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Short communication

Isolation and Identification of *Avibacterium paragallinarum* from Layer Chickens in Gazipur, Bangladesh

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ABSTRACT

An investigation was conducted for isolation, identification and determination of antibiotic sensitivity of *Avibacterium paragallinarun*, the causal agent of infectious coryza, from layer chickens. A total of 21 samples with characteristic symptoms of the disease were collected from a Hatchery of Gazipur. Tissue specimens obtained aseptically from swollen infra orbital sinus and tracheal swab were processed, of which, 3 were found positive while the rest 18 were negative. Isolation of bacteria was performed by first putting the specimen in Nicotinamide adenine dinucleotide (NAD) enriched phosphate buffer broth, anaerobically incubated for 24 hours followed by culturing loopful of broth on Blood agar (BA) and Chocolate agar (CA) plates enriched with NAD and streaked with feeder organism of *Staphylococcus. aureus.* On 24 hours of anaerobic incubation (candle jar method), dew drop satellite colonies of *A. paragallinarum* were visible on the culture plates. Cultural characteristics of bacteria as well as their staining, morphological, motility and biochemical properties such as sugar fermentation, MR and V-P tests, Indole production and catalase tests were recorded for identification. Further, antibiogram study revealed that the isolates were sensitive to Ciprofloxacin, Chloramphenicol and Gentamicin but resistant to Ampicillin, Amoxycillin, Oxytetracycline, Erythromycin and Sulphamethoxazole.

Key Words: Infectious coryza, Infraorbital swelling, Feeder organism, Antibiogram profiles.

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Introduction

Infectious coryza is an important disease of chicken caused by *Avibacterium paragallinarum* (Basonym; *[Haemophilus] paragallinarum*). The clinical signs associated with this disease include nasal discharge, conjunctivitis with swelling of the sinuses, face and wattles, diarrhoea, decreased feed and water consumption, increased number of culls and reduced (10-40%) egg production (Eaves *et al*., 1989; Calnek *et al*., 1991). Lesions of the disease also reflect an acute catarrhal inflammation of mucous membrane of nasal passage and sinuses of the upper respiratory tract.

Since the disease proved to be infectious and primarily affecting nasal passages, the name "infectious coryza" was adopted (Blackall *et al*., 1989) and described as rogue, cold, contagious or infectious catarrh and uncomplicated coryza (Yamamoto *et al*., 1991).The disease is usually transmitted through drinking water contaminated with infective nasal exudates (Page *et al.*, 1962). Infectious coryza is also accompanied with condemnation of carcasses due to upper respiratory disorders in broilers (Droual *et al.*, 1990).

The disease occurs worldwide and after recovering from infection, birds become carriers, therefore aiding the spread of *A. paragallinarum* (De Blieck, 1948). Secondly, the bacterial strains belong to one of nine serovars, which makes prophylaxis of the disease through inactivated vaccination ineffective especially due to low cross protection among these serovars (Rimler *et al*., 1977). Early workers identified the causative agent as "*Haemophillus gallinarum*," the organism that required both X (hemin) and V (NAD) factors for growth *in vitro*. On the other hand, from the 1960s to the 1980s, all the isolates producing the disease have been shown to require only V factor and were termed *Haemophilus paragallinarum* (Matsumoto and Yamamoto, 1975). However V-factor-independent *H. paragallinarum* isolates have also been encountered since 1989 (Mouahid *et al*., 1992). Thus, the causative agent of infectious coryza can be either V-factor dependent or independent.

In Bangladesh, infectious coryza is a notable disease of economic significance occurring in multiage layer flocks but diagnosis of the disease is mostly based on postmortem examination of the dead birds. Therefore, the present research work was undertaken to isolate and identify *Avibacterium paragallinarum* from infected layer chicken, determination of dependence on growth factor requirement to isolate *Avibacterium paragallinarum* and their antibiotic sensitivity profiles against commonly used antimicrobial agents.

