



## A Review on *Chlamydiales* in Indian Poultry and its Zoonotic Transmission

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**ABSTRACT:** This paper provides a comprehensive review of the *Chlamydiales* order, focusing on its prevalence in Indian poultry and the implications for zoonotic transmission to humans. The review examines the epidemiology of Chlamydial infections in various poultry species across India, highlighting the factors contributing to their spread. It discusses the clinical manifestations of these infections in both avian hosts and humans, underscoring the public health risks associated with zoonotic transmission. Additionally, the paper evaluates current diagnostic methods, preventive measures, and control strategies employed in poultry management. Through a synthesis of existing literature and field studies, this review aims to enhance understanding of *Chlamydiales* in Indian poultry and promote awareness of the potential health risks posed to humans, ultimately contributing to improved biosecurity practices in the poultry industry.

**Keywords:** *Chlamydiales*, Zoonotic transmission, Public health, Biosecurity, Indian poultry.

### INTRODUCTION

*Chlamydiales* infection in poultry is an important issue in poultry production and control programs because of its economic importance and public health concern. Although the prevalence of *Chlamydiales* in poultry in India is largely unknown, its zoonotic potential necessitates the study of these bacteria in poultry. The rural farming community largely practices backyard farming, which increases the rate of zoonotic transmission, due to unawareness among the rural people, and the area having high population of livestock, rich in wild animals that spread disease through close interaction with humans and livestock. Poultry can be a source for the transmission of zoonotic agents to humans, and people encountering them are the potential victims of such infections (Ravichandran *et al.*, 2021; Karthikeyan *et al.*, 2021; Stokes *et al.*, 2021). *Chlamydiales* are obligate intracellular pathogens with a unique cell wall lacking muramic acid, which leads to their failure to get stained with Gram's stain, but they are antigenically different and are studied as inclusions forming agents. They have high seroprevalence even in healthy or symptom-free birds, with recurrent outbreaks. In poultry and even in other species, clinical signs of disease may be either systemic or local with high mortality or morbidity. We address

epidemiological and clinical aspects of *Chlamydiales* in poultry to present the contemporary position on the role of birds as carriers of chlamydiosis threatening humans and to reflect on preventive measures that refer to such potentially hazardous sources (Stokes *et al.*, 2021; Ravichandran *et al.* 2021; Abd El-Ghany, 2020). In 2016, (Guo *et al.*, 2016) highlighted the prevalence of *Chlamydia gallinacea* as the endemic chlamydial species in chickens, distinguishing it from the more commonly known *C. psittaci*. Their research employed PCR techniques to analyze samples from oral and cloacal swabs, revealing a significant correlation between the isolation of *C. gallinacean* from domestic poultry and instances of atypical pneumonia among slaughterhouse workers. This study underscores the zoonotic implications of avian chlamydiosis and marks a critical step in understanding the epidemiology of *Chlamydia* spp. in poultry. Building on this foundation, (Ravichandran *et al.*, 2021) conducted a comprehensive review of avian chlamydiosis, emphasizing the complex etiology of this neglected zoonotic disease and elucidated the unique biphasic life cycle of *Chlamydiaceae* and identified *C. psittaci* as the primary zoonotic agent, while also recognizing the potential zoonotic risks posed by *C. gallinacean* and other species such as *C. avium*. Their findings highlight the

fecal-oral and vertical transmission routes among poultry, reinforcing the need for further genomic studies to better understand the virulence and evolutionary dynamics of these pathogens.

Stokes *et al.* (2021) expanded the discussion by reviewing chlamydial infections in wild birds, detailing the genetic variability of *C. psittaci* and its association with various avian species. The study documented the emergence of *C. gallinacean* in both domestic and wild birds, alongside other newly described Chlamydia species. This research points to the zoonotic significance of these emerging strains and the potential for cross-species transmission, emphasizing the need for vigilant monitoring of Chlamydia spp. in both wild and domestic avian populations. Further contributing to the understanding of *C. gallinacea*, (Szymańska-Czerwińska *et al.*, 2021) investigated the pathogenicity of this strain through experimental inoculation of chicken broilers. Their findings revealed that *C. gallinacea* primarily infects poultry and can lead to asymptomatic shedding, it also has the potential to induce reduced body-weight gains in infected birds. Importantly, Dustin *et al.* (2023) reported an outbreak of atypical pneumonia in slaughterhouse workers, reinforcing the plausibility of zoonotic transmission. This study highlights the urgent need for more comprehensive research to assess the zoonotic risks associated with *C. gallinacea* and its genetic diversity.

