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A Review on Infectious Coryza Disease in Chicken

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Abstract
Infectious Coryza (IC) is a serious respiratory tract disease of chickens caused by *Avibacterium paragallinarum*, formerly known as *Haemophilus paragallinarum*. The disease has complicated economic impact in poultry industry due to growth retardation and decreased egg production in layer flocks. Chicken (*Gallus gallus*) is the natural host for *A.paragallinarum* which are susceptible at all ages. The most prominent clinical sign of IC is edematous swelling of face and distension of infraorbital sinus due to highly accumulation of cheesy like exudates in conjunctival sac. *A. paragallinarum* is a slow-growing and fastidious bacterium. Most of its strains require V (NAD) factor for their growth in vitro. Three serotypes of *A. paragallinarum* (A, B and C) have been identified that are distributed throughout the world. Vaccination is the soundest method of preventive practice against infectious coryza. An indigenous coryza vaccine is the best preventive measure against both homologous and heterologous challenges because of those commercial vaccines are not protective against the local variants of *A.paragallinarum.*
Keywords: *A.paragallinarum*, chicken, infectious coryza, NAD, serotype, serovar, vaccine
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Introduction

Coryza referred to any respiratory disease of poultry. Since the disease proved to be infectious only in the nasal passages, the name "Infectious Coryza" was adopted. The disease is also called roup, snot or contagious catarrh. Avian infectious coryza (AIC) is a serious respiratory tract infection of chicken caused by an opportunistic pathogen *Avibacterium paragallinarum* (previously known as *Haemophilus paragallinarum*, *gallinarum*: from fowl) having an economic implication on poultry industry. *Avibacterium paragallinarum* is now believed to be evolving as a re-emerging disease of poultry birds [1], [2], [3].

As early as 1920, Beach believed that infectious coryza was a distinct clinical entity. In 1932, De Blieck isolated the causative agent and named it *Bacillus hemoglobinophilus coryzae gallinarum*. Schneider released the first report to draw attention to organisms that appear to resemble the *Avibacterium gallinarum*. The description of the species *P.gallinarum* occurred in 1955 and the species *Avibacterium paragallinarum* was originally described and validly published by Blackall *et al.* in 2005 [4][5],[6] [7].

Avibacterium paragallinarum isolates serotyped by two different methods. Page classified *A. paragallinarum* into three serovars (A, B, and C) based on the agglutination reaction and later these schemes were further divided in to nine Kume hemagglutinin serovars: A1–A4, B–1, and C1–C4 using hemagglutination inhibition [8]. However, these schemes might need to be revised and updated as new B variants have been discovered which are different from the standard strains (Spross, Modesto and 0083) in South Africa and Argentina. Cross protection with in Page serovars and Kume serogroups become difficult due to emergence of these new B variants [9],[10][11].

Occurrence and distribution

Infectious coryza occurs wherever chickens are raised. The disease occurs frequently from April to July and from October to November in the intensive chicken industry. Even though the disease can occur in birds of any age ,it is more common mainly in the laying period, as well as in the brooding period, at 30–40 days old just when they are stressed [12] [13].

Avian infectious coryza (AIC) disease had been reported from almost all the countries around the world and the causative agent of this disease *(A. paragallinarum)* has been isolated worldwide. AIC causes the greatest economic losses to the poultry industry of developing countries because of the presence of many variable pathogens or stress factors associated with poor environmental conditions for growth[14].

Table 1: distribution of infectious coryza in some countries of the world

Continent/region	Countries
Africa	Angola,Botswana,Cameroon, Egypt,Eritrea,Ethiopia,Ghana,Guinea ,Morocco, South Africa,Tanzania,Zimbabwe
Asia	Bahrain, Bangladesh, China, Honking, India, Indonesia, Iran, Jordan ,south korea,Taiwan,Thailand,Vietnam
Europe	German ,Ireland, United kingdom
North America	Canada ,Cuba,Jamaica, Mexico, United states
South America	Argentina, Bolivia, Brazil, Chile, Colombia, Peru, Uruguay
Oceania	Australia, French Polynesia, Caledonia ,New Zealand

Source: Uddab Poudel (2022) at https://vetnepal.com/article_details/Infectious-Coryza-in-poultry#.Y01PCyj1s8U.gmail

Transmission

Chickens (Gallus gallus) can be carriers of the disease and still appear healthy, which makes infectious coryza very hard to control, especially on farms without an "all-in, all-out" flock practice. Sneezing and coughing are frequently present which contributes to spreading the organism. The disease can be spread directly from chicken to chicken contaminated feed, water, equipment, and clothing [1].

