

Incidence of Mixed Infection in Coryza Cases

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Abstract

Diseases of the respiratory tract are a significant component of the overall disease incidence in poultry. In many cases, respiratory disease observed in a flock may be a component of a multi-systemic disease or it may be the predominant disease with lesser involvement of other organ systems. In some cases, such as infectious coryza the disease may be limited to the respiratory system, at least initially. Various pathogens may initiate respiratory disease in poultry, including a variety of viruses, bacteria, and fungi. Environmental factors may augment these pathogens to produce the clinically observed signs and lesions. During present study, an attempt was made to isolate *H. paragallinarum* from the nasal swabs, eye swabs (from live birds) and caseous infra orbital sinus and tracheal exudates swabs from dead birds from commercial poultry farms Anand, Kheda and Mahua region of Saurashtra area of Gujrat state. During the present study 6 *H. paragallinarum* isolates were obtained from 109 samples suspected of Infectious coryza infection.

Keywords: Poultry, Mixed infection, Respiratory Disease,

Introduction

Among infectious diseases of avian species Infectious coryza is one of the major problems affecting commercial poultry industry in the country. Infectious coryza is an upper respiratory disease of chickens caused by infection with *H. paragallinarum* (HPG). The disease is characterized by swollen infraorbital sinuses, nasal discharge, and depression. The disease is seen most commonly in adult chickens and can cause a very significant reduction in the rate of egg production. An infectious flock may potentially shed the organism for the rest of the life of flock. For this reason, HPG infection may endure indefinitely on farms having multiple-age chickens, particularly multiple-age layer or breeder farms.

Infectious coryza is a cosmopolitan disease which is present everywhere chickens are raised. When coryza is present without any other disease, it is characterized as an acute disease with a short course (of approximately two weeks) and spontaneous recovery. However, the involvement of other bacterial or viral agents is common. In this case the duration of the disease is prolonged (up to seven weeks) and this clinical outcome is known as complicated infectious coryza. Birds with complicated infectious coryza do not easily cure and usually remain with different consequences, which lead to important culling rates. While often seen

as a simple, mild upper respiratory tract infection can be much more complicated in poultry. Hence there is a need of understanding, the virulence and possible mechanism of disease caused by the organism.

Materials and methods

Test strains: Total one hundred and nine samples were collected from different age group of poultry birds suspected for Infectious coryza based on clinical signs (nasal discharge, conjunctivitis, face swelling) and post mortem examination (caseous or cheesy exudates in infra orbital sinus). Nasal swabs, eye swabs from live birds and infra orbital sinus cavity exudates from dead birds were collected on separate sterile cotton swabs from the commercial poultry farms in Anand, Kheda and Mahua region of Saurashtra area in Gujrat state. These samples were transported to the veterinary microbiology laboratory, Veterinary college, Anand on ice.

Reference strain: The reference strain of *H. paragallinarum* (strain A, C) was procured from Ventry Biologicals, Pune, Maharashtra.

Cultural isolation: The primary growth on HA was transferred to different media like Blood Agar (BA) without feeder culture and Nutrient Agar (NA). Two types of growth on blood agar were noted and then these two different colonies were again tested by smear examination and further growth on specialized media.

The growth on nutrient agar was observed for any pigmentation, smear examination and other colony characters.

The non-haemolytic colonies on blood agar were re-streaked on media like MacConkey agar (MCA), EMB agar and Brilliant Green Agar (BGA) for further differentiation of the colonies and then incubated at 37°C for 24 hour without reduction in oxygen tension. All the isolates were stained by Gram's Method and observed under microscope to check the purity of the growth.

The colonies on MacConkey agar (MCA) were examined for lactose fermenting and non lactose fermenting organisms. EMB agar was used for observation of greenish metallic shine specially for *E. coli* organisms. Colourless colonies on Brilliant Green Agar (BGA) were further used for reaction on TSI and agglutination reaction with Poly O anti Salmonella serum. The haemolytic colonies on blood agar were examined for smear examination.

Results

In the present study the isolates failed to grow on Nutrient Agar (NA), MacConkey agar (MCA), EMB Agar, and Brilliant Green Agar (BGA) indicating that either these media are inhibitory and / or they did not support the growth of *Haemophilus paragallinarum* due to lack of V factor.

Other organisms which were primarily isolated in this study include *E. coli*, *Pseudomonas*, *Salmonella*, *Staphylococci* and *Proteus*. The reports indicate that in infectious coryza cases, secondary organisms complicate the disease condition along with *H. paragallinarum* infection.

E. coli organisms were primarily presumed on the basis of their pink color lactose fermenting colonies on MacConkey agar and further confirmed by characteristic greenish metallic shiny colonies on EMB agar. Moreover these organisms have found to be gram negative having rod shape in smear examination.

For tentative identification of *Pseudomonas*, the growth of the organism on Nutrient Agar with greenish pigmentation, smear examination and oxidase test were carried out.

Salmonella organisms were identified by precipitation reaction with the Poly O antisera for Salmonella. Moreover reaction of the organisms on TSI slants (yellow butt, orange slant) and H₂S production were also observed.

Staphylococci were identified by smear examination, haemolytic colonies on BA and by catalase activity. The smear exam revealed gram positive cocci in grape like clusters.

The swarming type of colonial growth on Haemophilus Agar and Nutrient Agar were primarily

identified as *Proteus* organisms.