Together, these reported studies create a fine understanding of Chlamydiales in poultry, emphasizing the intricate relationships between avian hosts and the zoonotic risks posed by these pathogens. The ongoing research in this field is vital for developing effective monitoring and control strategies to mitigate the transmission of Chlamydia spp. to humans. The exploration of Chlamydiales, particularly in the context of Indian poultry and its zoonotic transmission, has garnered significant attention in recent years. This literature review synthesizes key findings from a selection of pivotal studies that illuminate the epidemiology, pathogenicity, and zoonotic potential of various Chlamydia species in avian populations.

#### A. Related work

The article titled "*Chlamydia gallinacean*, not *C. psittaci*, is the endemic chlamydial species in chicken (*Gallus gallus*)" by (Guo *et al.*, 2016) provides significant insights into the prevalence and implications of Chlamydia species in poultry, particularly focusing on the endemic nature of *C. gallinacea* and its potential zoonotic transmission to humans. The study employs PCR methodologies to assess the presence of Chlamydia spp. in oral and cloacal swabs, utilizing the Chi-Square Test for statistical analysis. This methodological rigor enhances the credibility of the findings, which reveal a concerning prevalence of chlamydial infections in domestic poultry.

One of the key contributions of this article is the identification of *C. gallinacea* as the predominant species in chickens, challenging the previously held notion that *C. psittaci* was the primary concern in avian populations. This distinction is crucial as it shifts the focus towards understanding the specific epidemiology and zoonotic potential of *C. gallinacea*, which has not been as thoroughly studied as *C. psittaci*. The authors highlight the importance of recognizing this endemic species to inform public health strategies and mitigate zoonotic risks associated with poultry handling.

The article also draws attention to the implications of chlamydial infections in slaughterhouse workers, linking the isolation of *C. gallinacean* with cases of atypical pneumonia. This connection underscores the potential for zoonotic transmission and emphasizes the need for surveillance and preventive measures in environments where humans interact closely with infected poultry. Furthermore, the characterization of acid-base abnormalities in pigs infected with Chlamydia suis provides a broader context for understanding the mechanisms of zoonotic transmission, suggesting that similar pathways may exist for avian chlamydiosis.

Additionally, the introduction of new real-time PCR tests for species-specific detection of *Chlamydomphila psittaci* and *Chlamydomphila abortus* is a noteworthy advancement in the field. These diagnostic tools are essential for monitoring and managing zoonotic risks, particularly in markets where adult chickens, ducks, and pigeons are sold. The reported seroprevalence of *C. psittaci* in these populations serves as a stark reminder of the zoonotic potential of chlamydial infections and the importance of rigorous health monitoring in poultry. The article titled "A comprehensive review on avian chlamydiosis: a neglected zoonotic disease" by Ravichandran *et al.* (2021) provides an in-depth examination of the Chlamydiaceae family, particularly focusing on its implications for avian health and zoonotic transmission. The authors effectively outline the taxonomic classification of this family, highlighting the obligate intracellular nature of these gram-negative bacteria, which possess a unique biphasic life cycle. This classification is critical for understanding the pathogenic mechanisms of Chlamydia species, particularly in the context of poultry.

Central to the article is the discussion of *Chlamydia psittaci* as the primary agent responsible for avian chlamydiosis, which poses significant zoonotic risks. The authors emphasize the importance of recognizing other species, such as *C. avium* and *C. gallinacea*, which contribute to the complex etiology of the disease. *C. gallinacean* is particularly noteworthy as it is commonly found in chickens and exhibits both feco-oral and vertical transmission routes. The documented zoonotic potential of this pathogen is underscored by the correlation between cases of atypical pneumonia in

slaughterhouse workers and the isolation of *C. gallinacean* from infected poultry in France, illustrating the direct implications for public health.

The authors also point out the relatively unexplored zoonotic potential of *C. avium*, indicating a gap in current research that warrants further genomic studies. This aspect is crucial, as understanding the virulence and evolutionary dynamics of these pathogens can inform both veterinary and human health strategies. The call for more comprehensive genomic investigations is a significant takeaway, as it highlights the need for ongoing research to better understand the zoonotic transmission pathways and the potential risks associated with different Chlamydia species.

The article titled "A Review of Chlamydial Infections in Wild Birds" by Stokes *et al.* (2021) provides a comprehensive overview of the Chlamydia species affecting avian populations, with particular emphasis on the genetic variability and host associations of these pathogens. The authors highlight *C. psittaci* as the most extensively studied avian Chlamydia species, noting its isolation from a diverse range of wild bird species. This species is characterized by its genetic diversity, with genotypes A through F and E/B primarily associated with avian hosts, while genotypes M56 and WC have been identified in mammals.

A critical evaluation of the material reveals that the article effectively consolidates existing knowledge regarding the genotypic classification of Chlamydia within avian populations. The authors provide valuable insights into the epidemiology of *C. psittaci*, particularly its prevalence in feral pigeons (genotypes E and B) and its broader distribution among various avian taxa, including parrots and passerines (genotype A). This information is crucial for understanding the potential reservoirs of Chlamydiales in wildlife, which can have implications for zoonotic transmission to domestic poultry and humans.