Clinical signs

The most prominent clinical sign may be edematous swelling of face and infraorbital sinus, secretion of watery nasal and ocular discharges, air sacculitis in secondary bacterial infection, conjunctivitis with a cheesy, fetid odor of exudate in the conjunctival sac and inflamed wattles. Pus-like excretes may be discharged from the nostrils and eyes, breathing difficulties, loss of appetite or decrease in feed consumption which lead to decreased weight and egg production in layers. However, death loss is usually low unless the disease is complicated with other agents, such as *Mycoplasma gallisepticum* [1],[6], [13], [14]. Besides, primarily involving upper respiratory tract, the infection also transcends down to trachea, air sacs as well as cause pneumonia in lungs in very extreme cases. In older and egg laying chicken, the organism predominantly can affect the reproductive organs that leads for poor egg quality and decreased egg production [3].

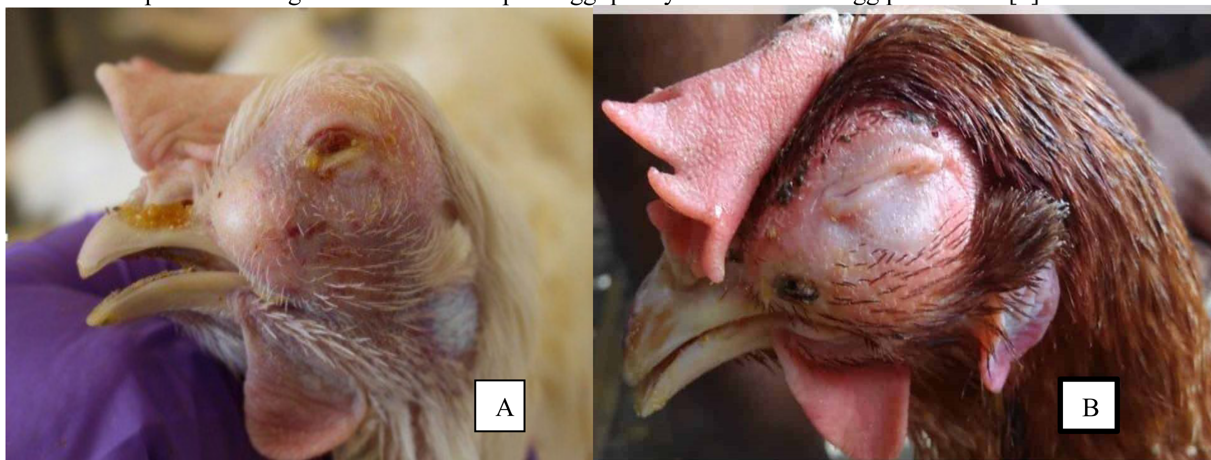


Figure 1: A) Chicken showing swollen infraorbital sinuses and open mouth breathing (https://extension.psu.edu/avian-coryza)
B) Chicken showing edema with sticky closures of eyes (https://cs-tf.com/coryza-in-chickens/)

Isolation of *Avibacterium paragallinarum*

Samples should be taken from two or three chickens in the acute stage of the disease. A sterile cotton swab is inserted deep into the sinus cavity where the organism is most often found in its pure (uncontaminated) form. Ocular, nasal and tracheal or air sac exudates may also be taken on a sterile swab. Samples should be transferred to Brain Heart Infusion (BHI) broth media supplemented with Nicotinamide Adenine Dinucleotide (NAD) [3].