Materials and Methods

A total of 21 samples (of which 15 were from live and 6 dead Chickens) were collected from Phenix Hatcheries Ltd. during the period of June 2012 to July 2013. Aseptically collected materials from swollen infraorbital sinus and tracheal swabs were used as laboratory specimens.

Isolation of Bacteria

Bacteriological samples obtained from nasal, tracheal and infraorbital sinus swabs inoculated separately in glycerol-enriched phosphate buffered broth supplemented with NAD was incubated anaerobically (candle jar method) at 37°C for 24hrs to allow maintenance and growth of only NAD dependent organism as followed by Byarugaba *et al*.(2007). A loopful of enrichment culture was then streaked onto Blood agar (BA) with NAD, blood agar with feeder colony of *S. aureus*, Chocolate agar (CA) with NAD and CA with feeder colony (*S. aureus*) followed by anaerobic incubation for 24 hour and examined for the growth of characteristics colonies.

Identification of bacteria

Colonial morphology of bacteria such as: shape, size, surface texture, edge, elevation and color was observed on pure culture; Gram staining and biochemical tests such as sugar fermentation, methyl red (MR), Voges-Proskauer (V-P), indole production, Catalase test and motility test in MIU medium were used foridentification of the bacteria (Buxton and Fraser, 1977).

Antimicrobial susceptibility testing

The Bauer disk diffusion method was used to test antimicrobial susceptibility of *Avibacterium paragallinarum* using freshly prepared Mueller Hinton agar (Oxoid, UK). Antimicrobial agents and their disc concentrations used were as follows: ampicillin (10 µg), ciprofloxacin (5 µg), gentamicin (10 µg), trimethoprim-sulphamethoxazole (25 µg) and tetracycline (30 µg). Results of antibiotic sensitivity tests were recorded as sensitive, intermediate and resistant following the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2007).

Results

Isolation of organisms

Enrichmend culture of glycerol-enriched phosphate buffered broth supplemented with NAD was streaked onto Blood agar with NAD and incubated anaerobically at 37 °C. After 24 hrs, dew drop like scattered colonies were found throughout the agar plate. A loopful of enrichment culture was streaked on BA which was then cross streaked with feeder colony (*S. aureus*) and incubated anaerobically at 37°C for 24hrs. Characteristic dewdrop satellite colonies were found adjacent to the feeder colony. Sample streaked on blood agar with NAD and cross streaked with feeder colony (*S. aureus*) followed by anaerobical incubation at 37°C produced more defined tiny dewdrop satellite colony (Fig. 1), than those produced on blood agar with NAD. No growth was found when cultured on blood agar without NAD or feeder organism after 48hrs of incubation. A loopful of enriched culture inoculated on CA containing NAD produced many tiny drop colonies after 48hrs of anaerobic incubation. No growth was found when cultured on CA with feeder organism after 48hrs of incubation as V-factors (NAD) were not available. Enriched samples inoculated on chocolate agar containing NAD and feeder organism produced dew drop like scattered colony. Samples were streaked on CA without providing additional NAD or feeder organism and incubated anaerobically in a candle jar at 37 °C. No growth was found on CA after 48hrs of anaerobic incubation. Organisms revealed Gram negative, small rod or cocco-bacilli with a tendency for filament formation. No turbidity and no changing of color of MIU medium were found indicating that the organisms were non motile. All the isolates fermented glucose, sucrose, maltose and mannitol and produced acid but did not ferment lactose. The organisms were found negative for MR, VP, indole and catalase tests. A total of 21 samples with clinical signs of infraorbital swelling, nasal exudates and decrease in egg production, were collected from Gazipur. Out of 21 samples3 were found positive while the rest 18 were found negative. From this study it could be mentioned that exudates of swollen infraorbital sinus was the main source of sample to be collected as the two isolates were obtained from them.