Moreover, the identification of newly described Chlamydia species such as *C. gallinacea*, first reported in domestic chickens, and *C. avium*, isolated from wild Columbiformes, underscores the emerging nature of chlamydial infections in birds. The article also touches upon the presence of other related families within the Chlamydiales order, referred to as 'Chlamydia-related bacteria' (CRB), which further complicates the landscape of avian chlamydiosis and its potential zoonotic implications.

However, while the review is thorough in detailing the genetic and ecological aspects of Chlamydia in wild birds, it could benefit from a more explicit discussion on the implications of these findings for poultry health in India. The article does not address the direct link between wild bird populations and the transmission of Chlamydia to domestic poultry, which is a critical aspect of the zoonotic transmission narrative. Additionally, a comparative analysis of the prevalence

and impact of these infections in Indian poultry, as well as the specific genotypes circulating within that context, would enhance the relevance of the article to the topic at hand.

The article titled "Experimental inoculation of chicken broilers with *C. gallinacean* strain 15-56/1" by Szymańska-Czerwińska *et al.* (2021) provides a significant contribution to the understanding of avian chlamydiosis, particularly focusing on the *Chlamydia gallinacea* strain and its implications for poultry health and zoonotic potential. The authors highlight that *C. gallinacean* primarily infects poultry species, including chickens, ducks, guinea fowl, and turkeys, and has also been identified in wild birds. This broad host range underscores the relevance of studying this pathogen in the context of both domestic and wild avian populations.

A critical aspect of the article is its discussion on the pathogenicity of *C. gallinacea*. The authors note that while field studies have predominantly reported asymptomatic shedding in birds, experimental inoculations have demonstrated adverse effects such as reduced body-weight gains in broilers. This observation is crucial as it suggests that *C. gallinacean* can impact poultry production, which is a significant concern for the poultry industry. Furthermore, the study mentions mortality in embryonated chicken eggs following inoculation with various *C. gallinacean* strains, indicating the potential lethality of this pathogen under specific circumstances.

The article also addresses the zoonotic transmission potential of *C. gallinacea*, which is particularly relevant for public health. The authors provide evidence of a possible zoonotic risk, citing an outbreak of atypical pneumonia in slaughterhouse workers who were exposed to infected poultry. This finding raises important questions about the transmission dynamics of *C. gallinacean* between birds and humans, suggesting that further research is warranted to assess the full extent of its zoonotic capabilities.

Moreover, the genetic diversity of *C. gallinacean* strains reported in the study adds another layer of complexity to understanding this pathogen. The identification of different strains suggests that regional variations may exist, which could influence the epidemiology and control measures for avian chlamydiosis. This genetic variability may also have implications for vaccine development and the effectiveness of control strategies in poultry farming.

## OVERVIEW OF CHLAMYDIALES BACTERIA

Chlamydiales is an order of a unique group of bacteria that multiply by intracellular parasitism and cause diseases in quite diverse vertebrate hosts, including humans. They are phylogenetically and ecologically diverse and share the same signature of asexual developmental cycle with a unique "di-twist"

alternating infectious biphasic form, the elementary body (EB), and the vegetative noninfectious reticulate body (RB) (Collingro *et al.*, 2020; Sixt, 2021). Combined with being fastidious in terms of nutrient requirements, a high rate of genetic recombination, and an inability to perform transformation or in vitro cell culture expansion of chlamydial isolates, the study and investigation of these bacteria have become very challenging. From the microbiology perspective, the rate of Chlamydiales research has accelerated significantly with the large number of whole-cell genome analyses and the development of genetic manipulations in some chlamydial species in recent years (Collingro *et al.*, 2020; Chen *et al.*, 2020). The taxonomy of the order Chlamydiales expanded rapidly after the recognition of various bacteria as obligate intracellular parasites. The order is divided by the chosen reference chromosomes into six families and currently has 28 genera, including 106 recognized species (Ahmad *et al.*, 2020). However, the genomic or host range diversity of members in the order is far from acceptable and is expected to increase as new Chlamydiales and new strains are discovered. All the Chlamydiales possess a significant number of predicted or hypothetical type III secreted effectors that play essential roles in bacterial pathogenesis, an invariable genome reduction, and an obligate intracellular developmental cycle, which may explain why attempts to establish the order Chlamydiales as a single genus or even united with the family Parachlamydiaceae have not been successful (Pereira *et al.*, 2024).

