorrhagic Bursa of Fabricius of bird suffering from Infectious Bursal Disease.



Bird affected with Marek's disease showing characteristic unilateral paralysis of the leg due to peripheral nerve involvement, resulting in recumbency and inability to stand.



Greyish-white tumorous infiltrates in the liver of bird suffering from Marek's Disease.



Enlargement and loss of striations of sciatic nerve of bird suffering from Marek's Disease.



Proliferative nodular lesions and scab formation on the comb of bird suffering from Fowl pox.



Fibrinous perihepatitis of bird suffering from colibacillosis.



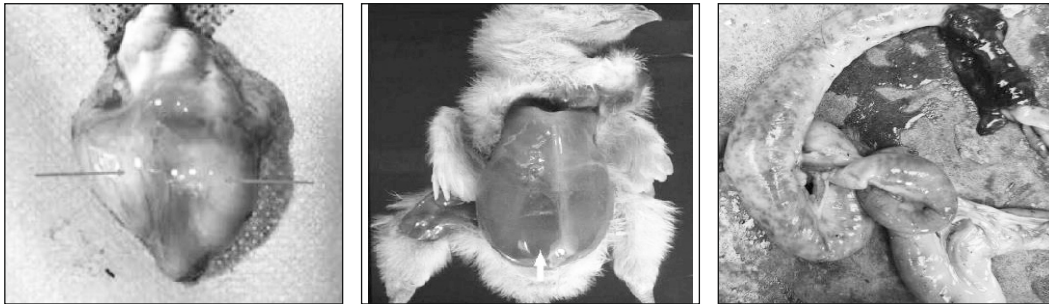
Airsacculitis with caseous exudate of bird suffering from colibacillosis.



Liver showing numerous necrotic foci on the surface of liver of bird suffering from Fowl cholera.



Enlarged Liver with congestion and pin point haemorrhages of bird suffering from salmonellosis.



Nodules on the myocardium giving shape as Bumps on the Heart of bird suffering from Salmonellosis. Accumulation of clear or straw-coloured fluid in the abdominal cavity of bird suffering from Ascites. Ballooning of caeca filled with blood clots of bird suffering from coccidiosis.

References

- Alexander D. J. Newcastle disease and other avian paramyxoviruses. 2000. *Rev Sci Tech.* **19**:443–462.
- Barnes H. J., Vaillancourt J. P. and Gross W. B. 2013. Colibacillosis. In: Saif YM, editor. *Diseases of Poultry*. 13th edn. Ames: Wiley-Blackwell. pp. 631–656.
- Calnek B. W., Barnes H. J., Beard C. W., McDougald L. R. and Saif Y. M. 2013. *Diseases of Poultry*. 13th ed. Ames: Wiley-Blackwell;
- Jordan F. T. W., Pattison M., Alexander D. and Faragher T. 2002. *Poultry Diseases*. 6th ed. London: Saunders Elsevier.
- Julian R. J. 2005. Production and growth-related disorders and other metabolic diseases of poultry. *Vet J.* **169**:350–369.
- McDougald L. R. 2003. Intestinal protozoa important to poultry. *Poult Sci.* **82**:1044–1050.
- OIE. 2023. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. Paris: World Organisation for Animal Health.
- Saif Y. M. 2008. *Poultry Diseases*. 6th ed. Philadelphia: Elsevier.
- Shivaprasad H. L. 2000. Pathology of salmonellosis in poultry. *Avian Pathol.* **29**:593–602.

SEROLOGY AND MOLECULAR DIAGNOSTICS FOR POULTRY DISEASES

Pradeep Kumar Ray

ICAR Research Complex for Eastern, Region, Patna

Poultry diseases pose a major threat to commercial bird health, food security, and international trade. Rapid intensification of poultry production system, coupled with increased bird movement and trade, has broadened the risk of infectious disease emergence and spread. Conventional diagnostic methods such as virus isolation, bacterial culture, and serology are although time consuming but remain valuable techniques for disease detection, outbreak management and surveillance. Serology plays a central role in the diagnosis, surveillance, and control of poultry diseases by detecting antibodies or antigens in serum and other body fluids. For veterinarians and veterinary laboratory personnel, serology remains an indispensable tool, particularly for large-scale monitoring and disease control programs. While serological methods provide information on exposure history, immune status, vaccine response, and flock-level infection dynamics, the molecular diagnostic techniques identify the presence of pathogen nucleic acids. Molecular diagnostic techniques have transformed the detection, characterization, and control of poultry diseases by offering rapid, sensitive, and specific pathogen identification. These techniques play a pivotal role in early disease detection, outbreak management, surveillance, and molecular epidemiology. Their ability to provide rapid and accurate results supports timely clinical decision-making and effective disease control strategies.

A. Serology

Importance of Serology in Poultry Health Management

Key roles of serology include:

- Monitoring flock exposure to infectious agents
- Assessing vaccine immune response
- Supporting disease surveillance and eradication programs
- Screening breeder and layer flocks
- Estimating herd immunity and infection prevalence

- Guiding vaccination schedules and biosecurity decisions

Unlike direct pathogen detection methods, serology reflects host immune response, which is especially valuable for chronic, endemic, or subclinical infections.

Principles of Serological assays

Serological assays are based on the specific binding between antigens and antibodies and it may involve:

- Antibody detection (**most common in poultry diagnostics**).
- Antigen detection (**used in some rapid assays**)

The presence, absence, or level of antibodies provides information on:

- Past exposure or infection
- Vaccination status
- Maternal antibody transfer
- Stage of infection (acute vs convalescent)

Common Serological Techniques Used in Poultry Diseases

1. Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is the most widely used serological test in poultry diagnostics.

- Principle: Antigens or antibodies are immobilized on a solid surface, and enzyme-labeled antibodies generate a measurable color change upon substrate addition. Intensity of color \propto antibody concentration

Types of ELISA:

- **Indirect ELISA:** Detection of antibodies (most common)
- **Sandwich ELISA:** Detection of antigens
- **Competitive ELISA:** for differentiating infected from vaccinated animals (DIVA)

Applications:

- Avian Influenza, Newcastle Disease, Infectious Bronchitis, IBD, MD
- Mycoplasma gallisepticum and M. synoviae

2. Hemagglutination Inhibition (HI) Test

Principle:

- Certain viruses agglutinate red blood cells while specific antibodies against these viruses inhibit this reaction.

- Highest serum dilution inhibiting HA = **HI titre**

Applications:

- Avian Influenza, Newcastle Disease
- **Reference test** for ND and AI serology, used for international trade certification

3. Agar Gel Immunodiffusion (AGID)

Principle:

Antigen and antibody diffuse through agar gel and form a visible precipitation line when they meet at optimal concentrations.

Applications:

- Avian Influenza (historical and surveillance use), Avian leukosis virus, IBD

4. Virus Neutralization Test (VNT)

Principle:

- Neutralizing antibodies prevent viral replication in cell culture or embryonated eggs.
- Virus is neutralized by specific antibodies
- Neutralized virus fails to infect cell culture or embryos

Applications: ND, IB, AI, AE

5. Latex Agglutination Test

Principle

- Antibodies or antigens coated on latex beads
- Visible agglutination indicates positivity

Applications: Mycoplasmosis, Salmonellosis etc

6. Rapid Serological Tests (Lateral Flow Assays)

Principle:

Immunochromatographic assays detect antibodies or antigens on test strips.

Applications:

- Avian Influenza, Newcastle Disease

Advantages:

- Rapid results (minutes), Field-friendly

Limitations:

- Lower sensitivity and specificity, Mainly screening tools

Sample Collection and Handling for Serology

Common Samples:

- Serum (primary sample for antibody detection)
- Plasma (EDTA or heparin)
- Egg yolk (for breeder immunity monitoring)
- Tracheal or cloacal swabs (for antigen detection tests)
- Avoid hemolysis
- Proper labelling and cold-chain maintenance
- Use of paired sera for acute and convalescent comparison

Interpretation of Serological Results

Serological results should be interpreted with consideration of:

- Age of birds, Vaccination history and maternal antibodies
- Clinical signs, Timing of sample collection and flock history

Important considerations:

- A single positive result indicates exposure, not necessarily active infection
- Rising antibody titers in paired samples suggest recent infection
- Vaccination can complicate interpretation

Common Poultry Diseases and Serological Tests

Disease	Pathogen	Common Serological Tests
Avian Influenza	AIV	ELISA, HI, AGID
Newcastle Disease	NDV	ELISA, HI, VNT
Infectious Bronchitis	IBV	ELISA, VNT
Infectious Bursal Disease	IBDV	ELISA
Marek's Disease	MDV	ELISA
Mycoplasmosis	Mycoplasma spp.	ELISA, Rapid tests
Avian Leukosis	ALV	AGID, ELISA

A. Principles and Advantages of Molecular Diagnostics

Molecular diagnostics rely on amplification, detection, or sequencing of DNA or RNA targets specific to infectious agents. Compared with conventional techniques,

molecular methods offer:

- High analytical sensitivity and specificity
- Reduced diagnostic turnaround time
- Detection of pathogens before sero-conversion
- Differentiation of strains, genotypes, and pathotypes
- Utility in both clinical diagnosis and surveillance programs

Major Molecular Diagnostic Techniques Used in Poultry Diseases

1. Polymerase Chain Reaction (PCR):

PCR remains the cornerstone of molecular diagnostics in poultry laboratories. Both DNA and RNA pathogens can be detected using PCR and RT-PCR, respectively. PCR is based on the enzymatic amplification of a target DNA sequence through repeated cycles of temperature changes. Each cycle theoretically doubles the amount of target DNA, leading to exponential amplification. The process relies on sequence-specific primers, a thermostable DNA polymerase and controlled temperature cycling. PCR technique has evolved into several variations for higher sensitivity and specificity

- **Conventional PCR:** End-point detection, useful for routine diagnosis
- **RT-PCR:** Detection of RNA viruses such as AIV, NDV, and IBV
- **Multiplex PCR:** Simultaneous detection of multiple pathogens
- **Semi-Nested and Nested PCR**

Key Steps of PCR: Each PCR cycle consists of three main steps i.e. Denaturation, Annealing and Extension (Elongation). These three steps are repeated for 30–40 cycles, resulting in exponential amplification of the target sequence.

2. Real-Time PCR (qPCR)

Real-time polymerase chain reaction (or quantitative PCR, qPCR) is one of the most powerful and widely used molecular diagnostic techniques which enables simultaneous amplification and detection of nucleic acids, providing rapid and specific identification of target microbes. In poultry disease diagnostics, real-time PCR is considered the **gold standard** for many viral and bacterial infections due to its accuracy, speed and suitability of the test for high-throughput testing.

Principle:

Real-time PCR monitors & measures the accumulation of amplified DNA

during each amplification cycle using **fluorescent signals**. The fluorescence intensity increases proportionally with the amount of amplified DNA fragment and is detected in real time by the PCR instrument. The key measurement in real-time PCR is the **cycle threshold (Ct) value**, which is the cycle number at which fluorescence exceeds a defined background threshold. Lower Ct values indicate higher initial target concentration.

Core Components of a Real-Time PCR Assay

- Template DNA or RNA
- Sequence-specific primers
- Fluorescent chemistry (dyes or probes)
- DNA polymerase (hot-start Taq polymerase)
- dNTPs, Mg²⁺, and reaction buffer
- Real-time PCR instrument

Fluorescent Detection Chemistries

(a) Intercalating Dye-Based Real-Time PCR

Example:

- SYBR Green I, Eva Green, **TB Green** (Proprietary dye from Takara Bio), SYTO Dyes (e.g., SYTO-13, SYTO-82)

Mechanism: The dye binds to ds DNA and emits fluorescence upon **excitation**.

Advantages:

- Simple and cost-effective, Suitable for screening assays

Limitations:

- Binds to non-specific products and primer-dimers
- Requires melting curve analysis for specificity confirmation

(b) Probe-Based Real-Time PCR

Example: TaqMan probes

Mechanism: A sequence-specific probe labeled with a reporter and quencher emits fluorescence only after probe cleavage during amplification.

Advantages:

- High specificity, Suitable for multiplex assays and No post-PCR analysis required High sensitivity and specificity
- Quantitative assessment of pathogen load

- Reduced contamination risk due to closed-tube systems

Limitations: Higher cost, Requires careful probe design

3. Loop-Mediated Isothermal Amplification (LAMP)

- Loop-mediated isothermal amplification (LAMP) is a nucleic acid amplification technique that amplifies DNA with high specificity, efficiency, and rapidity under **isothermal conditions**, typically at **60–65°C**. Unlike PCR, LAMP does not require thermal cycling and relies on a **strand-displacing DNA polymerase** (commonly *Bst* DNA polymerase).
- The high specificity of LAMP is achieved through the use of **four to six primers** that recognize **six to eight distinct regions** on the target gene, making it highly selective for the pathogen of interest.
- For RNA viruses, **Reverse Transcription LAMP (RT-LAMP)** is employed, in which reverse transcription and amplification occur in a single step.

Reaction components include:

- Target DNA or RNA
- *Bst* DNA polymerase
- Reverse transcriptase (for RT-LAMP)
- dNTPs, Mg²⁺ ions, Reaction buffer
- Primers (high concentration compared to PCR)

Detection of LAMP Products

LAMP amplification can be detected using multiple formats, making it versatile for laboratory and field applications.

a) Visual Detection (Colorimetric dyes e.g., hydroxynaphthol blue, phenol red)

b) Turbidity Measurement (Based on magnesium pyrophosphate precipitation)

c) Fluorescence Detection (Intercalating dyes (e.g., SYBR Green))

4. Nucleic Acid Sequencing

Sequencing techniques provide deeper insights into pathogen genetics.