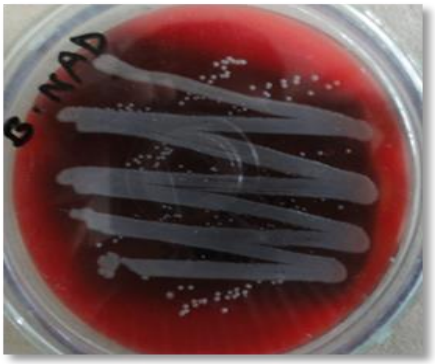

Fig 1. Predominant tiny dewdrop satellite colony on blood agar containing both NAD and feeder organism.

Antibiotics sensitivity tests

Antimicrobial sensitivity test was performed by disk diffusion method with eight chosen antimicrobial agents. The test isolates were sensitive to Ciprofloxacin, Chloramphenicle and Gentamicin but resistant to Ampicillin, Amoxycillin, Oxytetracycline, Erythromycin and Sulphamethoxazole.

Discussion

The identification of *A. paragallinarun*proved to be a cumbersome task. The present work also involved the determination of growth factor requirement of *A. paragallinarum* through inoculation on different culture media provided with variable amount of growth factors. The cultural medialike BA and CA were used with and without NAD and cultured with or without feeder organism of *S. aureus*. It was revealed that the organism grew more frequently with typical dewdrop colonies when cultured with BA containing both NAD and feeder organisms. These findings are in complete agreement with Soriano *et al.* (2004).

Scattered dewdrop like colonies with no hemolysis was found throughout the agar plate when cultured on BA with NAD which was similar to the findings of Sobti *et al.,* (2001). Characteristic dewdrop satellite colonies were observed adjacent to the feeder colony, when cultured on BA with feeder organism (*S. aureus*) which was in agreement with the findings of Sameera *et al.* (2001), Blackall *et al.* (1989), and Page *et al.* (1963). When cultured on BA with NAD and feeder organism (*S. aureus*), there was appearance of tinier dewdrop satellite colonies than that produced on BA with NAD. These results correlated with Soriano *et al.* (2004).

The growth of the organism on CA containing NAD and no growth of the organism on CA in the absence of NAD confirmed the bacteria as *Avibacterium paragallinarum*.Similar findings were reported by Chen *et al.* (1993), Quinn *et al.* (1994), Keslerk *et al.* (1997), Chukiatsiri *et al*. (2010) and Akthar *et al*. (2001). Further, on staining, the isolated bacteria appeared Gram negative small rod or cocco-bacilli with a tendency for filament formation which was also reported by Jaswinder *et al.* (2004), Sameera *et al.* (2001), Yamamoto *et al.* (1991) and Sawata *et al.* (1980). With regard to motility test, all the isolates were non motile in MIU medium which was in harmony with the findings of Blackall *et al.* (1989). The result of biochemical tests revealed negative reaction in MR-VP, Indole and Catalase tests and fermentation reaction with sugar were in agreement with the findings of Sameera *et al.* (2001) and Jaswinder *et al.* (2004).

Avibacterium paragallinarum isolates were detected as multidrug resistant (MDR) and were found resistant to Ampicillin, Amoxycillin, Sulphamethoxazole, Erythromycin and Tetracycline but sensitive to Ciprofloxacin, Chloramphenicol and Gentamicin.Almost similar antibiogram profiles were also recorded by Haunshi *et al.* (2006),Sameera *et al.* (2001) and Kurkure *et al.* (2001). There were two reported of cases of investigation of infectious coryza like one of Thalha *et al.,* (2001) at Mymensingh and the other by Islam *et al* (2004) at sylhet performed bypost-mortem examination and there was no isolation of the causal agent.

Conclusions

In Bangladesh infectious coryza is an important disease of economic significance occurring in multiage layer flocks but diagnosis of the disease still now based on postmortem examination of the dead birds.In the present study *A. paragallinarum*like organism was isolation and identification of from Layer Chickens in Gazipur, Bangladesh. This organism grew more frequently with typical dewdrop like satellite colony when cultured on BA containing both NAD and feeder organism. Further in depth study on serogroup determination, molecular detection and characterization of *A. paragallinarum* is warranted.

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