### **CHLAMYDIALES IN POULTRY: PREVALENCE AND IMPACT**

Chlamydiales are a group of Gram-negative, obligate intracellular bacteria. They occur in many mammals, birds, reptiles, and crayfish, including humans, as well. These bacteria in poultry have successively been detected in specimens worldwide in many studies. These infections reduced productivity and increased the case fatality rates in broilers and were involved in the complex etiology manifesting with a variety of clinical illness symptoms including chronic respiratory diseases and some forms of fatal fowl typhoid, such as septicemia, dropsy syndrome, crusty blue comb, or swollen head, in respective age classes. They cause symptoms of inflammation, skin thickening, and necrosis of the liver, and induce immune reactions in infected bodies, which caused white and keloid-like scars that slowly resolved, with calcification also seen in musculature (Marchino *et al.*, 2022; Stokes *et al.*, 2021).

Chlamydiales have been detected in all types of poultry like chickens, ducks, and turkeys maintained in free range, scavenging, as well as confined and in battery or colony cages. Regarding a comprehensive study of commercial chickens in the economically hindered,

semi-developed nation representative poultry-producing region in the subcontinent, our data had ample value validating the magnitude of potential zoonotic transmission of these bacterial agents from there to humans and for the delivery of evidence-based strategies for designing national One Health level controllers or eradicators for agriculturalists, especially in chicken farming, cattle farming, other large and companion animals, and breeders, the general public, hen collectors, and other poultry workers (Abd El-Ghany, 2020; Ravichandran *et al.*, 2021; Stokes *et al.*, 2021). Accompanying this report of poultry (which may carry strains also infecting humans) and drug resistance, the above example of statistical data of bacterial agents indicates that there is likely an epidemiological gap if only human disease databases are reported. In conclusion, scientific curiosity is increasing day by day in dealing with Chlamydia research in poultry. There is no signal of a low moving trend in the work of poultry dealing with chlamydial infections soon, as the field has, for a very long time, registered progressively expanded contributions beyond the four corners of our transaction house (Weng *et al.*, 2020). Moreover, recent studies have already shown that the zoonotic transmission of chicken-related Chlamydia has reached humans across different categories of occupational livelihood. This all underlines how we can hasten bidirectional global consolidation by addressing the problem of dealing with and monitoring chicken chlamydiosis and the interrelation backward.

### **ZOONOTIC POTENTIAL OF CHLAMYDIALES IN POULTRY**

Generally, Chlamydiales have been identified in birds, reptiles, and mammals, including livestock and companion animals. In the farming environment, the possibilities of interspecies interconnection can be very frequent. It is feasible for poultry to infect humans by way of direct contact with fecal matter, poultry, and the working environment. The main hosts reported for these bacteria are poultry, with their prevalence varying from country to country (Diaz *et al.*, 2022). Morphologically, Chlamydia and Chlamydia-like organisms have similar, if not identical, within-host developmental life cycles characteristic of obligatory intracellular bacteria, which are typically surrounded by two membranes and a modified peptidoglycan cell wall. It is very challenging to differentiate between these two organisms regarding immunological responses, immune modulatory reactions, and disease progression (Gitsels *et al.*, 2020). In humans, antibodies produced against Chlamydia were reported to cause many diseases such as pneumonia, meningitis, respiratory issues, cardiac problems, neurological disorders, and genital tract inflammation. The organisms are released from the infected cell by either lysis or extrusion (Rodrigues *et al.*, 2022).



Direct contact is the primary mode of transmission of *C. psittaci* from birds to humans, who can get infected even by inhaling the internal aerosols from infected birds (Hosseini, 2022). Other methods of transmission include urine, feces, or other infected material touching the mouth, skin, and conjunctivae, which instigate the onset of chlamydial diseases in humans (Venkatajothi, 2021). All the observations made suggest that the infection is not uncommon, although its occurrence might be underreported and awareness of the potential threat is necessary. Due to the high potential of *C. psittaci* along with other *C. spp.* for zoonotic transmission in humans, monitoring at the poultry industry level is significant (Marchino *et al.*, 2022). Additional research is essential to find the exact zoonotic potential of the reported bacteria from poultry. Various awareness programs should be organized to educate poultry industry workers and consumers about the zoonotic risks associated with *C. spp.* in poultry products. Management of zoonotic infections might be associated with several challenges due to their nonspecific clinical signs, and therefore, early diagnosis might be difficult. Openness and immediate reporting of the infection, along with the consolidation of biosecurity measures, can be practiced to minimize the zoonotic transmission of Chlamydiales from poultry. The impact of the occurrence of these bacteria in the poultry sector on public health is not known. A lack of information regarding Chlamydiales and their zoonotic transmissions to humans in the context of the poultry industry needs to be addressed. Information regarding the pathogenic potential of Chlamydiales in poultry for humans, potential hosts of Chlamydiales in poultry, and direct and indirect ways of Chlamydiales transmission from poultry to humans is discussed.