- Determination of nucleotide sequence
- Comparison with reference strains
- **Sanger sequencing:** Genotyping and confirmation

- **Next-Generation Sequencing (NGS):** Whole-genome analysis, detection of novel pathogens, molecular epidemiology, and antimicrobial resistance profiling

Applications and Importance

- NDV genotyping
- AI subtype and clade identification
- Tracing outbreak sources
- Understanding virus evolution
- Vaccine strain matching
- International reporting

5. **DNA Microarray Techniques in Poultry Disease Diagnosis**

- DNA microarray technology is a high-throughput molecular diagnostic approach that allows the simultaneous detection and analysis of thousands of nucleic acid targets in a single assay.
- The technique is based on **sequence-specific hybridization** between labeled nucleic acid targets (DNA or cDNA) from a sample and **immobilized oligonucleotide probes** fixed onto a solid surface, like glass slide or silicon chip.
- Hybridization between complementary sequences produces a detectable signal, enabling identification of multiple pathogens or genetic markers within a single experiment.
- Microarrays are particularly useful when clinical signs are non-specific and multiple pathogens are suspected.

Applications of Microarrays in Poultry Disease Diagnosis

- Simultaneous detection of respiratory pathogens (IBV, NDV, AIV, Mycoplasma spp.)
- Differential diagnosis of enteric diseases with genotyping and strain differentiation
- Detection of mixed or co-infections
- Surveillance of antimicrobial resistance genes
- Molecular epidemiology and outbreak tracing

6. Other Molecular Techniques In Poultry Disease Diagnosis

- Restriction Fragment Length Polymorphism (PCR-RFLP)
- *In situ* hybridisation
- Digital PCR (dPCR),
- High-Resolution Melting (HRM) Analysis - Detection of sequence variations based on DNA melting curves
- RPA [Recombinase Polymerase Amplification - Isothermal amplification at low temperature (37–42°C)]

Common Poultry Pathogens and Molecular Diagnostic Techniques

Pathogen	Disease	Target Gene	Molecular Test Used
Avian Influenza Virus (AIV)	Avian Influenza	M, H5, H7	RT-PCR, qPCR, Sequencing
Newcastle Disease Virus (NDV)	Newcastle Disease	F gene	RT-PCR, qPCR, Sequencing
Infectious Bronchitis Virus (IBV)	Infectious Bronchitis	S1 gene	RT-PCR, qPCR, Sequencing
Infectious Bursal Disease Virus	Gumboro disease	VP2	RT-PCR, qPCR
Marek's Disease Virus (MDV)	Marek's disease	Meq	PCR, qPCR
<i>Mycoplasma gallisepticum</i>	Chronic respiratory disease	16S rRNA	PCR, qPCR
<i>Mycoplasma synoviae</i>	Infectious synovitis	vlhA	PCR, qPCR
<i>Salmonella</i> spp.	Salmonellosis	invA	PCR, qPCR
<i>E. coli</i> (APEC)	Colibacillosis	virulence genes	PCR, qPCR

Pre-Analytical Considerations for Molecular Diagnostic Techniques

- Proper sample collection, cold-chain maintenance, and nucleic acid preservation are critical to avoid false-negative results.

Standard Molecular Diagnostic Laboratory Workflow

1. Sample receipt and accessioning
2. Nucleic acid extraction (manual or automated)
3. PCR/qPCR assay setup
4. Amplification and detection
5. Result analysis and validation
6. Reporting and interpretation

Quality assurance measures such as internal controls, positive/negative controls, and adherence to SOPs are essential at every step.

Interpretation of Molecular Diagnostic Results

Molecular results should always be interpreted alongside:

- Clinical signs and flock history
- Vaccination status
- Necropsy findings
- Epidemiological context

Detection of nucleic acid does not always equate to active disease, especially in vaccinated or carrier birds.

Limitations and Challenges

- Detection of non-viable organisms
- Risk of laboratory contamination
- Cost and infrastructure requirements
- Need for skilled personnel

Despite these limitations, molecular diagnostics remain indispensable tools in poultry disease control.

Future Directions

Emerging technologies such as CRISPR-based diagnostics, portable PCR platforms, and integrated genomic surveillance systems are expected to further enhance poultry disease diagnosis and control.

Conclusion

Molecular diagnostic techniques have revolutionized poultry disease diagnostics by enabling rapid, accurate, and comprehensive pathogen detection. Their integration into veterinary diagnostic laboratories strengthens disease surveillance, outbreak response, and overall poultry health management. Continued advancements and capacity building will further expand their impact in both commercial and small-scale poultry systems.

References

da Silva E.C., de Oliveira C.D., Ribeiro L.F.M., Casas M.R.T., Pereira J.G., Possebon F.S. and Junior J.P.A.. (2023) Salmonella detection with LAMP and qPCR and identification of serovars of interest by multiplex qPCR in poultry carcasses. *Brazilian Journal of Microbiology* Sep;54(3):2173-2182. doi: 10.1007/s42770-023-01095-y.

Diaz-Sanchez S., Hanning I., Pendleton Sean and D'Souza D., (2013), Next-generation sequencing: The future of molecular genetics in poultry production and food safety. Presented as part of the Next Generation Sequencing: Applications for Food Safety and Poultry Production Symposium at the Poultry Science Association's annual meeting in Athens, Georgia, 2012., *Poultry Science*. 92 (2): 562-572.

El-Tholoth M., Bau H.H. (2024) Molecular Detection of Respiratory Tract Viruses in Chickens at the Point of Need by Loop-Mediated Isothermal Amplification (LAMP). *Viruses*. 16(8):1248. doi: 10.3390/v1608124.

Espy, M.J. (January 2006). "Real-Time PCR in Clinical Microbiology: Applications for Routine Laboratory Testing". *Clinical Microbiology Reviews*. 19 (3): 165–256. [doi:10.1128/CMR.19.1.165-256.2006](https://doi.org/10.1128/CMR.19.1.165-256.2006)

POULTRY NECROPSY TECHNIQUES: IDENTIFICATION OF GROSS LESIONS & SAMPLE COLLECTION

Deepak Kr., Sanjiv Kr., Imran Ali, Kaushal Kr. and Neha Kumari

Department of Veterinary Pathology

Bihar Veterinary College, Bihar Animal Sciences University, Patna-14

Necropsy is the systematic post-mortem examination of the body of an animal after death. It helps in determining the cause of death, understanding disease processes, monitoring flock health, and guiding appropriate control and preventive measures. Due to the small size of birds and rapid post-mortem changes, poultry necropsy requires systematic techniques, careful observation, and proper sample collection to avoid autolysis and contamination. Accurate identification of gross lesions forms the basis for correlating clinical signs with pathological changes.

The main objectives of poultry necropsy include identifying the cause of morbidity and mortality, detecting infectious and non-infectious diseases, assessing management and nutritional problems, and collecting representative samples for laboratory diagnosis.

❖ Materials Required for Poultry Necropsy-

- Clean table or flat surface for necropsy
- Post-mortem knife or scalpel
- Scissors
- Forceps
- Poultry shears or bone cutter
- Tray or plate to keep organs
- Disposable gloves
- Apron or laboratory coat
- Face mask and cap
- Sample bottles or containers
- 10% neutral buffered formalin
- Sterile swabs (if required)
- Labels and marker pen
- Necropsy record sheet

- Measuring scale
- Disinfectant solution
- Waste disposal bags
- Facility for proper disposal of carcass

The bird is placed on its back with legs extended for systematic examination. Wings and legs are stretched and fixed using pins. Feathers over the abdomen and thorax are moistened with water or disinfectant to prevent aerosol formation. Steps in necropsy of poultry-

1. Preparation and History-

- Safety First: Wear gloves and a mask, especially if zoonotic diseases are suspected. Use a well-lit area with a washable surface.
- Flock History: Record bird age, breed, clinical signs (e.g., coughing, diarrhea), mortality rates, and recent treatments or vaccinations.
- Euthanasia: If necropsying a sick bird, use humane methods such as cervical dislocation or CO₂ chambers.
- Moistening Feathers: Wet the bird with a disinfectant solution or soapy water to prevent feathers from flying and contaminating the work area.

2. External examination-

- Check for external parasites (mites, lice), skin lesions, or bruising.
- Examine the head: inspect eyes, ears, nostrils (nares), comb, and wattles for discharge or swelling.
- Check joints (hock and stifle) for swelling or fluid accumulation.
- Body condition, evidence of trauma, swelling, or deformities are recorded before proceeding to internal examination.

3. Internal Examination-

(a) Opening of the Body Cavity-

The skin is incised from the cloaca to the keel bone, and the skin is reflected laterally. The breast muscles are examined for hemorrhages, emaciation, or necrosis. The keel bone is cut, and ribs are gently removed to expose the thoraco-abdominal cavity.

(b) Examination of Body Cavities-

The air sacs are examined first for cloudiness, thickening, exudate, or caseous material. The presence of fibrin, blood, or abnormal fluid in the body cavity is

recorded. Position, size, color, and consistency of organs are carefully observed before removal.

(c) Systematic Organ Examination-

- Liver: size, color, congestion, hemorrhages, necrotic foci.
- Spleen: enlargement or atrophy.
- Heart: pericardial fluid, hemorrhages, myocardium changes.
- Lungs: congestion, consolidation, nodules.
- Gastrointestinal tract: crop, proventriculus, gizzard, intestines for ulcers, hemorrhages, parasites, or contents.
- Kidneys: enlargement, pallor, urate deposition.
- Reproductive organs: ovary and oviduct for hemorrhage, regression, or egg peritonitis.
- Bursa of Fabricius and thymus: size and atrophy, especially in immunosuppressive diseases.

Identification of Gross Lesions-

Gross lesions are visible structural changes observed during necropsy. Identification of gross lesions involves careful observation of visible pathological changes in organs and tissues during necropsy. Lesions are assessed based on location, size, shape, color, consistency, and distribution to help in tentative diagnosis. Common types include congestion, hemorrhage, necrosis, ulceration, edema, and enlargement or atrophy of organs.

Disease	Key Gross Lesions Observed
Newcastle Disease (Ranikhet Disease)	Petechial hemorrhages in proventriculus, cecal tonsils & intestinal mucosa.
Marek’s Disease	Enlargement of peripheral nerves (sciatic, brachial). Gray eye (ocular form)
Fowl pox	Nodules on comb/wattles; diphtheritic oral lesions
Infectious bronchitis	Catarrhal tracheitis, cloudy air sacs urate-filled kidneys
Infectious Laryngotracheitis	Hemorrhagic tracheitis, -Caseous or bloody plugs in trachea
Coccidiosis	Hemorrhages in intestinal mucosa, -Blood-tinged or chocolate-colored contents
Fowl Typhoid	Enlarged, friable bronze- colored liver
Pullorum Disease	White pasty diarrhea around vent (chicks)
Aspergillosis (Brooder Pneumonia)	White to yellow caseous nodules in lungs

- ❖ Importance of Gross Lesion Identification-
 - i. Helps in tentative diagnosis
 - ii. Guides sample collection
 - iii. Assists in differential diagnosis
 - iv. Useful for disease surveillance and control

Sample Collection in Poultry Necropsy

General Principles-

Samples should be collected as early as possible using clean or sterile instruments. Representative portions of affected and adjacent normal tissue are preferred. Each sample must be properly labeled with bird details, organ name, date, and suspected disease.

- Samples for Histopathology

Small tissue pieces (0.5–1 cm thickness) from liver, spleen, kidney, heart, lung, intestine, bursa, and brain are collected and immediately fixed in 10% neutral buffered formalin in a ratio of at least 1:10 (tissue:fixative).

- Samples for Bacteriology and Virology

Aseptically collected tissues or swabs from liver, spleen, heart blood, trachea, or cloaca are placed in sterile containers. These samples should be kept cool and transported quickly to the laboratory.

- Samples for Parasitology

Intestinal contents, scrapings, and affected organs are collected in clean containers. Some samples may be preserved in suitable preservatives depending on the parasite suspected.

- Samples for Toxicology

Suspected feed, water, liver, kidney, and crop contents are collected in clean, chemical-free containers without preservatives and sent promptly for analysis.

Poultry necropsy is a vital component of veterinary diagnostic pathology. A systematic necropsy technique, accurate identification of gross lesions, and proper sample collection greatly enhance diagnostic accuracy. Regular practice and careful observation help veterinarians in effective disease diagnosis, control, and prevention in poultry production systems.

POULTRY SECTOR OVERVIEW AND HEALTH CHALLENGES IN BIHAR

Pawan Kumar¹ and Sanjiv Kumar²

¹MD, HiTech, Hajipur

²Department of Veterinary Pathology, BVC, BASU, Patna, Bihar

1. Introduction

Poultry farming has become an increasingly important agricultural enterprise in India, contributing significantly to rural livelihoods, nutritional security, and economic growth. In Bihar, a state with a large rural population and agrarian economy, the poultry sector plays a crucial role in income generation and employment, especially among smallholder farmers and vulnerable communities. However, despite its potential, the sector faces numerous challenges including disease outbreaks, infrastructure gaps, and market constraints that impact production, profitability, and sustainability.

This chapter provides a detailed overview of the poultry sector in Bihar, the current health challenges faced by poultry producers, and key factors affecting poultry productivity and development in the region.

2. Overview of the Poultry Sector in Bihar

2.1. Sector Profile

Poultry farming in Bihar includes both commercial and backyard poultry systems. The commercial sector typically involves larger broiler and layer units that focus on meat and egg production respectively. Backyard poultry, on the other hand, is practiced by small and marginal farmers and serves as a supplementary source of income and nutrition.

The state's poultry production has been growing due to increasing demand for poultry meat and eggs. According to government data, there are *over 1,200 medium and large poultry farms* and *more than 2,000 backyard units* across Bihar.

2.2. Importance to Rural Economy

Poultry farming contributes to livelihood diversification, especially in rural areas where farm incomes are often seasonal and uncertain. It supports self-employment and community nutrition through the local availability of eggs and poultry meat. Policy incentives, including subsidies for poultry farm establishment and support for

layer units, have been introduced by the Bihar government to promote sector growth.