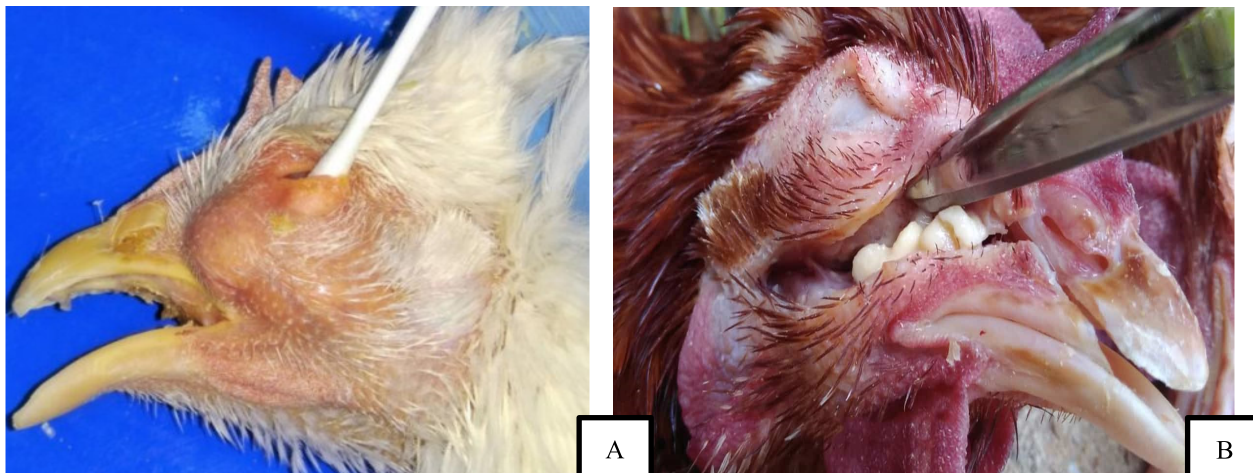
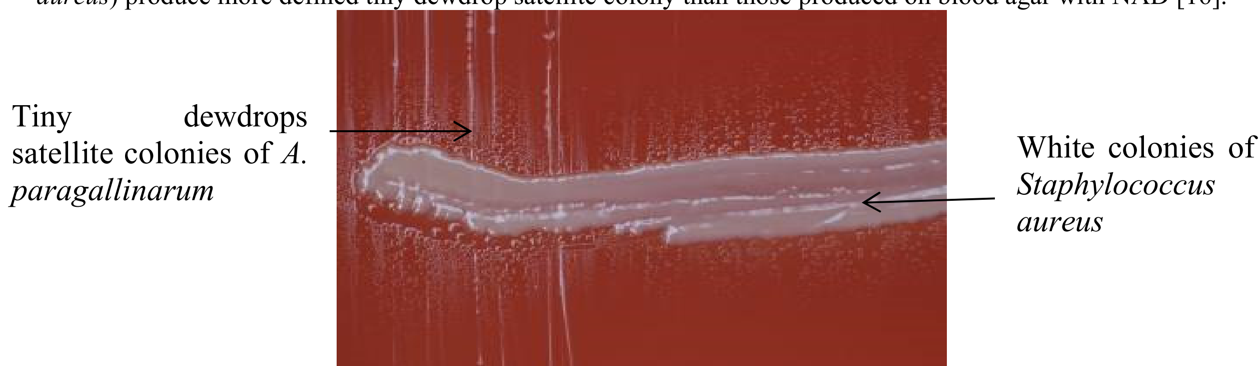


Figure 2: A) Collection of swab from conjunctival sac of affected layer chicken (Dwivedi et al., 2018) B) Chicken affected with IC having caseous material deposited in infra orbital sinus (https://cs-tf.com/coryza-in-chickens/)

Samples should be cultured with a swab streaked on 5% sheep blood agar supplemented with 1% heat-inactivated, sterile-filtered chicken serum and then followed by cross-streaking of *Staphylococcus aureus* as NAD provider for media. Bacterial growth needs to be supported by incubation at 37°C under micro aerobic or anaerobic conditions with increased levels of CO2 (5-10%) pressure for 24-48 hours. Bacterial colonies which indicated satellite growth should be selected to subculture in 7% horse blood Columbian chocolate agar for further purification and maintenance. Horse blood is considered as superior ingredients for the growth this fastidious organism [3], [15]. Sample streaked on blood agar with NAD and cross streaked with feeder colony (*S. aureus*) produce more defined tiny dewdrop satellite colony than those produced on blood agar with NAD [16].