### TRANSMISSION ROUTES OF CHLAMYDIALES IN POULTRY

The spread of Chlamydiales in mammalian hosts is poorly understood, but even less so in avian hosts. Most species of Chlamydiales can infect more than one genotype, but not all. Little is known about how Chlamydiales are transmitted within avian populations, although there is more information available for mammals.

In poultry, there are several ways to infect animals. The direct route is dominated by infected, orally shedding animals, direct beak-to-beak contact, parent to offspring contact during laying of infected eggs, or via oral shedding of fertile eggs during hatching. The indirect route is particularly important in hatch episodes and occurs through the respiratory tract by inhalation of infected airborne particles. Finally, insect vectors can also play a huge role in transmission irrespective of the season. Environmental conditions must be kept in mind for indirect and vector transmission; for litter-based thinning, such as dust, the drier the litter, the smaller

the infectious aerosol particle size. Density also makes a difference in the propagation of these fastidious bacteria, both between and within animals. Husbandry systems are determinant in bacterial shedding and transmission, and from an individual animal perspective, poor biosecurity increases the chances of introduction and transmission (Ravichandran *et al.*, 2021; Sachse & Borel 2020; Stokes *et al.*, 2021; Szymańska-Czerwińska *et al.*, 2023).

Avian species show a wide range of Chlamydiales susceptibility. Contrary to pigeons, macaws, ducks, geese, cranes, or great bustards, chickens, and turkeys can shed and harbor a large number of Chlamydia genotypes and strains. Additionally, the transmission of Chlamydiales does not have to happen between different species; it could proceed within a monotypic poultry flock. Such a high level of heterospecific transmission, in addition to its retransmission in unpredictable cycles, makes Chlamydiales a dangerous zoonotic agent (Sachse & Borel 2020; Stokes *et al.*, 2021; Riccio *et al.*, 2024; Turin *et al.*, 2022; Borel & Sachse 2023; Liu *et al.*, 2023; Heijne *et al.*, 2020).

### CHLAMYDIALES DETECTION METHODS IN POULTRY

Presently, there is no single gold standard testing method available for the routine diagnosis of Chlamydiales in poultry. Bacterial isolation by culture and microscopic examination of stained fixed smears is still important for large-scale *C. psittaci* infections in poultry. These traditional methods are genus-specific but do not have the ability to differentiate species and serovars. Molecular techniques have developed over the years and have significantly improved the rapid and accurate detection of the Chlamydiales family (Ravichandran *et al.*, 2021; Abd El-Ghany, 2020; Sykes, 2021; Koca, 2023). These assays are highly sensitive and can detect a wide range of chlamydial organisms from a variety of host and specimen sources. Moreover, many laboratories have successfully implemented these methods for the detection of animal chlamydial agents and clinical samples.

Serological assays have also been developed and are useful in large-scale surveillance for the diagnosis of *C. psittaci* in poultry, avian zoonotic diseases, and quarantine programs. These assays provide epidemiological information, as antibodies can be detected in infected birds by the time that shedding of the organism has terminated or been reduced due to host immune clearance (Hou *et al.*, 2024; Dembek *et al.*, 2023; Zhang *et al.*, 2022; Marchino *et al.*, 2022). A combination of antigen and antibody tests is more effective than either test performed independently. Clearly, one of the goals of chlamydial control in poultry is to determine the prevalence and distribution of these pathogens in nature, particularly in order to help predict the emergence of livestock containing

public health issues. Good diagnostic methods are powerful tools for epizootiological studies, terminating efforts to prevent or control the outbreaks of these diseases. A broader realization of the potential of chlamydial zoonosis and the ability to manage and prevent these outbreaks, including risk assessment, will depend on the routine use of efficient methods of diagnostics (Desquesnes *et al.*, 2022; Melnyk, 2022; Morelli *et al.*, 2024; Rudenko *et al.*, 2021). Some data suggest that rapid, accurate results in field tests can lead to more realistic and cost-effective control programs in the event of an outbreak.