2.3. Government Support and Schemes

The Government of Bihar has rolled out several initiatives aimed at developing the poultry sector:

- **Subsidy Programmes:** Financial incentives ranging from 30% to 50% for establishing broiler poultry farms to encourage new entrepreneurs.
- **Training and Extension Services:** Regular training programmes organized to improve farmer skills and knowledge on poultry management and health.
- **Feed and Input Support:** Availability of poultry feed through community organisations and feed analysis centres, including support from COMFED and private manufacturers.)

Despite these supportive efforts, the sector still confronts gaps in infrastructure and service delivery.

3. Poultry Health Challenges in Bihar

Poultry health remains one of the most significant barriers to sustainable production. Diseases not only reduce productivity but also inflict heavy economic losses and erode farmer confidence.

3.1. Major Disease Threats

Bihar's poultry sector frequently faces outbreaks of infectious diseases that affect both commercial and backyard flocks:

3.1.1. Avian Influenza (Bird Flu)

Avian influenza (commonly known as bird flu) is one of the most feared poultry diseases. Outbreaks have been reported in several areas, causing mass culling and substantial economic loss. For example, in one incident, *9,000 hens were culled* following a bird flu outbreak in a private farm, leading farmers to scale back operations due to repeated losses.

3.1.2. Other Viral Diseases

In addition to bird flu, poultry birds in Bihar are affected by diseases such as:

- **Newcastle Disease** (Ranikhet disease)
- **Marek's Disease**
- **Fowlpox**
- **Infectious Bursal Disease** (Gumboro Disease)

These diseases often lead to high mortality when not detected early or managed effectively. Farmers report that many new entrepreneurs lack the experience to diagnose or respond quickly, resulting in major losses before veterinary intervention occurs.

3.2. Impact of Disease Outbreaks

Disease outbreaks have multifaceted effects:

- **Economic Losses:** Mortality, reduced egg production, and culling lead to direct income losses for farmers. Compensation is often delayed and insufficient.
- **Market Disruption:** During outbreaks, meat shops and markets suffer lower sales or temporary closures, hampering supply chains.
- **Farmer Confidence:** Frequent disease events make farmers risk-averse, reducing investment in poultry expansion.

4. Other Key Challenges in Poultry Health and Production

4.1. Feed Quality and Cost

Feed represents the largest input cost in poultry production. High feed prices, coupled with inconsistent feed quality, limit profitability and can compromise bird health and growth.

4.2. Infrastructure and Technology Gaps

Bihar's poultry industry suffers from insufficient cold chain infrastructure, poor transportation, and limited access to modern production technologies. These gaps cause post-harvest losses and restrict the efficient movement of chilled meat and eggs to distant markets.

4.3. Biosecurity and Disease Control Management

Inadequate biosecurity measures on many farms allow rapid disease spread. Training farmers on simple biosecurity practices—such as controlled access, sanitation, and quarantine—is vital.

4.4. Service Delivery Constraints

Access to veterinary services, diagnostic laboratories, and rapid disease surveillance remains limited in rural areas. This results in delayed diagnosis and response, enabling disease spread.

5. Strategies for Improving Poultry Health in Bihar

To overcome existing challenges, coordinated efforts are needed across multiple fronts:

5.1. Strengthening Biosecurity

Farmers should be trained on best practices including isolation of new birds, regular disinfection, and proper waste disposal.

5.2. Enhanced Disease Surveillance

Improved monitoring systems and rapid response teams can help detect outbreaks early and limit spread.

5.3. Support for Insurance and Compensation

Timely compensation and affordable insurance programmes will encourage farmers to maintain flock sizes and invest in health measures.

5.4. Feed and Production Support

Ensuring access to quality feed, adopting balanced nutrition, and exploring cost-effective alternatives can increase production efficiency.

5.5. Technology Adoption

Encouraging the use of modern poultry management tools, including automated feeders, climate control, and record-keeping systems, can improve productivity and reduce health risks.

6. Conclusion

The poultry sector in Bihar holds significant potential for livelihood improvement, rural employment, and nutritional security. While positive developments such as government subsidies and training programs support sectoral growth, persistent health challenges—especially disease outbreaks like bird flu and other viral infections—continue to undermine productivity and profitability. Addressing these health and systemic challenges through improved biosecurity, infrastructure, veterinary services, and farmer support systems is essential for transforming poultry farming into a resilient and sustainable enterprise in the state.

PPE DEMONSTRATION, BIOSECURITY CHECKLIST PREPARATION AND IDENTIFICATION OF FARM-LEVEL GAPS IN POULTRY FARMS

**Kaushalendra Kumar, Ravi Ranjan Kumar Sinha and Dr. Ratnesh Kumar
Choudhary**

Bihar Veterinary College, BASU, Patna-14

Introduction:

Biosecurity is the foundation of successful and sustainable poultry production. It refers to the set of management practices designed to prevent the introduction, spread, and persistence of infectious agents within and between poultry farms (FAO, 2010). With increasing intensification of poultry farming, high bird density, and frequent movement of people, vehicles, and materials, the risk of disease transmission has significantly increased (OIE, 2019). Therefore, effective biosecurity is essential to protect poultry health, ensure food safety, and maintain farm profitability (Dorea et al., 2010).

Importance of Biosecurity in Disease Prevention:

Biosecurity plays a critical role in preventing infectious diseases in poultry farms by breaking the chain of disease transmission (Newell et al., 2011). Pathogens such as viruses, bacteria, fungi, and parasites can enter poultry farms through multiple routes, including contaminated footwear, clothing, equipment, vehicles, feed, water, wild birds, rodents, insects, and human visitors.

By implementing strict biosecurity measures such as controlled farm entry, use of personal protective equipment (PPE), proper sanitation and disinfection, and separation of clean and dirty areas, the risk of pathogen entry is significantly reduced. Biosecurity also limits the spread of disease within the farm by preventing cross-contamination between poultry houses, age groups, and flocks (OIE, 2019).

Effective biosecurity reduces dependence on antibiotics and medications, thereby supporting antimicrobial resistance (AMR) control and ensuring safer poultry products for consumers. Moreover, strong biosecurity improves bird welfare, enhances growth performance, and increases overall production efficiency (Gelaude et al., 2014).

Common Poultry Diseases Linked to Poor Biosecurity:

Poor biosecurity practices are directly associated with the occurrence and spread of several economically important poultry diseases. Some of the most common diseases linked to inadequate biosecurity include:

- **Newcastle Disease (ND):** A highly contagious viral disease, causing high mortality, respiratory distress, and severe production losses. It spreads rapidly through contaminated equipment, footwear, and human movement.
- **Avian Influenza (AI):** A serious viral disease transmitted through wild birds, contaminated water, and poor farm hygiene. Outbreaks often result in mass culling and strict movement restrictions.
- **Infectious Bursal Disease (IBD/Gumboro):** A viral disease affecting the immune system of young birds, leading to immunosuppression and increased susceptibility to secondary infections.
- **Infectious Bronchitis (IB):** A viral respiratory disease that spreads easily through air, personnel, and equipment, causing reduced growth and egg production.
- **Salmonellosis:** A bacterial disease associated with contaminated feed, water, rodents, and poor sanitation. It also poses a public health risk.
- **Coccidiosis:** A parasitic disease linked to poor litter management and hygiene, resulting in poor feed conversion and increased mortality.
- **Chronic Respiratory Disease (CRD):** Often exacerbated by poor ventilation, overcrowding, and inadequate biosecurity measures.

These diseases can be effectively prevented or minimized through consistent application of biosecurity protocols.

Economic Impact of Disease Outbreaks in Poultry Farms:

Disease outbreaks in poultry farms cause substantial economic losses, affecting both small-scale and commercial producers. The economic impact arises from multiple direct and indirect factors, including:

- Increased mortality and morbidity
- Reduced growth rate and feed efficiency
- Decline in egg production and egg quality
- Higher expenditure on medicines, vaccines, and veterinary services
- Cost of culling, disposal, and disinfection

- Production downtime and delayed restocking

Additionally, disease outbreaks damage farmer confidence, disrupt supply chains, and negatively impact employment and allied industries. In contrast, investment in biosecurity is cost-effective and yields long-term benefits by ensuring consistent production, reduced losses, and improved profitability (Dorea et al., 2010; Gelaude et al., 2014).

PPE Demonstration in Poultry Farms:

Personal Protective Equipment (PPE) is a critical component of biosecurity in poultry farming. PPE acts as a physical barrier between farm personnel and potential disease-causing agents, thereby reducing the risk of pathogen entry, spread, and cross-contamination within and between poultry farms (FAO, 2010; OIE, 2019). Proper selection, use, removal, cleaning, and disposal of PPE are essential to ensure its effectiveness.

Types of PPE Used in Poultry Farms:

1. Face Mask

Face masks help prevent the transmission of airborne pathogens, dust particles, and respiratory droplets that may carry viruses and bacteria.

- Protect workers from inhaling dust, ammonia, and infectious agents
- Prevent contamination of birds by human respiratory droplets
- Recommended types include disposable surgical masks or N95 masks during disease outbreaks

2. Gloves

Gloves act as a barrier against direct contact with contaminated surfaces, litter, feces, sick birds, and dead birds.

- Disposable latex, nitrile, or rubber gloves are commonly used
- Reduce the risk of hand-borne transmission of pathogens
- Should be changed between poultry houses and after handling sick birds

3. Coveralls/Apron

Coveralls or aprons protect clothing and skin from contamination.

- Made of washable or disposable material
- Prevent pathogens from being carried on clothes to other poultry sheds or farms
- Separate coveralls should be used for different poultry houses

4. Boots

Boots protect feet from contaminated litter, feces, and wet surfaces.

- Preferably made of rubber or plastic for easy cleaning
- Should be used exclusively inside the poultry farm
- Must be disinfected using footbaths at entry and exit points

5. Head Caps

Head caps prevent contamination of hair, which can carry dust and pathogens.

- Especially important in closed housing systems
- Reduce the risk of disease spread through hair and scalp contact

6. Hand Sanitizers

Hand sanitizers are essential for maintaining hand hygiene before and after farm activities.

- Alcohol-based sanitizers (minimum 60–70% alcohol) are recommended
- Used after glove removal and when handwashing facilities are not immediately available

Step-by-Step Demonstration of PPE Use:

A. Proper Wearing (Donning) of PPE

1. Wash hands thoroughly with soap and water or use hand sanitizer
2. Wear clean coveralls or apron
3. Put on boots and ensure proper fitting
4. Wear face mask covering nose and mouth securely
5. Place head cap properly covering hair
6. Put on gloves as the final step
7. Check PPE for proper fit and comfort before entering the poultry house

B. Safe Removal (Doffing) of PPE

1. Remove gloves first without touching the outer surface
2. Perform hand hygiene using sanitizer
3. Remove coveralls/apron carefully, avoiding contact with the outer surface
4. Remove boots and place them in designated cleaning area
5. Remove head cap
6. Remove face mask by holding the straps only
7. Perform thorough handwashing or sanitization after complete removal

Improper removal of PPE can lead to self-contamination; therefore, strict

adherence to doffing procedures is essential.

C. Cleaning and Disposal of PPE

- **Disposable PPE** (masks, gloves, caps) should be collected in designated biohazard bags and disposed of as per farm waste management protocols
- **Reusable PPE** (boots, washable coveralls) should be:
 - Washed with detergent
 - Disinfected using approved disinfectants
 - Dried completely before reuse
- Boots should be scrubbed and disinfected daily
- PPE storage areas should be clean and separate from poultry housing

Role of PPE in Preventing Cross-Contamination:

PPE plays a vital role in preventing cross-contamination by limiting the transfer of pathogens between:

- Different poultry houses
- Different age groups of birds
- Sick and healthy flocks
- Poultry farms and the outside environment

Consistent use of PPE reduces the risk of farm-to-farm disease transmission caused by personnel, equipment, and visitors. When combined with other biosecurity measures such as disinfection, restricted access, and proper sanitation, PPE significantly strengthens the overall biosecurity system of poultry farms.

Biosecurity Checklist Preparation:

Components of a poultry biosecurity checklist:

- Entry and exit control
- Visitor and vehicle management
- Footbath and disinfection procedures
- Equipment and housing sanitation
- Feed and water hygiene
- Litter management
- Rodent, insect, and wild bird control
- Isolation of sick birds
- Dead bird disposal methods
- Record keeping and documentation

- Hands-on preparation of a farm-specific checklist

Identification of Farm-Level Gaps

- On-farm assessment of existing practices
- Identification of critical biosecurity gaps such as:
 - Irregular use of PPE
 - Inadequate disinfection facilities
 - Poor visitor control
 - Lack of quarantine/isolation area
 - Improper carcass disposal
 - Absence of SOPs
 - Group discussion on risk factors

Corrective Measures and Recommendations

- Practical and low-cost biosecurity improvements
- Strengthening compliance with SOPs
- Role of farm workers in maintaining biosecurity
- Monitoring and follow-up strategies

Conclusion:

Biosecurity and proper use of personal protective equipment are essential pillars of sustainable and profitable poultry production. Effective biosecurity practices prevent the introduction and spread of infectious diseases, reduce economic losses, and ensure flock health and food safety. PPE serves as a critical barrier against cross-contamination, protecting both poultry and farm workers when used correctly. Preparation of a biosecurity checklist and identification of farm-level gaps enable systematic assessment and targeted improvements in farm management. Adoption of practical corrective measures, regular training, and strict compliance with standard operating procedures collectively strengthen biosecurity, minimize disease risks, and enhance productivity and long-term viability of poultry farms.

References:

- Centers for Disease Control and Prevention (CDC). (2020). Guidance for the selection and use of personal protective equipment (PPE) in healthcare settings. CDC, Atlanta, GA, USA.
- Dorea, F. C., Berghaus, R., Hofacre, C., & Cole, D. J. (2010). Survey of biosecurity

protocols and practices adopted by growers on commercial poultry farms in
G e o r g i a , U S A .
Preventive Veterinary Medicine, 95(3–4), 253–261.