Courtesy of E. Soriano-Vargas *et al.* (2013)

Figure 3: Avibacterium paragallinarum, cultivated on bovine blood agar together with a streak of Staphylococcus aureus. A. paragallinarum has increased growth with dewdrop like larger colonies (called satellite colonies) around the streak of Staphylococcus aureus, which cause hemolysis and release of the V factor (NAD) from the erythrocytes.

The causative agent in the earlier period of recognition was identified as *Haemophillus gallinarum* that requires X factor (hemin) and V factor (NAD) for its in vitro growth. However, over a period of time, changes in growth requirement of the bacteria were realized that resulted in the re-naming of the bacteria as *Haemophillus paragallinarum* that requires only V factor (NAD) for growth. Later on, nearing to early 90s, an evolution of further new kind to the existing type of *Haemophillus paragallinarum* was understood, that was NAD independent requiring no V factors for their growth. This continual process of periodic evolutionary changes in organism is apparently thought to be due to the vaccine pressure/adaption [3].

Morphological identification of the organism

A smear of sinus exudates or culture should be made and Gram stained. It should reveal Gram-negative bipolar-staining and pleomorphic rod or coco-bacilli morphology with a tendency to form filament like arrangement with short chains. Ordinarily, the size of bacterium ranges from 1 to 3µm in length and from 0.4 to 0.8µm in width [3]. Another efficient diagnostic procedure is to inoculate the sinus exudates or culture into two or three young normal chickens by the infraorbital sinus (intra sinus). The typical signs and lesion associated with coryza may develop in 24-28 hrs or longer (3-5 days); however, the incubation period may be delayed up to 1 week if only a

few organisms are present in the inoculum [11],[17],[18].

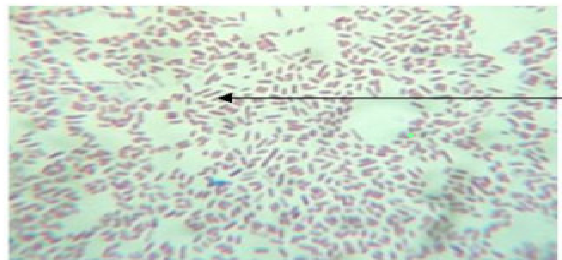


Figure 4: Gram's staining of A. paragallinarum (arrow) (Akter et al.,2016)

Molecular identification and serotyping of *Avibacterium paragallinarum*

The species-specific PCR of HPG-2 gene would appear to be a suitable alternative to screen *A. paragallinarum* positive samples collected from IC suspected cases [19], [20]. The homology of region 2 is a serovar-specific region in the hyper variable region (HMTp210) of *A. paragallinarum.* Using multiplex PCR, the hypervariable region could be amplified 0.8, 1.1 and 1.6 kbp fragments for serovars A, B and C, respectively [21].

Table 2: Species and serovar specific primers for identification of A. paragallinarum

Target gene	Primer	Primer sequence	Product size	References
HPG-2 gene (species-specific)	R1 - F	5'-CAAGGTATCGATCGTCTCTCTACT-3'	500 bp	[19], [22][23]
	N1 - R	5'-TGAGGGTAGTCTTGCACGCGAAT-3'		
HMTp210 (serovar-specific)	A, B, C -F	GGCTCACAGCTTTATGCAACGAA		[21]
	A -R	CGCGGGATTGTTGATTTTGTT	0.8 kbp	
	B -R	GGTGAATTTCACCACACCAC	1.1 kbp	
	C -R	TAATTTTCTTATTCCCAGCATCAATACCAT	1.6 kbp	