The evaluation of assay performance in terms of sensitivity, specificity, predictive values, etc., has shifted into the evaluation of the ability of these assays to predict outcomes, such as clinical or financial impacts, and has helped investigators make decisions about managing or preventing disease. The shift towards addressing questions about the performance of the assay with diagnostics could be considered a second or third stage development in the realm of performance diagnostics. Although genetic studies are useful for understanding the epidemiology of *C. psittaci*, they are not cost-effective for use in many laboratories, and fewer researchers are using serological methods because of doubts regarding specificity, animal diversity and age, and the specificity of antigens (Duan *et al.*, 2022; Anstey *et al.*, 2021; Ko *et al.*, 2024) (Sachse & Borel 2020; Kasimov *et al.*, 2022). It has been found that many antigens have reduced specificity in other species of animals, and this fact can result in many false-negative and/or false-positive reactions. There are some potential properties available by different techniques in order to speed up the detection and isolation of *C. psittaci* shedding, particularly from the egg or reproductive tract of the host, including tissue culture, direct fluorescent antibody, immunofluorescent antibody, enzyme-linked immunosorbent assay, and PCR (Kappler & Henne 2020; Reyneveld *et al.*, 2020; Desquesnes *et al.*, 2022; Costa *et al.*, 2022). None of these techniques have been currently fully standardized. The methodology shows a gain in speed and simplicity, becoming a possible optional substitute for the other ones. Detection methods are used to manage the spread of infection from pregnant female birds as well as to reduce the shedding of the bacteria or virus to the other parts, particularly the eggs and meat of laying birds.

#### **ONE HEALTH APPROACH IN CHLAMYDIALES CONTROL**

The One Health approach, as the name suggests, considers the holistic interconnectedness of human, animal, and environmental health. Appropriate strategies for controlling Chlamydiales infections require close cooperation between veterinarians, public health professionals, and environmental scientists.

Wildlife conservationists and biologists are also part of such strategic paradigms to prevent the introduction of avian Chlamydiales into the ecosystem. Given the significance of a One Health approach in controlling bacterial zoonoses, infectious diseases with complex epidemiology, the impact and acceptability of each One Health approach will vary, depending on the particular social and cultural circumstances (Jorwal *et al.*, 2020; Zinsstag *et al.*, 2023; Davis & Sharp 2020; Erkyihun & Alemayehu 2022).

Efforts to control zoonotic infectious diseases in wild and domestic animal populations benefit from intersectoral collaboration. One Health integration of disease control strategies for domestic animals and their wildlife hosts has successfully controlled bacterial zoonoses, such as bovine tuberculosis in brushtail possums and Spotted Fever in falcons. However, achieving intersectoral collaboration in for-profit, resource-poor domestic animal industries remains elusive, particularly when the benefits predominantly accrue to public health. Public policymaking and risk communication should be used to influence harmful practices, such as unsafe and illegal disposal of culled poultry, including those infected with Chlamydiales, into waste-recovery and aquatic habitats. These approaches principally examine what makes One Health efforts effective. Furthermore, the application of One Health principles to Chlamydiales infection control programs will not only be beneficial for the health of the poultry population and the guarantee of their welfare, but above all for the general health of the human population.

In fact, integrating control measures at the animal–human–environment interface can undoubtedly prevent eventual exposure to zoonotic agents. This is particularly true for Chlamydiales in poultry, considering the existing evidence of zoonotic transmission and the risks of exposure to domestic and wild birds and bird habitats. At this ceremony, some examples of successful projects are mentioned. They are based on cooperative interventions involving professionals and stakeholders from different disciplines and were specifically implemented in some areas to control outbreaks of infectious diseases. The major strategies used include the integrated conduct of surveillance in humans, animals, vectors, and environments, the tracking and notification to the health and veterinary services of infectious diseases motivated by regulatory measures, and the sharing of information and diagnostic data between the professionals involved (Wilson *et al.*, 2020; Pley *et al.*, 2021; Zinsstag *et al.*, 2023). In conclusion, these results stress the importance of adopting this approach in future research and the introduction of specific policies for infectious disease control and prevention. The following recommendations are aimed at the adoption of a "One Health" paradigm.

## CHALLENGES AND OPPORTUNITIES IN CHLAMYDIALES RESEARCH IN INDIAN POULTRY

Research in Chlamydiales in Indian poultry, particularly farmed poultry, is meager. Several hurdles exist in this context.

1. Economic Challenges Funding for research studies in developing countries is severely constrained.
2. Infrastructural Bottlenecks Research that involves advanced technologies necessitates access to high-end instruments that are routinely available in P4-level facilities, which are not present in India. However, the Government of India has already taken steps to establish such facilities in collaboration with BSL-4 laboratories for maintaining diseases.
3. Advanced diagnostics such as reverse transcription-polymerase chain reaction with phylogenetics cannot be performed in a routine diagnostic setup in India.
4. Ethical Issues A research project aimed at the Indian poultry sector should not be overly ambitious; instead, it should underscore the need for epidemiological studies for the improvement of disease conditions in Indian poultry.
5. Opportunities Presently, research in the microbiome of Indian poultry is gaining rapid impetus by several research groups due to the availability of funds through a flagship program of the Government of India. Moreover, pharmaceuticals, diagnostics, and vaccines are growing at a rate of 16% CAGR (Sood *et al.*, 2020).

