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AVIAN BORDETELLOSIS: A SIGNIFICANT BACTERIAL RESPIRATORY DISEASE OF TURKEYS (*Meleagris gallopavo*)

Wafaa A. ABD EL-GHANY

Poultry Diseases Department, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt

Email: wafaa.soliman@cu.edu.eg

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ABSTRACT: This review was designed to spotlight on avian bordetellosis regarding the bacterium pathogenesis, susceptibility, transmission, pathology, laboratory diagnosis, and prevention and control measures. Bordetellosis (moreover called turkey coryza) is a contagious bacterial upper respiratory disease of poultry, especially turkey poults. The disease is characterized by high morbidity and low mortality with terrible economic losses for turkeys industry. Bordetellosis is caused by *Bordetella avium* (*B. avium*) bacterium which colonizes and destructs the cilia of the respiratory tract. Concurrent infection during bordetellosis outbreaks is common and contributes to the poor performance of *B. avium*-infected flocks. Domesticated and wild birds are susceptible to bordetellosis. All ages can get infection with bordetellosis, however, young ages are more susceptible than adult. Infection and transmission of *B. avium* occurs through aerosol, water, and reservoirs, but not vertically. The clinical picture of bordetellosis is usually upper respiratory, except in complications with other bacteria or stressors, the infection extends to the lower respiratory tract. The bacterium is isolated aerobically on 10% sheep blood agar and appears as Gram negative bacilli. Endotoxin, tracheal cytotoxin, heat-labile dermonecrotic toxin, and osteotoxin are produced by *B. avium*. Antibiotic treatment of *B. avium* shows variable results and it is usually ineffective. Different types of living and inactivated vaccines are used to prevent bordetellosis.

Keywords: *Bordetella avium*, Diagnosis, Domesticated birds, Pathogenesis, Turkey coryza.

INTRODUCTION

Avian bordetellosis or turkey coryza is a highly contagious bacterial respiratory disease of different avian species, mainly turkeys (Ehsan et al., 2020). *Bordetella avium* (*B. avium*) bacterium is the causative agent of bordetellosis in domesticated turkeys (Skeeles and Arp, 1997; Boulianne et al., 2020) and chickens (Boulianne et al., 2020). Infection with *B. avium* is disseminated in domestic and wild birds (Stenzel et al., 2017). Since the 1970s, bordetellosis has been considered a major health problem in young birds especially turkey poults. The disease affects all ages of birds with a significant economic losses in poultry industry. Although bordetellosis causes low mortality, its high morbidity could be problematic for the production performance of broiler turkeys especially in early weeks of life. In the United States of America (USA), the disease is ranked as the third of the most important challenging diseases affecting turkeys industry (Beach et al., 2012) and causes several million dollars losses (Skeeles and Arp, 1997). Coinfections of turkeys with *B. avium* and other bacterial infections such as *Escherichia coli* (Van Alstine and Arp, 1987), *Ornithobacterium rhinotracheal* (El-Sukhon et al., 2002; Szabó et al., 2015), *Chlamydia psittaci*, *Pseudomonas fluorescens*, and *Klebsiella pneumoniae* (Hinz et al., 1992) were reported.

Bordetellosis was first described in Canada in 1967 (Filion et al., 1967). Simmons et al. (1976) also described bordetellosis as turkey rhinotracheitis. In Germany, Hinz et al. (1978) found a respiratory disease of turkeys as that previously observed in Canada and USA. Later on, bordetellosis has been widely distributed in many countries all over the world such as USA (Gentry-Weeks et al., 1991; Raffel et al., 2002; Beach et al., 2012), Australia (Blackall et al., 1995), Hungary (Szabó et al., 2015), Poland (Smialek et al., 2015; Stenzel et al., 2017), Brazil (Grespan et al. 2012), Turkey (Ozbey and Muz, 2006; Türkyilmaz et al., 2009), India (Balouria et al., 2019), Iran (Ehsan et al., 2020), Jordan (El-Sukhon et al., 2002), and Egypt (Abo-State et al., 2018; Erfan et al., 2018; Eldin et al., 2020). For instance, high prevalence rates of *B. avium* (54.54% and 49.18%) were detected in turkey flocks of Poland and Egypt, respectively (Stenzel et al., 2017; Eldin et al., 2020).

Accordingly, this article was designed to spotlight on avian bordetellosis regarding the bacterium pathogenesis, susceptibility, transmission, pathology, laboratory diagnosis, and prevention and control measures.

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The causative agent and pathogenesis

Previous preliminary studies designated the causative agent of turkey coryza as a *Bordetella*-like bacterium (Hinz et al., 1978). Later, this bacterium was known as *Alcaligenes faecalis* based on biochemical identification (Simmons et al., 1980). *Bordetella avium* is the causative of bordetellosis or turkey coryza. It is a bacterium belongs to the genus *Bordetella*, which contains 15 species. Moreover, *B. bronchiseptica*, *B. holmesii*, *B. parapertussis* and *B. pertussis* are present in mammalian hosts, but they are completely distant from *B. avium* that isolated from avian hosts. Other types of *Bordetellae* such as *B. ansorpii, B. bronchialis, B. flabilis, B. muralis, B. petrii, B. sputigena, B. trematum, B. tumbae* and *B. tumulicola* have not well studied.