Food and Agriculture Organization of the United Nations (2010). Biosecurity for highly pathogenic avian influenza: Issues and options. FAO Animal Production and Health Paper No. 165. Rome, Italy.

Gelaude, P., Schlepers, M., Verlinden, M., Laanen, M., & Dewulf, J. (2014). Biocheck.UGent™: A quantitative tool to measure biosecurity at broiler farms and the relationship with technical performances and antimicrobial use. *Transboundary and Emerging Diseases*, 61(Suppl. 1), 45–55.

INTEGRATED DISEASE MANAGEMENT IN POULTRY AND VACCINATION PROTOCOL

Kaushal Kumar, Deepak Kumar, Imran Ali and Sanjiv Kumar

Department of Veterinary Pathology, BVC, BASU, Patna, Bihar

Introduction

Intensive poultry production systems are highly susceptible to infectious diseases owing to high stocking density, rapid flock turnover, and frequent movement of birds. Disease outbreaks lead to significant economic losses through increased mortality, reduced growth rate, poor feed efficiency, and decreased egg production. Integrated Disease Management (IDM) represents a holistic and preventive approach that integrates biosecurity, management practices, nutrition, disease surveillance, vaccination, and rational use of therapeutics to sustain flock health and ensure productive poultry farming.

Components of Integrated Disease Management

Integrated Disease Management (IDM) emphasizes prevention rather than curative treatment and is based on the coordinated and systematic application of multiple disease control measures. This approach integrates strict biosecurity protocols, sound management practices, balanced nutrition, continuous disease surveillance, strategic vaccination programs, and the judicious use of therapeutics. By addressing disease risks at various stages of production, IDM minimizes the likelihood of disease introduction, spread, and persistence, thereby reducing economic losses and promoting sustainable and efficient poultry production. Various aspects of Integrated Disease Management (IDM) are as follows:

1. Biosecurity
2. Housing and environmental management
3. Balanced nutrition and water hygiene
4. Disease surveillance and early diagnosis
5. Strategic vaccination
6. Judicious therapeutic interventions
7. Record keeping and monitoring

1. Biosecurity Measures

External biosecurity aims to prevent entry of pathogens into the farm and includes restriction of visitors and vehicles, use of footbaths and protective clothing, sourcing chicks from disease-free hatcheries, quarantine of newly introduced birds, and effective control of rodents, insects, and wild birds.

Internal biosecurity focuses on preventing disease spread within the farm through All-in–All-out systems, segregation of age groups, routine sanitation of equipment, proper disposal of carcasses and litter, and thorough cleaning and disinfection between batches.

2. Management and Nutritional Support

Adequate ventilation, optimal stocking density, proper temperature and litter moisture control, balanced feed with vitamins and minerals, mycotoxin-free feed, clean drinking water, and use of probiotics help reduce stress and improve disease resistance.

3. Disease Surveillance and Early Diagnosis

Continuous disease surveillance enables early detection and rapid intervention. Key indicators include sudden increase in mortality, reduced feed or water intake, poor growth rate, uneven flock performance, and drop in egg production. Early warning signs include sudden increase in mortality, reduced feed or water intake, poor growth, and drop in egg production. Diagnosis involves post-mortem examination, serology (ELISA, HI), molecular assays (PCR), bacteriology, and histopathology. Prompt post-mortem examination and laboratory confirmation are essential for accurate diagnosis.

4. Vaccination in Integrated Disease Management

Vaccination is a cornerstone of IDM and provides flock-level immunity by reducing disease severity, mortality, and pathogen shedding. Vaccines must be selected based on local disease prevalence and administered correctly to achieve optimal protection. It reduces disease severity, mortality, and pathogen shedding. Birds must be healthy at vaccination, cold chain (2–8°C) maintained, correct dose and route followed, and stress avoided before and after vaccination. Schedules should be based on regional disease prevalence.

Indicative Broiler Vaccination Schedule

Age	Disease	Vaccine	Route
Day 0	Marek's disease	Live (HVT)	Subcutaneous
5–7 days	Newcastle disease	Live	Eye drop / Water
10–12 days	IBD	Live	Drinking water
18–21 days	IBD (Booster)	Live	Drinking water
21–24 days	ND (Booster)	Live	Drinking water

Indicative Layer Vaccination Schedule

Age	Disease	Vaccine	Route
Day 0	Marek's disease	Live	Subcutaneous
5–7 days	ND	Live	Eye drop
2–3 weeks	IBD	Live	Drinking water
6–8 weeks	ND	Live	Drinking water
8–10 weeks	Fowl pox	Live	Wing web
12–14 weeks	ND	Inactivated	IM
16–18 weeks	EDS-76	Inactivated	IM

1. Therapeutic Use and AMR Prevention

Therapeutic use of antibiotics in poultry farming should be based on scientific principles and responsible practices to prevent the development of antimicrobial resistance (AMR). Antibiotics should be administered only after a confirmed diagnosis of bacterial infection, preferably supported by laboratory tests and veterinary advice. Indiscriminate or routine prophylactic use of antibiotics must be avoided, as it promotes the emergence of resistant pathogens and compromises the effectiveness of available drugs. When antibiotics are used, the correct drug, dose, and duration should be strictly followed, and mandatory withdrawal periods must be observed to ensure that poultry meat and eggs are free from harmful residues. In addition, the use of alternatives such as probiotics, prebiotics, and immune modulators plays an important role in improving gut health, enhancing natural immunity, and reducing dependence on antibiotics. These supportive measures help maintain a balanced microbial population and strengthen disease resistance, thereby

contributing significantly to the prevention of antimicrobial resistance and promoting sustainable and safe poultry production.

2. Role of Veterinary Officers

Veterinary officers play a vital and multifaceted role in the effective implementation of Integrated Disease Management (IDM) in poultry farming. They provide expert guidance on planning and executing IDM strategies, including the selection and scheduling of appropriate vaccination programmes tailored to local disease risks and farm conditions. Veterinary officers regularly conduct post-mortem examinations to identify the causes of mortality and carry out detailed investigations during disease outbreaks to determine the source, mode of spread, and risk factors involved. They coordinate with diagnostic laboratories for timely and accurate laboratory confirmation of diseases, which helps in adopting targeted control and treatment measures. In addition, veterinary officers train and educate farm staff on biosecurity practices, early disease recognition, hygienic management, and proper handling of birds and equipment. They also ensure the timely reporting of notifiable and emerging diseases to the concerned authorities, which is essential for disease surveillance, rapid response, and prevention of wider spread, thereby safeguarding poultry health and productivity at both farm and regional levels.

Conclusion

Integrated Disease Management (IDM) is very important for maintaining healthy poultry flocks and achieving sustainable poultry production. IDM focuses on preventing diseases rather than only treating them after they occur. It is supported by several key components, including effective vaccination programmes, strict biosecurity measures, good farm management practices, and the careful and responsible use of medicines.

Vaccination helps protect birds against common and serious infectious diseases by strengthening their immunity. Strict biosecurity measures, such as controlling the movement of people, vehicles, equipment, and birds, help prevent the entry and spread of disease-causing agents on poultry farms. Good management practices, including proper housing, balanced nutrition, clean drinking water, adequate ventilation, and regular sanitation, reduce stress in birds and improve their resistance to diseases. Judicious use of therapeutics, under veterinary guidance, ensures that medicines are used only when necessary, reducing the risk of drug resistance and

residues in poultry products.

Veterinary officers play a crucial role in the successful implementation of Integrated Disease Management. They guide farmers in planning and carrying out vaccination schedules, maintaining biosecurity, identifying early signs of disease, and adopting proper treatment protocols. Through regular monitoring, disease surveillance, and farmer education, veterinary officers help minimize disease-related losses, improve flock health, and enhance overall productivity. Thus, effective IDM, supported by veterinary expertise, is essential for healthy poultry, economic stability of farmers, and safe poultry production.

VIRAL DISEASES OF POULTRY: FIELD DIAGNOSIS AND SAMPLING

Imran Ali, Sanjiv Kumar, Kaushal Kumar and Deepak Kumar

Department of Veterinary Pathology, Bihar Veterinary College, Patna

1. Introduction

Viral diseases constitute one of the most important constraints affecting poultry production systems worldwide. They are responsible for heavy economic losses due to high morbidity, mortality, reduced growth rate, decreased egg production, poor feed conversion efficiency, and increased expenditure on disease control measures. In developing countries, including India, the impact is further aggravated by high poultry density, variable biosecurity standards, and limited access to advanced diagnostic facilities at the field level.

Early and accurate diagnosis of viral diseases is essential for timely implementation of control measures such as movement restriction, biosecurity enhancement, emergency vaccination, and strategic depopulation where required. While laboratory confirmation remains the gold standard, accurate field diagnosis based on clinical signs, flock history, and gross lesions provides the first and most critical step in disease investigation. Veterinary officers, therefore, play a pivotal role in recognizing disease patterns, collecting appropriate samples, and ensuring their safe and timely submission to diagnostic laboratories.

2. Common Viral Diseases of Poultry of Field Importance

2.1. Newcastle Disease (ND)

- **Etiology:** Newcastle disease virus (Avian Paramyxovirus type-1).

Newcastle disease is one of the most devastating viral diseases of poultry, affecting birds of all ages. The severity of disease varies with the virulence of the virus strain, immune status of the flock, and management conditions.

- **Field diagnostic clues:** Sudden onset of illness, high morbidity and mortality, respiratory distress such as gasping and coughing, greenish diarrhea, nervous signs including torticollis, tremors, and paralysis, and a sharp decline in egg production in layers.

- **Gross lesions:** Petechial hemorrhages at the tips of proventricular glands, hemorrhagic Caecal tonsils, congestion of trachea and lungs, and splenic enlargement.

- **Field Diagnostic Kits:**

Kit: NDV Rapid Antigen Test (Lateral Flow)

Principle: Immunochromatographic detection of ND virus antigen.

Sample: Tracheal / cloacal swab

Procedure: Swab → buffer → cassette → read in 5–10 minutes.

Interpretation: Two lines – Positive; control line only – Negative.

Remarks: Rapid screening; confirm by RT-PCR.

2.2. Highly Pathogenic Avian Influenza (HPAI)

- **Etiology:** Avian Influenza A virus, Orthomyxovirus (H5N1) subtypes.

HPAI is a transboundary and zoonotic disease of major public health and economic importance.

- **Field diagnostic clues:** Per-acute deaths without premonitory signs, cyanosis of comb and wattles, facial edema, respiratory distress, nervous signs, and sudden drop in egg production.
- **Gross lesions:** Widespread petechial hemorrhages in visceral organs, pulmonary congestion and edema, hemorrhages in shank and breast muscles. As HPAI is a notifiable disease, suspected cases must be immediately reported and handled following national guidelines.

- **Field Diagnostic Kits:**

Kit: Influenza A / H5 / H7 Rapid Antigen Test

Principle: Lateral flow immunoassay detecting Influenza A nucleoprotein antigen.

Sample: Tracheal and cloacal swabs

Procedure: Swab → buffer → cassette → read in 5–15 minutes.

Interpretation: Positive indicates Influenza A virus presence.

Remarks: Does not differentiate LPAI/HPAI; immediate reporting mandatory

2.3. Infectious Bursal Disease (IBD / Gumboro Disease)

- **Etiology:** Infectious bursal disease virus. (*Birna Virus*)

IBD primarily affects young chickens between 3 and 6 weeks of age and is of great significance due to its immunosuppressive effects.

- **Field diagnostic clues:** Depression, ruffled feathers, vent picking, whitish diarrhea, and increased susceptibility to secondary infections.
- **Gross lesions:** Enlarged, edematous, and hemorrhagic *bursa of Fabricius* during early stages, followed by bursal atrophy in later stages, and hemorrhages in thigh and breast muscles.
- **Field Diagnostic Kits:**
 - Kit:** IBD Antigen / Antibody Rapid Test
 - Principle:** Immunochromatographic detection of viral antigen or antibodies.
 - Sample:** Bursa tissue / cloacal swab (antigen); serum (antibody)
 - Procedure:** Sample + buffer → cassette → read in 5–10 minutes.
 - Interpretation:** Antigen positive – active infection; antibody positive – exposure/vaccination.

Remarks: Antibody kits cannot differentiate vaccine from field infection.

2.4. Infectious Bronchitis (IB)

- **Etiology:** Corona virus belongs to the family *coronaviridae*.

IB is a highly contagious respiratory disease with multiple serotypes, making diagnosis and control challenging.
- **Field diagnostic clues:** Respiratory rales, sneezing, nasal discharge in young birds, poor weight gain, and in layers, production of misshapen, thin-shelled, or soft-shelled eggs.
- **Gross lesions:** Catarrhal tracheitis, cloudy air sacs, and in nephropathogenic strains, pale and swollen kidneys with urate deposition.
- **Field Diagnostic Kits:**
 - Kit:** IBV Rapid Antigen Test
 - Principle:** Lateral flow immunoassay detecting IB virus antigen.
 - Sample:** Tracheal swab
 - Procedure:** Swab → buffer → cassette → read in 5–10 minutes.
 - Interpretation:** Positive indicates IB virus involvement.
 - Remarks:** Serotype differentiation requires laboratory testing.

1.2. Marek's Disease

- **Etiology:** Caused by cell-associated DNA Herpes Virus

Marek's disease is a lymphoproliferative disease characterized by tumors and nerve involvement.

- **Field diagnostic clues:** Progressive paralysis of legs or wings, uneven flock mortality, weight loss, and poor response to antibiotic therapy.
- **Gross lesions:** Enlargement of peripheral nerves, presence of tumors in liver, spleen, gonads, and gray eye due to ocular lymphomatosis.
- **Field Diagnostic Kits:**

Kit: Portable PCR (no rapid antigen strip)

Principle: PCR amplification of Marek's disease virus DNA.