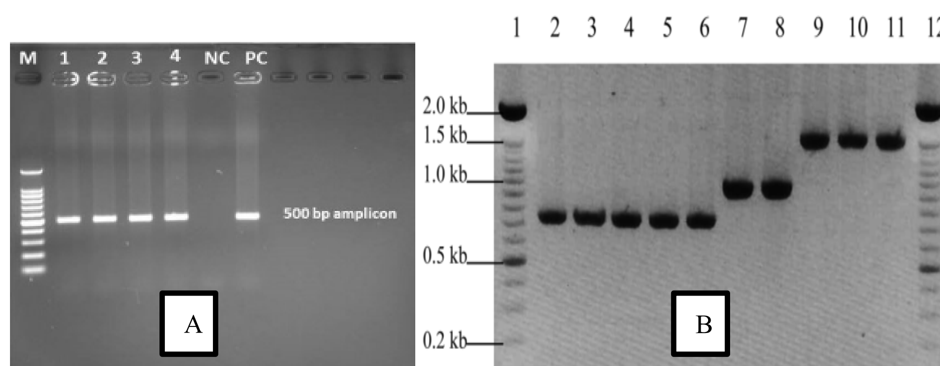


Figure 5: A) Species-specific PCR (HPG-2 gene of *A. paragallinarum*) (Umar *et al*., 2020)
B) Agarose gel electrophoresis of multiplex PCR products of *A. paragallinarum* strains, Marker (lanes 1 and 12); serovar A (lanes 2-5); serovar B (lanes 7 and 8); serovar C (lanes 9- 11) (Sakamoto *et al*., 2011)

Susceptibility to Chemical and Physical Agents

A.paragallinarum is a delicate organism that is inactivated rather rapidly outside the host. Infectious exudate suspended in tap water is inactivated in 4 hours at ambient temperature; when suspended in saline, the exudate is infectious for at least 24 hours at 22°C. Exudate or tissue remains infectious when held at 37°C for 24 hours and, on occasion, up to 48 hours; at 4°C, exudate remains infectious for several days. At temperatures of 45-55°C, hemophili are killed within 2-10 minutes. Infectious embryonic fluids treated with 0.25% formalin are inactivated within 24 hours at 6°C[18].

Biochemical identification tests

The organism can ferment sugars like glucose, sucrose, maltose and mannitol and produced acid but do not ferment some of the sugars such as lactose and trehalose. It is negative for MR, VP, indole ,catalase and some other tests [3],[24].

Table 3: Biochemical characteristics of Avibacterium paragallinarum

Test	*A. paragallinarum*	References
oxidase, urease, ß-Galactosidase	+	[3],[24],[18],[25],[26]
catalase, ornithine decarboxylase	-	
MR, VP, indole	-	
motility	-	
nitrate reduction	+	
CO2 requirement	+	
NAD requirement	+	
sucrose	+	
maltose	+	
glucose	+	
fructose	+	
D-mannitol	+	
D-sorbitol	+	
L-arabinose	-	
D-galactose	-	
dulcitol	-	
trehalose	-	