There were some other bacteria showing growth only in Haemophilus agar and no growth in any other medium. These organisms have shown catalase activity but these were further not analyzed.

Discussion

In India there is very scanty information available on *H. paragallinarum* because the laboratory diagnosis of *H. paragallinarum* infection is based mainly on demonstration and confirmation by isolation and identification of the organisms. This diagnosis proves to be a difficult task for routine isolation and identification.

During present study, an attempt was made to isolate *H. paragallinarum* from the nasal swabs, eye swabs (from live birds) and caseous infra orbital sinus and tracheal exudates swabs from dead birds from commercial poultry farms Anand, Kheda and Mahua region of Saurashtra area of Gujrat state. In the same study, other commensal and fast growing organisms were also observed which were then tentatively identified and compared with the findings of other workers.

The vastly different nature of infectious coryza when complicated by other pathogens and stress factors has been demonstrated by reports from countries such as Argentina, India, Morocco and Thailand (Hoerr *et al.*, 1992) Unique clinical presentations such as arthritis and septicemia, presumably complicated by presence of pathogens detected, such as *Mycoplasma gallisepticum*, *M. synoviae*, *Pasteurella spp.* *Salmonella spp.* and infectious bronchitis virus, have been found in broiler and layer flocks in Argentina. The isolation of *H. paragallinarum* from non respiratory sites such as liver, kidney and tarsus was reported in some outbreaks (Sandoval *et al.*, 1994).

Reports for the occurrence of *Pasteurella* have been reported by Adlakha, (1967) Similar reports have been shown by Verma *et al.*, (1985) that *E. coli*, *Mycoplasma* were the primary pathogens found in a study for isolation of *H. paragallinarum* in infectious coryza cases in Hissar.

Sandoval *et al.*, 1994 reported presence of other pathogens such as *Mycoplasma gallisepticum*, *M. synoviae*, *Pasteurella spp.* *Salmonella spp.* and infectious bronchitis virus in broiler and layer flocks in Argentina while isolating *H. paragallinarum* from the suspected cases of Infectious coryza.

The findings of Blackall *et al.* (1997) states that there is a high possibility that commensal bacteria, which are typically vigorous growers, can mask the presence of the small colonies of *H. paragallinarum*. The other bacteria found in this study also reveal the

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Table-1. Tentative identification of Other Organism on Different Media.

Sr.	Suspected Oxidase	No. of	Growth on					Reaction anti-Salmonella sera	Agglutination on TSI	Smear by Poly C	Catalase Exam	
			organism isolates BA	MCA	EMB	BGA	NA					
1	<i>E. coli</i>	40	+	Pink	Greenish metallic Shine	NT	NT	NT	Gram negative rods	+	-	
2	<i>Salmonella</i>	13	+	Colour less	NT	Pink discoloration	NT	Yellow but, H ₂ S Production	Agglutination	Gram negative rods	+	-
3	<i>Proteus</i>	6	+,foul smelling	Colour less	NT	NT	Swarming	NT	NT	Gram negative rods	+	-
4	<i>Pseudomonas</i>	5	Dark discoloration	+	NT	NT	Greenish pigmentation	NT	NT	Gram negative rods	+	+
5	<i>Staphylococci</i>	15	Haemolysis	NT	NT	NT	+	NT	NT	Gram positive Cocci in Clusters	+	-

+ = Growth observed, NT = Not tested

same thing that as they are fast growing and routinely found in birds, the presence of *H. paragallinarum* might have been masked.

Similar findings about the other pathogens have been reported by Sobti *et al.* (2001) who isolated *H. paragallinarum* from cases of infectious coryza. Out of 253 samples 28 were avian *haemophili*, 17 *Pasteurella*, 10 *Streptococcus*, and 11 *Corynebacterium* isolates.

Jaswinder-Kaur *et al.* (2004) also identified other organisms as *Proteus*, *Staphylococcus*, *E. coli* and *Pasteurella multocida* from the samples suspected for infectious coryza.

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References

1. Adlakha, S. C. (1967): Role of *Pasteurella multocida* in infectious coryza of fowls. *Indian Vet J.*, 44, 828 - 833.
2. Blackall, P. J., et.al.(1997): Infectious coryza, In B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, L. R. McDougald and Y. M. Saif (eds), (*Diseases of Poultry*) 10th edn, pp 179-190, Ames: Iowa State University Press.
3. Hoerr, F. J., et.al.(1994): Case report: Infectious coryza in broiler chickens in Alabama., *Proceedings of 43rd Western Poultry Disease conference.*, 62-63.
4. Jaswinder Kaur, et.al.(2004): *Indian Journal of Animal Sciences.* 74 (5): 462-465.
5. Sandoval, V. E., Terzolo, H. R., (1994): *Corizza Infeciosa, Primera Parte, description de la enfermedad, el agente, y los brotes de campo, Avicult Profes,* 14, 29-35.
6. Sobti,-D-K; Dhaneas,-N-S; Chaturvedi,-V-K (2001): *JNKVV-Research-Journal.* 34 (1/2): 54-56.
7. Verma R., Kumar, A., Chandiramani, N. K. and Kulshreshtha, R. C., (1985): *Coryza in chickens associated with mixed infection, Indian J. Poult. Sci.,* 20(1): 68 -70.