Sample: Feather follicle, nerve tissue, tumors

Procedure: Sample extraction → portable PCR → result in 30–90 minutes.

Interpretation: Positive amplification confirms MD virus presence.

Remarks: Requires equipment and trained personnel.

Diagnosis of Viral disease of Poultry

Diseases	Primary field signs	Hallmark Gross lesions	Diagnostic Kits
Avian Influenza (HPAI)	Sudden high mortality, cyanosis of comb/wattles, edema of the head.	Pinpoint hemorrhages (petechial) on visceral fat and proventriculus.	Rapid antigen tests (lateral flow assays) LAMP (Loop-mediated isothermal amplification)
Newcastle disease (ND)	Respiratory distress, greenish diarrhea, twisting of neck. (torticollis)	Petechial hemorrhages at the tips of proventricular glands. Hemorrhagic ulcers in the intestinal lymphoid patches. (Peyer's patches)	rapid antigen/antibody test kits and real-time PCR (qPCR) kits
Infectious bursal disease (IBD)	Ruffled feathers, vent pecking, depression in dead chicks.	Swollen, "grape like" or hemorrhagic <i>Bursa of Fabricius</i> .	Rapid antigen tests (lateral flow assays)/ ELISA kits/ RT-PCR kits
Marek's disease	Progressive paralysis (range paralysis), one leg forward one leg backward.	Enlargement of sciatic nerves, "Grey eyes" (iris discoloration).	LAMP (Loop-mediated isothermal amplification)
Infectious bronchitis (IB)	Sneezing, rales, watery egg whites, misshapen eggs.	Mucoid exudate in trachea, pale swollen kidneys (nephrotic strains)	ELISA kits (antibody detection)/ Real time RT-PCR kits

3. Field Diagnosis: Stepwise Approach

Field diagnosis of viral diseases requires a systematic and logical approach. Veterinary officers should begin with detailed history taking, including age of birds,

vaccination status, mortality pattern, production records, recent introduction of birds, and biosecurity practices. This should be followed by careful clinical examination of affected birds and flock-level assessment to determine morbidity, mortality, and uniformity of clinical signs.

2. Sampling for Viral Disease Diagnosis

Proper sample collection is critical for successful laboratory diagnosis. Samples should ideally be collected from birds in the early or acute stage of disease. Live birds or freshly dead birds are preferred, while autolysed carcasses should be avoided.

In viral poultry diseases, proper sample preservation and transport are critical because the viability of viruses depends heavily on maintaining the cold chain. To ensure both biosafety and sample integrity, the triple packaging system should always be followed. This includes placing the sample in a leak-proof primary container, such as a cryovial containing viral transport medium (VTM), which is then sealed inside a secondary leak-proof bag or plastic container with absorbent material. Both are finally placed in a tertiary insulated Styrofoam or thermocol box with frozen gel packs.

Disease suspected	Preferred samples
ND/ AI	Tracheal and cloacal swabs, lungs, spleen
IBD	<i>Bursa of Fabricius</i>
IB	Trachea, lungs, kidneys
Marek's disease	Peripheral nerves, tumors
Mixed infection	Trachea, spleen, liver

For field storage, samples should be refrigerated at 4°C for short-term storage (less than 24 hours), while long-term storage (more than 48 hours) requires deep freezing at -70°C or transport using dry ice; standard -20°C freezers should be avoided for virus isolation as they can damage the viral envelope.

In contrast, formalin-fixed tissue samples should be kept at room temperature and never frozen. Strict biosafety precautions must be followed in the field, including wearing appropriate personal protective equipment (gloves, mask, and apron), disinfecting instruments and footwear, avoiding farm-to-farm contamination, ensuring safe disposal of carcasses, and adhering to national guidelines for notifiable diseases.

While field diagnosis helps guide immediate action, laboratory confirmation using tests such as RT-PCR, virus isolation, ELISA, and histopathology is essential to confirm the causative agent, differentiate between similar viral diseases, support surveillance and outbreak control, and evaluate vaccination strategies effectively.

1. Conclusions:

Effective control of viral diseases in poultry begins at the field level. Accurate flock history, careful clinical examination, and recognition of characteristic gross lesions enable veterinary officers to arrive at a sound tentative diagnosis. However, definitive diagnosis and epidemiological surveillance depend on proper sample selection, aseptic collection, appropriate preservation, and timely transport to diagnostic laboratories. Strengthening field diagnostic and sampling skills among veterinary officers is therefore essential for early disease detection, outbreak containment, and sustainable poultry production

COLLECTION AND HANDLING: BLOOD, SWABS AND TISSUE SAMPLES OF POULTRY

Imran Ali, Sanjiv Kumar, Kaushal Kumar and Deepak Kumar

Department of Veterinary Pathology, Bihar Veterinary College, Patna

Introduction

Accurate laboratory diagnosis of poultry diseases depends largely on proper sample collection, handling, storage, and transport. Errors at any of these stages can lead to false-negative results, contamination, or sample rejection, ultimately compromising disease control programs.

Veterinary officers play a vital role in field-level sampling, especially during outbreaks of respiratory, enteric, septicemic, and immunosuppressive diseases. This section provides standardized guidelines for collection and handling of blood, swab, and tissue samples from poultry.

1. General Principles of Sample Collection

2.1 Selection of Birds

For disease diagnosis, freshly sick birds showing early clinical signs should be selected, as they provide the best quality samples. Decomposed carcasses and birds dead for more than 6–8 hours should be avoided due to tissue autolysis, except when tissues are immediately preserved for histopathology. For serological tests, samples should be collected from apparently healthy birds in affected flocks, as they had better reflect the immune status of the flock.

2.2 Aseptic Technique

Proper aseptic technique is essential to avoid contamination. Sterile instruments and containers must be used at all times. Gloves should be changed between birds, especially during outbreak investigations. Instruments should be flamed or disinfected between tissue collections to prevent cross-contamination and ensure reliable laboratory results.

2.3 Labeling and Documentation

Each sample must be clearly and correctly labeled with the farm/flock No., birds number, sample type, date and time of collection, and suspected disease. Accurate

labeling and documentation are crucial, as incomplete labeling is a common reason for laboratory rejection and delayed diagnosis.

2. Blood Sample Collection and Processing

Blood collection is an important part of disease diagnosis in poultry and is commonly done for serology, hematology, and molecular diagnostic tests. In most of the birds, the wing (brachial) vein is the preferred site for blood collection because it is easy to access and causes minimal stress to the bird. Blood samples are mainly used for serological tests, which help in detecting antibodies against diseases such as Newcastle disease (NDV), Infectious bronchitis (IBV), and Infectious bursal disease (IBD), and for molecular diagnostics, which are used to identify viral or bacterial DNA or RNA. Proper blood collection helps in accurate diagnosis and effective disease monitoring in poultry flocks.

3.1 Sites of blood collection

Wing vein	The most common site for adult poultry
Jugular vein	Preferred in small birds or chicks as it provides larger volume quickly
Heart Puncture	Generally reserved for terminal sampling or very small avian species (requires high skills to avoid immediate mortality)

3.2 Handling Procedures

Sample Goal	Container	Handling instructions
Serum	Plain tube (No anticoagulant)	Allow blood to clot at an angle for 1 -2 hrs. Do not refrigerate until the clot has retracted.
Whole blood	EDTA tube (Purple top)	Gently invert 8-10 times. Never freeze whole blood if meant for hematology. Keep at 4°C.in refrigerator
Blood spot	FTA cards	Apply 1-2 drops to specialized filter paper; allow to air dry. Excellent for molecular transit in heat.

4. Swab Sample Collection and Handling

Swab samples play a crucial role in the diagnosis of respiratory and enteric diseases in poultry. They are commonly used because they allow direct collection of pathogens from the site of infection. Dacron or nylon swabs are preferred, particularly for PCR-based tests, as they do not inhibit molecular reactions and help in accurate detection of pathogens. Swab samples are considered the gold standard

for monitoring respiratory viruses such as avian influenza (AI) and Newcastle disease virus (NDV), as well as enteric pathogens like Salmonella and Campylobacter. Proper collection and handling of swab samples greatly improve the reliability of laboratory diagnosis.

4.1 Types of Swabs

Oropharyngeal	ND, AI, IB
Tracheal	Respiratory pathogens
Cloacal	Enteric viruses, AI
Lesion swabs	Bacterial infections

4.2 Transport media

Purpose	Transport medium
Virology	Viral Transport medium (VTM)
Bacteriology	Stuart/ Amies medium
PCR	VTM or sterile PBS

4.3 Procedure and technique

During swab collection, the swab should be gently inserted into the required site to avoid injury to the bird. Once inserted, the swab should be rotated at the sampling site to ensure adequate collection of secretions and epithelial cells. Immediately after collection, the swab must be placed into the appropriate transport medium, and the stick should be broken carefully without contaminating the sample. For oropharyngeal (OP) swabs, the swab is inserted into the choanal slit at the roof of the mouth and into the trachea, and the mucosal surface is rubbed firmly to collect sufficient epithelial cells. For cloacal swabs, the swab is inserted into the vent and rotated firmly against the mucosal wall, and proper collection is indicated by visible fecal staining on the swab.

4.4 Storage and Transport

After collection, swab samples should be stored at 4°C if they are to be transported to the laboratory within 48 hours, as this helps preserve the viability of pathogens and the integrity of nucleic acids. For longer storage periods, especially for virological investigations, swab samples should be kept at -70°C to prevent degradation of viral particles and genetic material. Throughout storage and transport, it is essential to maintain a strict cold chain by using ice packs or insulated containers. Any break in

the cold chain can reduce sample quality and may lead to false-negative results, so careful handling during transport is critical for accurate laboratory diagnosis.

4. Tissue Sample Collection

For advanced and confirmatory disease diagnosis, tissue samples are collected during necropsy for multiple diagnostic purposes, including histopathology, bacteriology, virology, and molecular assays. Tissues must be collected for two distinct purposes: fresh or frozen tissues for microbiological and molecular diagnosis, and properly fixed tissues for histopathological examination. It is essential that all tissue samples are collected aseptically to avoid contamination and are preserved according to the specific diagnostic requirement. Proper tissue sampling helps in confirming the disease, identifying the causative agent, and understanding the tissue-level changes associated with infection.

Errors	Consequences
Improper labeling	Sample rejection
Hemolyzed blood	Invalid serology
Delayed transport	False negative results
Formalin contamination	PCR failure
No cold chain	Viral degradation

5.1 Histopathology

For histopathological examination, tissue samples should be fixed in 10% neutral buffered formalin to preserve normal tissue architecture. To allow proper and rapid penetration of the fixative, tissue pieces should be thin, ideally between 0.5 cm and 1.0 cm in thickness. A strict 10:1 ratio of formalin to tissue must be maintained; meaning the volume of fixative should be at least ten times the volume of the tissue sample. In cases of systemic disease, it is essential to collect and fix tissues from all crucial organs, including the brain, trachea, lungs, liver, spleen, kidneys, *Bursa* of Fabricius, and any visible lesions, as this increases the chances of reaching an accurate diagnosis.

5.2 Microbiology and PCR

For microbiological culture and PCR testing, tissue samples must be collected using sterile instruments to avoid contamination. Instruments should be changed or properly flamed between different organ systems so that organisms from one tissue do not contaminate another. After collection, samples should be placed in sterile Whirl-Pak® bags or other leak-proof sterile containers. For short-term storage and immediate transport to the laboratory, samples should be kept at 4°C. If transport is expected to take more than 48 hours, samples can be frozen at –20°C or –80°C for molecular tests; however, freezing should be avoided when bacterial culture is required, as some bacteria may be killed by freezing, leading to false-negative results.

1. Sample Handling, Storage and Transportation:

Proper storage and transport of samples are critical for accurate laboratory diagnosis, especially for virology and molecular testing, where sample integrity is highly sensitive to temperature. Maintaining a strict cold chain is essential, and this can be achieved by using ice packs, thermocol (insulated) boxes, and a triple-layer packaging system. In the triple packaging system, the primary container should be leak-proof and securely closed, the secondary container should be a sealed plastic bag to contain any leakage, and the outer container should be a rigid, well-labeled box to protect the samples during transit. All samples should ideally be delivered to the laboratory within 24 hours of collection. During disease outbreaks, it is important to inform the laboratory in advance before dispatching samples, so that timely processing and appropriate biosafety measures can be ensured.

2. Common Errors to Avoid

Purpose	Preservation
Histopathology	10% Neutral Buffered Formalin
Bacteriology	Sterile container, no preservative
Virology/PCR	Sterile container, chilled
Toxicopathology	Frozen, No preservative

7. Conclusions:

Accurate diagnosis of poultry diseases begins at the field level, where the quality of sample collection and handling plays a decisive role in laboratory outcomes. Proper selection of birds, aseptic collection of blood, swab, and tissue samples, and their preservation according to the intended diagnostic purpose are essential to prevent contamination and sample deterioration. Equally important are correct labeling, maintenance of the cold chain, and timely transport to the laboratory, which ensure that samples reach the diagnostician in optimal condition. When these steps are followed systematically, laboratory diagnostic accuracy is greatly improved, enabling early detection, effective disease control, and better flock health management in poultry production systems.

References:

1. OIE (2021). *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. World Organisation for Animal Health, Paris.
2. *Diseases of Poultry*. Saif, Y.M., Fadly, A.M., Glisson, J.R., McDougald, L.R., Nolan, L.K., & Swayne, D.E. (2019). *Diseases of Poultry (14th ed.)* Wiley-Blackwell, USA.
3. *Veterinary Clinical Pathology*. Stockham, S.L., & Scott, M.A. (2018). *Fundamentals of Veterinary Clinical Pathology (3rd ed.)* Wiley-Blackwell.
4. *Veterinary Diagnostic Virology*. Cunningham, C.O. (2010). *Veterinary Diagnostic Virology*. Cambridge University Press.