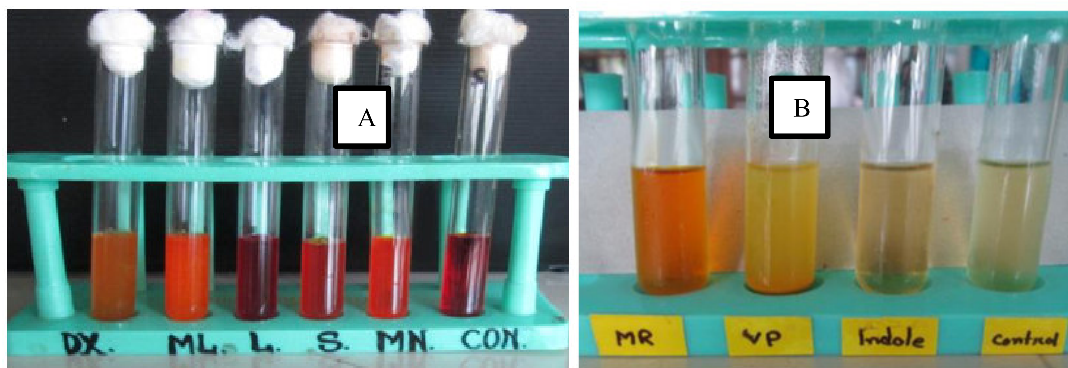


Figure 6: A) sugar fermentation tests *A. paragallinarum* produces acid by fermenting dextrose (DX), maltose (ML), sucrose (S) and mannitol (MN). No fermentation in the cases of lactose (L) and control (CON). B) Biochemical tests for *A. paragallinarum*. The bacteria produce orange and yellow colors in Methyl Red (MR) and Voges-Proskauer (VP) test indicating negative results, respectively. No color is produced in indole test and control. (Khatun, 2016)

Antimicrobial Susceptibility Testing

A. paragallinarum is a slow-growing and fastidious bacterium. Most of its strains require V-(nicotinamide adenine dinucleotide, NAD) factor for their growth and there is no recommended standardized medium for susceptibility testing. Columbia blood agar (CBA) with 7% horse blood can be used to perform antibacterial susceptibility test by Kirby-Bauer disk diffusion method[15].

The *A.paragallinarum* isolates are sensitive against erythromycin, gentamycin, licomycin, neomycin, pectinomycin, oxytetracycline, tylosin, ciprofloxacin and azithromycin. However, high levels of antimicrobial resistance to neomycin, streptomycin, tetracycline, doxycycline, ampicillin, cephalexin and erythromycin have been reported [27][28][29][30].

Prevention and control of *A.paragallinarum*

Prevention is the only sound method of control for infectious coryza disease. All-in/all-out flow of animals as part of sound farm management and biosecurity practices are important disease prevention measures. Replacement chickens should be raised on the same farm or obtained from clean flocks. If replacement chicks are to be placed on a farm that has a history of infectious coryza, bacterins/vaccines are available to help prevent and control the disease. Commercial vaccines against IC that produced from standard internationally recognized strains of inactivated *A.paragallinarum* are widely used around the world [11].

Previous studies showed that the use of autogenous vaccines containing the prevalent serotypes in an area and incorporating aluminum hydroxide gel as an adjuvant appear to be more effective in controlling infectious

coryza [31][32]. Abd El-Ghany from Egypt reported that autogenous *A.paragallinarum* vaccine containing either aluminum hydroxide or mineral oil adjuvant were found to be effective and safe when given as double I/M shots in layer chickens at 6 and 9 weeks of age. A research done in India also indicated that field isolates were found to be substantially variable in genetic makeup from standard vaccine strains. Therefore, indigenous coryza vaccine is the best preventive measure against both homologous and heterologous challenges [26]. On the contrary, commercial vaccines provided good protection against homologous challenge but showed poor performance against heterologous challenge [33].

The three Page serovars are distinct from each other as the antibodies from each serovar are unable to protect chickens from two other serovars, while they can provide protection against the serovars within the same group. All three serotypes of *A. paragallinarum* (A, B and C) must be considered when immunizing the chickens against infectious coryza disease and vaccines should be matched with reported serovars as there is no guaranteed cross protection between different serovars [34].

The trivalent inactivated vaccine produced from standard internationally recognized strains did not completely protect chickens against three serovars of *A.paragallinarum* field isolates. The IC vaccine is protective against *A.paragallinarum* when the serovars of the vaccinal strains are matched with the local /field strains. Double vaccination showed better protection efficacy than single vaccination. The recombinant fusion peptide derived from HMTp210 could be useful for producing effective ,safe and low-cost vaccines against infectious coryza in chickens [35][36].

Currently, there are commercially produced infectious coryza vaccines are available on the market. Nobilis® Coryza, Poulvac® Coryza ABC IC3 and Virsin 336 Oil emulsion are examples of inactivated trivalent coryza vaccines contain A, B and C serotypes.

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