## PUBLIC HEALTH IMPLICATIONS OF CHLAMYDIALES IN POULTRY

Effective and safe poultry production has public health significance besides economic value for any nation. Poultry are considered excellent pets and are often kept like pets, especially in rural areas. Keeping this close association in mind, zoonoses have a high potential to break out in the human-poultry population. Apart from pet-keeping, in India, the workforce at various poultry farms is directly involved with the birds. Public health implications may be linked at first with the awareness that birds may be a source of Chlamydiales and that a highly obscure serovar of *Pasteurella* might play an important part in co-infection. Each factor adds significant risk, such as close worker contact and workers engaged in unsafe animal husbandry or flock management practices, posing a risk for consumers of poultry products. Consumption of contaminated non-vegetarian food products has been a source of infection with *Campylobacter*, *Chlamydia* spp., *M. avium* tubercle bacilli, and rarely *E. coli* O157, and domestic refrigerators are also occasionally verified sources of foodborne infections (Temesgen *et al.*, 2020) (Mwamba, 2020). Awareness about the health risk that the non-discriminating consumer faces, especially in India, through consuming non-vegetarian or egg

products without cooking and handling food hygienically, is important. All the preventive measures are closely linked with policy decision-making dimensions of public health.

Historically, abattoir/service personnel working on farms and disease control officers have also been presumed to acquire zoonotic mycoplasma infections, and public health strategies are focused at the policy level to define meat hygiene and food safety parameters. Veterinary public health is a service structured on a one-directional approach of emanating human diseases from animals (including birds) to humans, but if pathogens exhibit reciprocal zoonotic potentials, multidisciplinary work must be guided by public health concerns (Mubareka *et al.*, 2023). For prevention and control through the ability to warn targeted populations about potential risks and manage such outbreaks, realistic surveillance, reporting, and response systems are of no use unless incidents are communicated without delay and interventions are carried out in a coordinated fashion and in a timely manner. Given the scenario, it appears that the “stand-alone” approach of developing a human database and/or an animal disease reporting system will not help to confine an outbreak in poultry leading to a human outbreak in Indian conditions.

## REGULATORY FRAMEWORKS FOR CHLAMYDIALES CONTROL IN INDIAN POULTRY

Laws, policies, and guidelines pertaining to Chlamydiales control are the major tools in this direction in all countries. The sectoral ministries and departments in the governments and international organizations have framed various rules, regulations, policies, and guidelines for the Chlamydiales infection and its spread prevention. In India, regulatory and policy initiatives, and guidelines for Chlamydiales control are governed by: (1) Ministry of Agriculture and Farmers Welfare, Department of Animal Husbandry and Dairying; Department of Animal Husbandry, Dairying, and Fisheries have framed various programs and notified offices for Chlamydiales control/eradication. (2) Ministry of Health and Family Welfare is responsible for zoonotic diseases in India in close coordination with the Ministry of Environment and Forests (Sood *et al.*, 2021; Kulshreshtha *et al.*, 2024; Dar *et al.*, 2010; Kumar *et al.*, 2022).

Various departments and ministries of the Government of India have made several policy initiatives and notified offices for the control of chlamydiosis in India to control Chlamydiales infection of poultry origin for the effective prevention of outbreak and spread of the disease to humans from animal sources. Regulatory and policy initiatives, program directions, frameworks, and guidelines need to be purpose-oriented for effective control of Chlamydiales infection in the common

interest of all stakeholders. A study comparing the present policy initiatives and control programs of the Indian government and international bodies further indicates that from the regulatory perspective, the farming and veterinary practices require convergence for the successful prevention of the spread of disease and people involvement at the grassroots level. While envisioning integrated solutions to the Chlamydiales problem, various areas need to be focused on for future consideration or relook at policy initiatives related to Chlamydiales control practices, public health, and regulatory frameworks in India (Kasimov *et al.*, 2022; Dembek *et al.*, 2023; Dembek *et al.*, 2023).

## RESEARCH GAP AND FUTURE DIRECTIONS IN CHLAMYDIALES RESEARCH

Given the advances in research tools and technologies in the last 30 years, we may conduct large-scale comparative genomics, metagenomics, and transcriptional profiling to rule out the exact pathogens among different species of animals and to understand the genetic and immune/higher physiological reasons for differential susceptibility among members of Chlamydiales. The development of stable chicken models and field-based natural infection studies would be in the second plane of applications of this pathogen. Good quality and large-scale gene silencing studies may help to understand the hypothesis of E+ dependent host killing by chlamydiae. We need to develop better in vitro and in vivo irreversible transforming strains with translational potential as onco-vaccine candidates for oncological studies. The Chlamydiales veterinary vaccine is already market-ready, but significant regulatory issues should be systematically addressed. We have, until today, better antibiotics, but not a single efficient therapeutic to ensure 'one dose protection' against avian infections of Chlamydiae. We must appreciate the need for longitudinal studies to know the exact life-long impact of Chlamydiales in the poultry industry. Certainly, genomics and proteomics are real tools to understand the exact moment of pathogenesis at micro-niche level depth, which are expected to be exploited during less comprehensive studies as well. A more cross-disciplinary attitude is a must in future Chlamydiales research, where basic and translational research scientists need to collaborate with more molecular and microbial biologists, as well as theoretical and field epidemiological modelers, to achieve time-bound chlamydia research outputs. Poultry farmers have significant inputs to be shared for applying these research outputs in real field-level scenarios. Additionally, based on the global prevalence and newer genotypic clustering and metagenomics of different species of Chlamydiales scleroproteins, it is essential to keep investigating Chlamydiales in the next 5–10 years.