POULTRY FARM AND HATCHERY BIOSECURITY, DISINFECTION AND SANITATION

Anjay, Purushottam Kaushik, Seuli Saha Roy, Bhoomika and Sudha Kumari

Department of Veterinary Public Health & Epidemiology,
Bihar Veterinary College, Bihar Animal Sciences University, Patna

Introduction

Biosecurity, disinfection, and sanitation form the cornerstone of modern poultry production. In intensive poultry systems, infectious agents can spread rapidly due to high stocking density, shared equipment, frequent human movement, and close interaction between farms and live bird markets. Such conditions create ideal pathways for the introduction, amplification, and dissemination of pathogens within and between flocks.

Failures in biosecurity not only result in significant economic losses due to increased mortality, reduced productivity, and trade restrictions, but also pose serious risks to public health through zoonotic diseases and the emergence and spread of antimicrobial resistance. Inadequate sanitation and indiscriminate use of antimicrobials to compensate for poor hygiene further exacerbate these risks, compromising both animal and human health.

Effective biosecurity relies on a preventive, risk-based approach that integrates farm design, controlled access, hygienic management practices, and systematic cleaning and disinfection. Timely implementation of these measures enables early interruption of disease transmission, limits pathogen build-up in the production environment, and reduces the likelihood of outbreak escalation. Sustainable disease control in poultry production therefore depends on continuous adherence to biosecurity principles, trained personnel, and regular monitoring, aligning poultry health management with food safety and One Health objectives.

Concept and objectives of biosecurity

Biosecurity refers to an integrated set of management practices designed to prevent the introduction, establishment, and spread of infectious agents within and between

poultry farms and hatcheries.

The primary objective of biosecurity is to prevent the entry of pathogens into poultry production systems and to minimize the spread of infection within the farm once birds are placed. Effective biosecurity practices also aim to protect poultry workers and the surrounding community from exposure to zoonotic pathogens, thereby safeguarding public health. In addition, biosecurity ensures the production of safe and wholesome poultry products by maintaining high standards of hygiene throughout the production chain. By reducing disease incidence and pathogen load, biosecurity measures help limit the reliance on therapeutic antimicrobials, thereby contributing to antimicrobial stewardship and sustainable poultry production.

Basic principles of poultry biosecurity

Operational biosecurity in poultry farms is based on three fundamental principles: isolation, traffic control, and sanitation.

Isolation involves keeping birds physically separated from potential sources of infection, including other flocks, wild birds, animals, and contaminated environments. Traffic control refers to the regulation and monitoring of movement of people, vehicles, equipment, and materials within and between poultry farms to prevent mechanical transmission of pathogens. Sanitation focuses on systematic cleaning and disinfection of facilities, equipment, and surroundings to reduce the overall pathogen load in the production environment. These principles must be applied consistently and effectively at the farm level, shed level, and hatchery level to ensure comprehensive disease prevention and control.

Poultry farm and hatchery location and design

Poultry farms should be located away from other poultry farms, live bird markets, slaughterhouses, and water bodies frequented by migratory birds, which are significant sources of infection. Proximity to residential areas and main roads should be avoided to reduce unnecessary human traffic and environmental contamination. Ideally, farms should be at least 1–2 km away from other commercial facilities to minimize airborne and mechanical transmission of pathogens through people, vehicles, and equipment.

A secure boundary wall with controlled entry and exit points is essential for

biosecurity. Minimum perimeter security of 30 meters is recommended. Display SOPs and biosecurity signage in regional and local languages at every unit. Signs should indicate “Restricted Area” or “Visitors Not Allowed,” especially near breeding stocks and hatcheries. Single-window systems should be implemented for the sale of poultry and poultry products, with counters placed at the farm entrance to prevent commercial vehicles or clients from entering the farm premises.

The farm layout should follow a logical flow: brooder sheds → grower sheds → adult bird sheds, with a corresponding drainage system following the same direction. Roads and floors should be made of concrete to facilitate easy cleaning and disinfection. Poultry houses should have concrete floors and proper drainage to prevent water stagnation and reduce attraction of wild birds and pests. Distance between sheds: 30 ft between similar types and 100 ft between different types. Hatcheries should be located at least 500 ft from grow-out sheds.

Adequate ventilation and access to sunlight are crucial to reduce the buildup of infectious agents and stress from accumulated gases. The orientation of the long axis of sheds should consider climate: Cold regions: North–South; Hot and humid regions: East–West; Extremely hot summer regions: South–East.

Install bird-proofing nets in all units to prevent entry of feral birds. Prune or remove overhanging branches to avoid contamination from wild birds. Avoid dense foliage near poultry run areas. Cover open drains and remove potential roosting sites for wild birds. Use bird reflectors where necessary to discourage wild bird presence.

Hatcheries must have strict separation of clean and dirty areas with unidirectional workflow to minimize cross-contamination. Dedicated spaces should exist for egg receiving, fumigation, incubation, hatching, chick processing, and dispatch. Proper ventilation with filtered air supply is essential to maintain air quality and reduce microbial load.

Foot dips of uniform size must be installed at the entry of all sheds, preferably using a 50:50 mixture of lime and bleaching powder. Concrete flooring and well-planned drainage help in effective cleaning and sanitation.

A post-mortem facility near incinerators and a separate laboratory with trained manpower are recommended for disease monitoring and surveillance.

Demonstration sheds can be constructed near laboratory areas for farmer training, keeping training and residential facilities away from production sheds to maintain biosecurity.

Restricted access and movement control

Farms should have a single, controlled entry gate, and entry should be permitted only with the authorization of the farm manager or designated responsible person. Registers must be maintained to record the entry of visitors, vehicles, and materials to ensure traceability. Footbaths for personnel and wheel dips for vehicles containing effective disinfectants should be installed at the farm entrance and maintained regularly.

At the shed level, strict biosecurity measures are required to minimize cross-contamination between flocks. Poultry sheds should be kept locked when not in use to restrict unauthorized access. Separate boots, protective clothing, and equipment should be designated for each shed and should not be interchanged. Foot dips containing fresh disinfectant must be provided at both the entry and exit of every shed. Movement of personnel and equipment should always proceed from younger flocks to older flocks to reduce the risk of transmitting infections to more susceptible birds.

Control of vehicle movement within the farm premises is equally critical. Entry of external vehicles should be limited as much as possible, and all vehicles entering the farm must undergo thorough cleaning and disinfection of wheels, undercarriage, and cargo areas. Drivers should be restricted from entering poultry sheds under any circumstances. Feed, chick, and other essential delivery vehicles must strictly follow approved sanitation protocols to prevent mechanical transmission of pathogens.

Personnel hygiene and worker biosecurity

Farm workers must receive regular training on biosecurity principles to ensure awareness and compliance with established protocols. The use of farm-specific clothing, boots, and protective gear should be compulsory to prevent the introduction and spread of pathogens. In breeder farms and hatcheries, shower-in and shower-out practices are strongly recommended to further reduce biosecurity risks. Proper hand washing with soap or suitable disinfectants must be carried out before and after

entering poultry sheds. Workers should be strictly advised against keeping poultry at home or visiting other poultry farms, as such activities increase the risk of disease transmission. Regular medical check-ups of farm staff are also essential to safeguard worker health and to minimize the potential spread of zoonotic infections.

Cleaning, disinfection and sanitation

Disinfectants are largely ineffective in the presence of organic matter; therefore, thorough cleaning involving the removal of dirt, manure, feathers, and other debris must always precede disinfection. The use of appropriate detergents along with water under pressure is necessary to ensure effective removal of organic material from surfaces.

All farm equipment, including feeders, drinkers, trays, crates, and tools, should be cleaned on a daily basis. The recommended procedure involves washing with detergent, followed by rinsing with clean water, application of a suitable disinfectant, and complete drying before reuse. Sharing of equipment between farms should be strictly avoided, and wherever possible, plastic or metal equipment should be preferred over wooden materials, as they are easier to clean and disinfect effectively.

Poultry house sanitation can be categorized into terminal and partial cleaning procedures. Terminal or complete cleaning is carried out after the removal of a flock and involves the removal of litter, manure, feathers, and dust from the shed. All internal surfaces should then be thoroughly washed and disinfected using approved disinfectants, and fumigation may be carried out where required. Following terminal cleaning, the shed should be kept empty for a minimum period of ten days before restocking to allow effective downtime and reduction of residual pathogens.

Partial or concurrent cleaning is performed while birds remain in the house and focuses on maintaining hygiene without disturbing the flock. This includes regular removal of caked litter, cleaning of fans, vents, and equipment, and routine disinfection of feeders and drinkers. Sanitization of drinking water should be carried out regularly to maintain water quality and reduce the risk of waterborne infections.

Disinfectants: selection and use

The selection and proper use of disinfectants play a vital role in effective poultry farm and hatchery biosecurity. An ideal disinfectant should possess broad-spectrum

activity against bacteria, viruses, fungi, and protozoa and should remain effective even in the presence of limited organic matter. It must be non-toxic to birds and humans when used at recommended concentrations, easy to apply under field conditions, and economical for routine use without compromising efficacy.

Several classes of disinfectants are commonly used in poultry production systems. These include chlorine compounds, iodophores, quaternary ammonium compounds, and peroxygen compounds such as peracetic acid, which are known for their wide antimicrobial activity. Phenolic disinfectants may also be used in restricted situations, taking into account their limitations and safety concerns. Regardless of the type of disinfectant selected, it is essential to follow the manufacturer's recommendations regarding dilution, application method, and contact time, as improper use can significantly reduce effectiveness and compromise biosecurity.

Hatchery sanitation and biosecurity

Hatchery sanitation and biosecurity are critical for preventing the vertical and horizontal transmission of infectious agents within poultry production systems. Hatching eggs should be subjected to fumigation or appropriate disinfection soon after collection and before setting to reduce surface contamination and microbial load. Separate equipment must be used for setter and hatcher rooms to avoid cross-contamination between different stages of incubation.

Chick processing areas should be cleaned and disinfected daily to maintain high hygienic standards and ensure the production of healthy day-old chicks. Hatchery waste, including eggshells and infertile or unhatched eggs, should be disposed of promptly in a biosecure manner to prevent the buildup and spread of pathogens. In addition, regular microbiological monitoring of the hatchery environment is essential to assess sanitation effectiveness, detect potential contamination early, and support continuous improvement of hatchery hygiene practices.

Waste management and disposal

Poultry manure should be stored away from poultry sheds in designated areas to minimize contamination and odor problems. Before field application, manure should be composted or otherwise treated to reduce pathogen load. Access of wild birds, rodents, and other animals to stored manure must be prevented to avoid disease

transmission.

Dead birds should be removed from poultry sheds immediately to eliminate potential sources of infection and prevent contact with healthy birds. Disposal should be carried out using approved methods such as burial, incineration, or rendering, in accordance with local regulations. All dead birds must be treated as potentially infectious material, and appropriate biosecurity precautions should be followed during handling and disposal to protect both poultry workers and the farm environment.

Feed and water hygiene

Only good quality, pathogen-free feed should be used, and it must be protected from contamination by rodents, insects, and moisture during storage and handling. Feed bins and storage areas should be cleaned regularly to prevent spoilage, mold growth, and buildup of contaminants.

A continuous supply of potable water is essential for poultry health and performance. Drinking water should be sourced from safe supplies and maintained free from microbial and chemical contamination. Regular testing of water quality, along with routine water sanitation practices, should be carried out to ensure consistent water hygiene and to reduce the risk of waterborne diseases.

Record keeping and monitoring

Detailed records should be maintained for visitor and vehicle entry, cleaning and disinfection schedules, mortality rates and disease occurrence, vaccination and medication programs, feed and water testing results, and rodent and insect control activities. Proper documentation enables traceability of inputs and movements, facilitates auditing and compliance with biosecurity standards, and supports early detection and timely response to disease outbreaks, thereby strengthening overall flock health management.

Biosecurity during disease outbreaks

Affected sheds should be isolated at once, and all movement of birds, eggs, manure, equipment, and personnel from the affected area should be stopped. Disinfection procedures must be intensified, and enhanced personal protective measures should be implemented for all staff working in or near affected units. Any unusual increase

in mortality or signs of disease should be reported promptly to the appropriate veterinary and regulatory authorities. All actions during an outbreak must strictly follow national disease control guidelines to ensure effective containment, protect public health, and minimize economic losses.

Conclusion

Effective biosecurity, disinfection, and sanitation are continuous processes, not one-time activities. Success depends on commitment, training, discipline, and regular monitoring. When properly implemented, these measures significantly reduce disease risk, improve productivity, protect public health, and ensure sustainability of the poultry industry.

References

Department of Animal Husbandry and Dairying, Government of India. (2024). Poultry disease action plan 2024.

Government of India. (2019). General Guidelines for Biosecurity at Central Poultry Development Organizations (Basic Tenets can be applied to State poultry farms also).

World Organisation for Animal Health (WOAH/OIE). (2021). Terrestrial Animal Health Code: Chapter 6.5 — Biosecurity in poultry production.

Department of Agriculture, Water and the Environment, Government of Australia. (2020). Commercial poultry farm biosecurity manual.

Poultry Dairy Feed. (2020). Biosecurity in poultry farms.

TRANSPORTATION MORTALITY IN HEAVY WEIGHT BIRDS IN BIHAR SCENARIO

Sanjiv Kumar, Imran Ali, Deepak Kumar and Kaushal Kumar

Department of Veterinary Pathology, Bihar Veterinary College, Patna

Transportation of heavy weight birds such as commercial broilers, broiler breeders, turkeys, waterfowl, and other large poultry breeds is an indispensable component of modern poultry production systems. Birds are routinely transported from farms to processing plants, live bird markets, or between rearing units. Although unavoidable, transportation remains one of the most stressful events in the production cycle and is frequently associated with considerable morbidity and mortality if not properly managed. Transportation mortality thus represents a critical animal welfare issue as well as a significant economic loss to poultry producers and processors.

Transportation mortality encompasses deaths occurring during catching, loading, transit, unloading, and lairage prior to slaughter. Mortalities may be apparent, with birds found dead on arrival, or concealed, where birds succumb shortly after transport due to physiological damage incurred during transit. Heavy weight birds are particularly susceptible owing to their elevated metabolic heat production, limited mobility, increased body mass, and relatively compromised cardiopulmonary capacity.

In the context of Bihar, transportation mortality assumes greater importance due to the structural and environmental characteristics of the state's poultry sector. Poultry production in Bihar is largely dominated by small- to medium-scale farms, many of which are located in rural or peri-urban areas, often far from organized slaughterhouses or large urban markets. Consequently, birds frequently undergo long-distance transport under suboptimal conditions, thereby increasing exposure to multiple stressors. In many cases, birds are transported in non-specialized vehicles such as pickup vans or modified trucks with inadequate ventilation and poor crate design, further aggravating mortality risk.

Bird-related factors constitute a major determinant of transportation mortality. Heavy bodyweight and muscular conformation increase metabolic demand and impair

thermoregulation, predisposing birds to heat stress, circulatory compromise, and physical injury. Older birds and those with excessive fat deposition exhibit reduced tolerance to heat and stress, while birds with underlying respiratory, cardiovascular, or musculoskeletal disorders show diminished resilience during transport. These vulnerabilities are particularly relevant in Bihar, where limited access to routine veterinary screening may result in subclinically affected birds being transported without prior health assessment. Furthermore, the increasing use of fast-growing commercial broiler strains in the state has intensified the mismatch between body mass and cardiopulmonary capacity, making birds more prone to stress-induced mortality.

Environmental and climatic conditions play a decisive role in transportation mortality, especially in Bihar's subtropical climate. Summers are characterized by prolonged periods of high ambient temperature often exceeding 40°C, accompanied by high relative humidity, while the monsoon season brings persistent moisture and poor ventilation conditions. Such an environment severely restricts evaporative cooling, the primary mechanism of heat dissipation in birds. During summer transport, especially in poorly ventilated vehicles, birds rapidly develop hyperthermia, dehydration, and respiratory distress, leading to increased mortality. Conversely, during winter months, particularly in northern and riverine areas of Bihar, cold stress and hypothermia may occur when birds are transported without adequate protection, increasing metabolic demand and weakening already stressed individuals.

Handling and management practices during catching and loading significantly influence survival during transport. In Bihar, catching is often performed manually by untrained labor, sometimes during daytime hours to match market schedules. Rough handling, excessive compression of birds, and overcrowding in crates are common practices that lead to fractures, bruising, internal hemorrhage, and compromised respiration. Improper loading density further exacerbates stress, as overcrowded crates promote heat accumulation and suffocation, while under-filled crates allow excessive movement, resulting in falls and injuries, particularly on uneven rural roads. Transport infrastructure in Bihar adds another layer of complexity to transportation mortality. Although major highways have improved in recent years, many poultry-producing regions remain connected by rural roads with potholes, uneven surfaces, and heavy traffic congestion. These conditions increase journey duration, vibration

stress, and the likelihood of vehicular accidents. Several reported incidents in the state involving overturned poultry-loaded vehicles highlight the contribution of road safety issues to transport-related losses. Delays during transit and prolonged waiting times at markets or slaughter points further elevate stress, particularly during hot and humid conditions.

Physiologically, transportation stress activates the hypothalamic–pituitary–adrenal axis, leading to elevated corticosterone levels, increased metabolic rate, and redistribution of blood flow away from non-essential organs. In heavy weight birds, this stress response rapidly overwhelms thermoregulatory capacity. Heat stress induces excessive panting, leading to respiratory alkalosis, dehydration, and electrolyte imbalance. Peripheral vasodilation aimed at heat dissipation reduces central blood pressure, predisposing birds to circulatory shock. These effects are often exacerbated in Bihar due to prolonged exposure to high ambient temperatures and humidity during transport. Birds with pre-existing subclinical disease or poor nutritional status are particularly vulnerable and may die during transit or shortly after arrival.

Pathological findings in transport-related mortality typically reflect systemic hypoxia, circulatory failure, and trauma. Gross lesions commonly observed include congestion of visceral organs, pulmonary edema, ascites, fatty liver changes, and extensive muscle hemorrhages. Fractures of long bones and keel bone injuries are frequently encountered, especially when birds are handled roughly or transported on uneven roads. Histopathological examination often reveals myocardial degeneration, renal tubular necrosis, and pulmonary congestion, indicative of severe physiological stress and hypoxia.

Monitoring and assessment of transportation mortality remain limited in Bihar due to the largely unorganized nature of poultry transport. Pre-transport health checks and environmental risk assessment are not consistently practiced. During transport, real-time monitoring of temperature, humidity, and airflow is rarely implemented, and signs of distress such as excessive panting or prostration often go unnoticed until mortality occurs. Post-transport evaluation is generally restricted to visible dead-on-arrival counts, with limited documentation or analysis of trends, thereby hindering corrective interventions.

Preventive strategies for reducing transportation mortality in Bihar must be adapted to

local realities. Gentle handling practices and training of catching and transport personnel are critical to minimizing trauma. Transport scheduling should prioritize early morning or nighttime movement during summer months to reduce heat exposure. Improving ventilation in transport vehicles, even through low-cost modifications such as increased side openings and elevated crate placement, can significantly reduce thermal stress. Optimizing loading density, ensuring access to water until loading, and avoiding unnecessary transport delays are particularly important under Bihar's climatic conditions. During extreme weather events, emergency interventions such as stopping vehicles in shaded areas and enhancing airflow can prevent catastrophic losses.

From a welfare and regulatory perspective, transportation mortality in Bihar highlights the need for stronger implementation of national animal welfare guidelines and increased involvement of veterinary authorities in transport oversight. While regulations exist, enforcement and awareness remain limited, particularly among small-scale operators. Strengthening extension services, promoting awareness of welfare-friendly transport practices, and encouraging data recording and reporting can substantially improve outcomes.

Economically, transportation mortality imposes direct losses through bird deaths and indirect losses through carcass downgrading, reduced market value, and increased labor and logistical costs. For small and medium poultry farmers in Bihar, even modest transport losses can significantly affect profitability. Therefore, reducing transportation mortality is not only an animal welfare imperative but also a critical component of sustainable poultry development in the state.

In conclusion, transportation mortality in heavy weight birds is the result of complex interactions among bird physiology, environmental stressors, handling practices, and transport infrastructure. In Bihar, these factors are further intensified by climatic extremes, infrastructural limitations, and largely unorganized transport systems. Addressing transportation mortality in the state requires a combination of improved management practices, climate-adapted transport strategies, enhanced training, and stronger veterinary oversight. Through targeted interventions and welfare-oriented transport planning, significant reductions in mortality can be achieved, improving both animal welfare and economic sustainability of Bihar's poultry sector.

OUTBREAK INVESTIGATION IN POULTRY

Sumant Kumar¹ and ²Sanjiv Kumar

¹Venkys india limited, Manager technical, Bihar and Jharkhand,

²Bihar Veterinary College , Bihar Animal Sciences University, Patna-14

INTRODUCTION

Poultry farming is one of the fastest-growing sectors of animal husbandry; however, it is highly vulnerable to infectious disease outbreaks due to high stocking density, rapid turnover of birds, and continuous movement of people, equipment, and vehicles. Disease outbreaks in poultry cause severe economic losses through high mortality, poor growth rate, reduced feed efficiency, decreased egg production, condemnation of carcasses, and increased expenditure on treatment, vaccination, and biosecurity measures. In addition to economic losses, certain poultry diseases pose serious threats to public health and international trade.

In this context, **outbreak investigation** becomes a critical responsibility of veterinarians. Timely and systematic investigation enables early detection of disease, identification of causative agents, understanding of transmission dynamics, and implementation of effective control and preventive measures. A well-conducted outbreak investigation not only controls the current episode but also helps prevent recurrence in the future.

1. POULTRY DISEASE OUTBREAK

A **poultry disease outbreak** is defined as the occurrence of disease cases, morbidity, or mortality in a poultry flock at a level significantly higher than what is normally expected for a particular farm, geographic area, or time period. An outbreak may involve infectious, parasitic, nutritional, toxic, or management-related causes, although infectious diseases are most commonly responsible.

In poultry, outbreaks often spread rapidly due to close contact among birds and shared feed, water, and airspace. High-density housing and inadequate biosecurity further accelerate disease transmission.

Common Poultry Diseases Causing Outbreaks

Several infectious diseases are frequently responsible for outbreaks in poultry:

- **Avian Influenza (AI):** A highly contagious viral disease causing sudden high mortality, respiratory signs, and severe economic and public health consequences.
- **Newcastle Disease (Ranikhet Disease):** Characterized by respiratory, nervous, and digestive signs with high mortality in unvaccinated flocks.
- **Infectious Bursal Disease (IBD):** Affects young birds, causing immunosuppression and increased susceptibility to secondary infections.
- **Infectious Bronchitis:** A viral respiratory disease affecting growth, egg production, and egg quality.
- **Fowl Cholera:** A bacterial disease caused by *Pasteurella multocida*, often resulting in sudden death.
- **Salmonellosis:** Causes enteritis, septicemia, and zoonotic risk.
- **Coccidiosis:** A parasitic disease leading to diarrhea, poor weight gain, and mortality, particularly in young birds.

Signs Suggestive of a Poultry Outbreak

Certain warning signs strongly indicate the onset of an outbreak and demand immediate veterinary attention:

- Sudden or unexplained increase in mortality
- Sharp decline in feed and water intake
- Sudden drop in egg production or deterioration of egg quality
- Respiratory signs such as coughing, sneezing, nasal discharge
- Nervous signs including tremors, torticollis, or paralysis
- Diarrhea, ruffled feathers, and depression
- Rapid spread of illness within a shed or across multiple sheds

2. POULTRY OUTBREAK INVESTIGATION

Outbreak investigation in poultry is a structured epidemiological process undertaken to confirm the existence of an outbreak, identify the causative agent, determine the source and route of transmission, assess risk factors, and implement effective control and preventive strategies. The investigation integrates field observations, laboratory diagnostics, and epidemiological analysis.

Objectives of Poultry Outbreak Investigation

The major objectives of outbreak investigation include:

- Accurate identification of the etiological agent

- Determination of the source and mode of disease transmission
- Identification of management, environmental, or biosecurity risk factors
- Implementation of immediate control measures to limit disease spread
- Development of long-term preventive strategies
- Reduction of economic losses and protection of public health
- Fulfillment of legal obligations for reporting notifiable diseases

3. STEPS IN POULTRY OUTBREAK INVESTIGATION

Step 1: Preparation for Field Investigation

Preparation is the foundation of a successful outbreak investigation. Before visiting the farm, the veterinarian should collect preliminary information such as flock size, age, breed, production type (broiler, layer, breeder), vaccination history, feed source, water supply, and recent changes in management. Necessary arrangements should be made for personal protective equipment (PPE), disinfectants, necropsy tools, sample collection materials, and transport media. Proper preparation ensures biosecurity and prevents disease spread to other farms.

Step 2: Confirmation of the Outbreak

The next step is to confirm whether an outbreak truly exists. This is done by comparing current morbidity, mortality, and production parameters with historical farm records or standard benchmarks. Seasonal variations and normal background mortality should be considered. Confirmation avoids unnecessary panic and ensures that resources are appropriately utilized.

Step 3: Clinical Examination and Diagnosis

Affected birds should be carefully observed for clinical signs such as respiratory distress, diarrhea, nervous symptoms, or lameness. Representative sick and freshly dead birds should be selected for post-mortem examination. Gross lesions provide valuable clues regarding the probable disease. Appropriate samples including blood, tracheal swabs, cloacal swabs, feces, and organs (liver, spleen, bursa, lungs) should be collected aseptically and sent to the laboratory for confirmation.

Step 4: Case Definition and Case Identification

A **case definition** is formulated to standardize identification of affected birds. It is usually based on clinical signs, age group, time period, and location. Using this definition, cases are identified and recorded systematically. Line listing of affected birds or sheds helps in understanding the extent and pattern of the outbreak.

Step 5: Descriptive Epidemiology

Descriptive epidemiology involves analyzing the outbreak in terms of **time, place, and bird-related factors**. The time of onset, peak, and duration of the outbreak are evaluated to understand disease dynamics. Spatial distribution among sheds, houses, or neighboring farms helps identify focal points of infection. Bird-related factors such as age, breed, sex, and vaccination status provide insight into susceptibility and immunity gaps.

Step 6: Hypothesis Generation

Based on field observations and descriptive analysis, possible hypotheses are developed regarding the source of infection, mode of transmission, and contributing factors. These may include introduction of infected birds, contaminated feed or water, failure of vaccination, poor sanitation, rodent or wild bird exposure, or movement of personnel and equipment.

Step 7: Hypothesis Testing

Hypotheses are tested using laboratory diagnostic techniques. Molecular methods such as PCR, serological tests like ELISA or HI, bacterial culture and sensitivity tests, and environmental sampling help confirm the causative agent and transmission pathways. Laboratory results provide scientific validation of field findings.

Step 8: Control and Preventive Measures

Immediate control measures aim to limit further spread of disease and reduce losses. These include isolation of affected sheds, restriction of movement of birds, people, and vehicles, emergency medication or vaccination, and humane culling where necessary. Long-term preventive measures focus on strengthening biosecurity, improving sanitation, implementing proper vaccination schedules, ensuring quality feed and water, and controlling rodents, insects, and wild birds.