## CONCLUSIONS

The literature review presents a comprehensive analysis of the current understanding of Chlamydiales in Indian poultry, with a particular emphasis on the zoonotic transmission of Chlamydia species. The introduction establishes the significance of this topic, highlighting the growing concern surrounding avian chlamydiosis and its implications for both poultry health and public health. The reviewed studies collectively underscore the prevalence of *Chlamydia gallinacea* as a primary agent in domestic poultry, contrasting with the traditionally recognized *C. psittaci*. This shift in focus is critical for understanding the epidemiology of chlamydial infections in poultry and their potential transmission to humans (Guo *et al.*, 2016).

The main body of the review delves into various studies that elucidate the complexities of chlamydial infections in avian populations. The review by (Ravichandran *et al.*, 2021) highlights the biphasic life cycle of Chlamydiaceae and the fecal-oral and vertical transmission routes among poultry, emphasizing the zoonotic risks posed by *C. gallinacea* and other species. The genetic variability of *C. psittaci* in wild birds, as discussed in (Stokes *et al.*, 2021), further complicates the understanding of zoonotic transmission pathways, suggesting that both domestic and wild avian populations must be monitored closely.

The experimental work conducted by (Szymańska-Czerwińska *et al.*, 2021) provides evidence of the pathogenicity of *C. gallinacea*, noting its capacity to cause reduced body-weight gains in infected poultry and its association with atypical pneumonia in slaughterhouse workers. This reinforces the zoonotic potential of this strain and illustrates the need for heightened surveillance and preventive strategies in environments where humans and infected poultry interact closely.

In conclusion, the reviewed literature offers a nuanced understanding of Chlamydiales in Indian poultry, emphasizing the importance of recognizing *C. gallinacea* as a significant zoonotic agent. The studies collectively highlight the need for ongoing research to elucidate the transmission dynamics, pathogenicity, and genetic diversity of Chlamydia species in both domestic and wild birds. This knowledge is essential for developing effective monitoring and control strategies to mitigate the zoonotic risks associated with avian chlamydiosis.

This review provides key information about the prevalence of Chlamydiales in Indian poultry and their possible transmission to humans. It also shows that the existing chlamydial and chlamydophila vaccines are not effective against chlamydiosis; hence, it is high time to come up with a new generation of vaccines. Chlamydiales species, as well as chlamydial and chlamydophila serovars circulating in Indian poultry,



are also summarized in this review. The Indian poultry industry has a current bird population of more than 3,000 million, making a significant impact on the domestic and international markets. The products include chicken, table eggs, and hatch eggs. The Indian poultry network has increased over the last decade, with small farmers running half of the industry. Furthermore, India is the second-largest egg producer and the fifth-largest broiler producer in the world. Additionally, there are many wild birds, such as waterfowl, migratory, and water-bound birds, that are carrying these Chlamydiales and spreading them further.

The findings from this review indicate that Chlamydiales infect the gut of Indian poultry and are also present in the respiratory system. These Chlamydiales can infect humans in and around poultry farms, leading to flu-like symptoms. Therefore, the Government of India should take proper steps according to the One Health strategy by involving all stakeholders, which include the Ministry of Agriculture, the Ministry of Environment, the Ministry of Health, and the Ministry of Animal Husbandry. Research should focus on the development of a new generation of multivalent vaccines, diagnosis, and their costly clinical manifestations, reservoirs, sources, and carriers; genotyping and markers for genetics; and epigenetic effects on humans, poultry, and the environment. Strategies for a regular surveillance program for the detection of Chlamydiales DNA and RNA should be implemented. In addition, DNA vaccines can be evaluated alongside RNAs. This complexity makes controlling Chlamydiales in human and veterinary medicine furthermore difficult. The development of international collaborative research programs can target future policing of chlamydiosis. The development of an interactive system, involving all stakeholders including the environment, is expected to eradicate chlamydiosis from all hosts by vaccinating from egg to infinity, by the concept of infinite vaccination, and by providing public awareness and knowledge.

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