Step 9: Reporting and Communication

A comprehensive outbreak investigation report should be prepared, documenting findings, laboratory results, actions taken, and recommendations. Effective communication with farm owners is essential for compliance and future prevention. In cases of notifiable diseases such as Avian Influenza or Newcastle Disease, timely reporting to veterinary authorities is mandatory to initiate regional or national control measures.

CONCLUSION

Outbreak investigation in poultry is a vital component of veterinary public health and poultry disease management. A systematic, timely, and scientifically sound investigation enables early diagnosis, effective control of disease spread, prevention of future outbreaks, and minimization of economic losses. The role of the veterinarian as a disease investigator, advisor, and communicator is central to safeguarding poultry health, farmer livelihoods, and public health.

PARASITIC DISEASES OF POULTRY: PREVENTION & CONTROL

Pankaj Kumar¹, Sanjiv Kumar², Deepak Kumar²

¹Department of Veterinary Parasitology, ²Department of Veterinary Pathology,
Bihar Veterinary College, BASU, Patna-14

Parasitic infestations represent a significant and persistent challenge to poultry health, welfare, and productivity worldwide. These diseases are caused by a diverse range of organisms: including ectoparasites (external parasites) and endoparasites (internal parasites)—that live on or within the host bird, deriving sustenance at its expense.

The impact of parasitic diseases extends beyond direct illness and mortality. Subclinical infections, often overlooked, can lead to severe economic losses through reduced weight gain, decreased egg production and quality, impaired feed conversion efficiency, and increased susceptibility to secondary infections. Furthermore, parasites can act as vectors for other serious bacterial and viral pathogens, complicating disease management.

The main categories of poultry parasites include:

End-parasites: Primarily helminths (worms) and protozoa. Among these, coccidiosis, caused by *Eimeria* species, is arguably the most economically important parasitic disease in poultry production due to its ubiquity and potential for rapid, devastating outbreaks in intensive rearing systems.

Ecto-parasites: Such as lice, fleas, mites, and ticks, which cause irritation, feather damage, anemia, and stress

Effective control requires an integrated approach combining good biosecurity, proper housing and litter management, strategic anti-parasitic treatments, and, where available, vaccination. Understanding the life cycles, transmission routes, and clinical signs of these parasites is fundamental to implementing successful prevention and control programs, ensuring flock health, and safeguarding the economic viability of poultry operations.

GIT HELMINTHS.

About 100 helminths have been identified in both domestic and wild birds.

TRANSMISSION

Nematodes are transmitted horizontally by ingestion of infective eggs, larvae or infected intermediate hosts (e.g. insects, snails or slugs) depending on the species of the helminths. Eggs of many nematodes are resistant to low temperatures and disinfectants, but may be susceptible to heat and desiccation.

CLINICAL FEATURES

1. *Heterakis* sp., *Ascaridia* sp. and *Capillaria* sp. produce non-specific signs that include inactivity and unthriftiness, depressed appetite, retarded growth and death.
2. Other helminths like *Dispharynx nasuta* and *Tetrameres americana* inhabit the proventriculus and gizzard causing thickening and necrosis of mucosa.
3. Pathogenic tapeworms such as *Raillietina cesticulus* and *Davainea proglottina* are found in the small intestine causing mild and often non-fatal lesions, weight loss and decreased production.

POST MORTEM FEATURES

- Presence of helminths in the affected organ
- Wasted carcass
- Thickening and sometimes nodules in the intestine
- Lesions depend on the helminth responsible

DIAGNOSIS

- Clinical features
- Simple floatation technique for identification of helminth eggs in faeces
- Recovery of parasites from intestine at post mortem

TREATMENT AND CONTROL

- **Piperazine compounds:** Widely used against Ascarids in chicken and turkeys orally in feed or water.
- **Phenothiazine compounds:** Widely used against caecal worms.
- **Hygromycin B:** Incorporated into poultry feed for the control of Ascarids, caecal worms and *Capillaria* sp.
- **Coumaphos:** Administered via feed or water over a 10-14 day period for capillariasis.
- **Ivermectin:** Injected subcutaneously for the control of both hook and round

worms.

- **Other agents used include:** Pyrantel tartrate, mebendazole, levamisole, thiabendazole, hexachlorophen, oxfendazole, and nicosamide.

Summary of Parasitic Diseases Caused by Helminths in Poultry

Organ Affected	Name of Parasite	Intermediate Host	Symptoms	Lesions
Trachea	<i>Syngamus trachea</i>	None	Suffocation and emaciation	Red worms found in trachea.
Crop	<i>Gangylonema ingulvicola</i>	Beetle and cockroaches	Almost no signs	Worms under the mucosa
Proventriculus	<i>Disharynx nasuta</i>	Pill bug, sow bug, cockroaches, and grasshoppers	Diarrhoea and emaciation	Worms partly burrowed in the proventricular glands; haemorrhages
Gizzard	<i>Cheilospirura hamulosa</i> <i>C. spinosa</i> <i>Amidostromum anseris</i>	Beetles and grasshoppers	No symptoms, or loss of appetite and emaciation	Necrosis of lining of gizzard or haemorrhages; nodules in gizzard muscles
Small Intestine	<i>Ascaridia galli</i>	None	Diarrhoea, slow growth, mortality	Bunches of worms in small intestine; catarrhal enteritis
Small Intestine	<i>Raillietina tetragona</i> <i>R. echinobothrida</i> <i>R. cesticillus</i> <i>Davainea proglottina</i>	Ants (for <i>Raillietina</i> spp.) Beetle (for <i>D. proglottina</i>)	Weakness, convulsion, paralysis (cestodes) Reduced growth and egg production (cestodes)	Intestinal walls show granulomatous nodules (cestodes) Catarrhal or haemorrhagic enteritis
Caecum	<i>Heterakis gallinarum</i>	Cockroaches, beetles, and grasshoppers	Slight emaciation	Inflammation of the caeca

PROTOZOAN DISEASES OF POULTRY:

Among the protozoan diseases of poultry, only coccidiosis is the most important one. Others are only seen occasionally. They include:

1. Coccidiosis
2. Histomoniasis
3. Trichomoniasis
4. Hexamitiasis

5. Avian malaria
6. Leucocytozoon infection
7. Toxoplasmosis

AVIAN COCCIDIOSIS

A parasitic protozoan disease of birds primarily affecting the GIT. As soon as there is favourable condition that favours its multiplication (e.g., high temperature and moisture) beyond a certain threshold the disease is precipitated. It has a rapid infection process of 4–7 days. Poultry coccidia are known to be host-specific, and different species parasitize specific parts of the intestine.

ETIOLOGY:

It is caused by *Eimeria* species of family *Eimeriidae* and order *Eucocciida*. Some species of importance include

TRANSMISSION:

- Ingestion of relatively large number of sporulated oocysts by a susceptible host.
- Oocyst may be mechanically transmitted via feed, water, and personnel.

CLINICAL AND P.M FEATURES

E. tenella

Primarily affects the ceca resulting in bloody fecal droppings, accumulated blood in the ceca on P.M. Ceca core—which is an accumulation of clotted blood, tissue debris, and oocyst—may be found in birds surviving the acute stage.

E. necatrix

Affects the anterior and mid portions of the small intestine manifesting as: dehydration, white and reddish spots scattered on the intestinal serosa, thickening of the intestinal wall, there may be blood, mucus, and fluid in the intestinal lumen. With the infected area dilated 2–2.5 times the normal diameter. Although the damage is usually in the small intestine, the oocyst are found only in the ceca since the sexual phase is completed in the ceca.

E. acervulina

Often produces chronic disease, poor growth with low mortality, gray or whitish patches in upper half of the small intestine.

E. brunetti* and *E. mitis

These produce lesions in the lower small intestine, rectum, ceca, and cloaca. In severe infections with *E. brunetti*, there is extensive coagulative necrosis and sloughing of the mucosa throughout most of the small intestine.

E. adenoides* and *E. gallopavonis

Primarily affect the ileum, cecum, and the rectum, poor growth with low mortality, thickening of intestinal wall, caseous cast in the intestines of turkeys.

E. meleagridis* and *E. dispersa

Lesions are produced in the upper small intestine of turkeys. General signs associated with this disease are: reduced feed intake, weight loss, dropiness, ruffled feathers, severe diarrhoea, and variable mortality.

DIAGNOSIS

- Demonstration of oocysts in faeces or intestinal scrapping.
- Clinical and post mortem features.

T R E A T M E N T A N D C O N T R O L

Oral medications administered through drinking water are more readily used:

- Amprolium and amprolium plus ethopabate combination.
- Sulfonamides
- Furazolidone
- Pyrimethamine plus sulphoquinoxaline
- Vitamin and antibacterial medications improve recovery rate
- Though complete prevention of infection may not be feasible, raising birds on wire floors minimize the occurrence of the disease.

Other control measures include:

- Improved hygiene measures, use of proper waterers and feeders to prevent entry of faeces into them, proper litter management to ensure dryness especially during the rainy season.
- 5–7 kg of lime powder may be sprinkled and mixed with moist litter covering 100 ft². The lime withdraws moisture and generates enough heat to kill the oocyst and other parasitic eggs or even bacteria.
- Recently, attempts have been made to produce vaccines against coccidiosis. A live attenuated vaccine containing oocysts of *E. acervulina*, *E. maxima*, *E. mitis* and *E. tenella* was found to be successful in the UK

MITES INFESTATION:

Mites are like ticks but smaller in size and they are also said to transmit some diseases. There are different classes of mites in poultry. They include:

- **Red mites** e.g., *Dermanyssus gallinae*. Nymph and adults feed on blood at night causing anaemia, reduced growth, egg production and death in heavy infestation.
- **Feather Mites** e.g., *Syringophilus bipectinatus*. Found in quills of feathers resulting in loss of feathers, itching of skin and fall in egg production. *Knemidocoptes gallinae*. Enter into shaft or roots of feathers causes depiluming, itching of skin.
- **Scaly leg mites** e.g., *Knemidocoptes mutans*. It burrows into epidermal portions of the leg and unfathered parts of the skin, causing exudations that makes the parts affected to become thick.
- **Air sac mites** e.g., *Cytodites nudus*. They are very tiny mites of about 0.5mm size and found in bronchi, lungs or air sacs of poultry, and other domestic animals. They are considered to be non-pathogenic.



बिहार पशु विज्ञान विश्वविद्यालय पटना-800014, बिहार

नामांकन नोटिस

बिहार पशु विज्ञान विश्वविद्यालय, पटना के अधीन बिहार पशु चिकित्सा महाविद्यालय, पटना एवं संजय गाँधी गव्य प्रौद्योगिकी संस्थान, पटना में शैक्षणिक सत्र 2025-2026 में नये पाठ्यक्रम शुरू किए जा रहा है, नामांकन हेतु विवरणी निम्नवत् है:-

बिहार पशु चिकित्सा महाविद्यालय, पटना

कोर्स का नाम	अवधि
बी.एस.सी. (पोल्ट्री प्रोडक्शन)	3 वर्ष (6 सेमेस्टर)

पैरा वेटेनरी साइंसेज

कोर्स का नाम	अवधि
डिप्लोमा इन वेटेनरी एंड लाइवस्टॉक डेवलपमेंट (डी.वी.एल.डी.)	2 वर्ष (4 सेमेस्टर)
डिप्लोमा इन वेटेनरी लेबोरेटरी टेक्नोलॉजी (डी. वी. एल. टी.)	2 वर्ष (4 सेमेस्टर)
सर्टिफिकेट कोर्स इन आर्टिफिशियल इन्सेमिनेशन	3 माह

पोस्ट ग्रेजुएट डिप्लोमा

ऑनलाईन – वेटेनरी होम्योपैथी, एथ्नोवेटेनरी मेडिसिन, वन हेल्थ,
ऑफलाईन – बोवाइन क्लिनिकल प्रैक्टिस, कैनाइन एंड फेलाइन क्लिनिकल प्रैक्टिस ।

एडवांस ट्रेनिंग कोर्स ऑन इम्पोर्टेंट वेटेनरी क्लिनिकल प्रोसीजर
अवधि: 3 सप्ताह, प्रवेश क्षमता: 6

सर्टिफिकेट कोर्स

वेटेनरी फॉरेंसिक साइंस, सीमन हैंडलिंग एवं आर्टिफिशियल इन्सेमिनेशन, मॉलिक्यूलर डायग्नोसिस ऑफ इन्फेक्शस डिजीजेस,
वेटेनरी डायग्नॉस्टिक इमेजिंग, एम्ब्रायो ट्रांसफर टेक्नोलॉजी (आईवीएफ) इन बोवाइन।

ऑनलाइन पाठ्यक्रम

फीड एवं फॉडर टेक्नोलॉजी पर ऑनलाइन शार्ट कोर्स
प्रसार एवं उद्यमिता विकास पर ऑनलाइन शार्ट कोर्स

संजय गाँधी गव्य प्रौद्योगिकी संस्थान, पटना

कोर्स का नाम	अवधि
बी.टेक. (एफ.टी.)	4 वर्ष (8 सेमेस्टर)

प्रवेश क्षमता, अवधि एवं सभी कोर्स की विस्तृत जानकारी हेतु विश्वविद्यालय की वेबसाइट
www.basu.org.in पर अपलोड किए गए विवरण पुस्तिका को देखें।


Training Program on "Advanced Disease Diagnosis in Poultry"
(19 to 23 January, 2026)



प्रसार शिक्षा निदेशालय, बिहार पशु विज्ञान विश्वविद्यालय, पटना-14


Bihar Animal Sciences University Social Networking Platforms

 **Facebook:** www.facebook.com/basu.org

 **X (formerly Twitter):** <https://x.com/basupatna>

 **Instagram:** <https://www.instagram.com/basupatna>

 **LinkedIn:** <https://www.linkedin.com/in/biharasu>

 **YouTube:** <https://youtube.com/@basupatna>

**Department of Veterinary Pathology, BVC
Directorate of Extension Education
Bihar Animal Sciences University, Patna-14**