In USA, the genome of 197N of *B. avium*, a spontaneous nalidixic acid-resistant variant of strain 197, was detected in a turkey (Gentry-Weeks et al., 1991), and then it was sequenced (Sebaihia et al., 2006). Besides, Ehsan et al. (2020) demonstrated that the sequences analysis of suspected culture in Iran were 98%, 96%, and 98% similar to *B. avium* 197N (AM167904.1), 4142 (AY925058.1), and 4156 (AY925068.1) sequences, respectively. Early in 70th, the preliminary studies designated the bacterial causative agent of coryzs in turkey as a *Bordetella*-like organism (Hinz et al., 1978). Simmons et al. (1980) biochemically identified *Bordetella*-like bacterium as *Alcaligenes faecalis*. Later, *B. avium* was defined as the causative agent of bordetellosis or turkey coryza disease. This bacterium was classified under the genus *Bordetella* that contains 15 species. Other *Bordetella* species such as *B. bronchiseptica*, *B. holmesii*, *B. parapertussis* and *B. pertussis* that were detected in mammalian hosts are completely distant from *B. avium* in avian hosts. Moreover, *B. ansorpii, B. bronchialis, B. flabilis, B. muralis, B. petrii, B. sputigena, B. trematum, B. tumbae* and *B. tumulicola* have not well studied in different avian species. The genome map of 197N of *B. avium*, a spontaneous nalidixic acid-resistant variant of strain 197, was detected in a turkey early in USA (Gentry-Weeks et al., 1991), and then the bacterium was full sequenced (Sebaihia et al., 2006). The recent Iranian study of Ehsan et al. (2020) showed that the sequences analysis of suspected culture were 98%, 96%, and 98% similar to *B. avium* 197N, 4142, and 4156 sequences, respectively.

Virulence key factors for *B. avium* pathogenesis can include specific adhesion to the respiratory epithelium cilia and local mucosal injury (Knab et al., 2020). *Bordetella avium* colonizes and adheres specifically to the local ciliated epithelium of the upper respiratory tract upon infection (Temple et al., 1998). Then, the bacterium induces ciliostasis, apoptosis, and extrusion of ciliated cells from the epithelium (Miyamoto et al., 2011). There was an association between the *in-vitro* ability of *B. avium* to adhere the tracheal ciliated epithelium and its ability to colonize the respiratory tracts of turkeys (Temple et al., 1998). *Bordetella* adhesion, represented by filamentous haemagglutinin, is present on the bacterial surface and plays an essential role in the adhesion and the colonization process of the host respiratory epithelium (Edwards et al., 2005). Therefore, fimbriae and hemagglutinin may contribute to *B. avium* pathological effects (Arp et al., 1988). Many *B. avium* isolates can produce toxins such as endotoxin, tracheal cytotoxin, heat-stable dermonecrotic toxin, and osteotoxin, besides hemagglutinine which are involved in the pathogenesis of bordetellosis (Figure 1; Rumińska and Koncicki, 1999). Gentry-Weeks et al. (1988) reported that both dermonecrotic toxin and tracheal cytotoxin are the main virulence factors for *B. avium in-vivo* pathogenicity as they concerned with the damage of tracheal tissue.

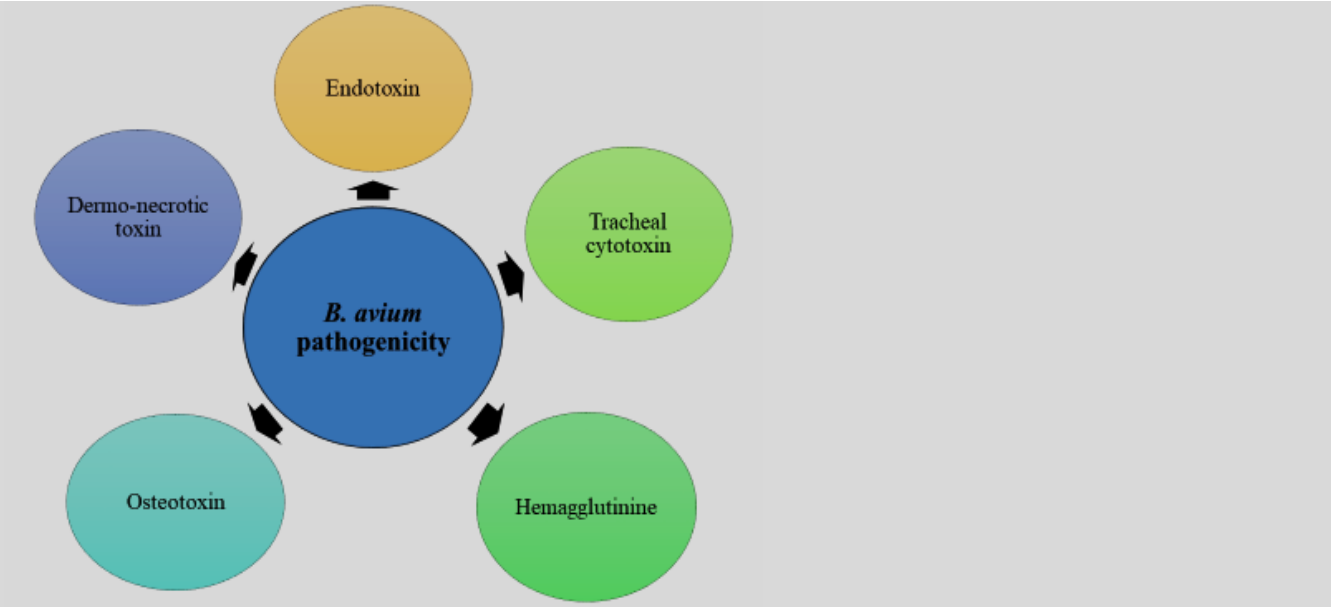

Figure 1 - Factors associated with the pathogenicity of *B. avium* infection

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Virulence-associated expression genes such as *bvgA*, *fhaB*, and *fimA* are crucial for the pathogenicity of *B. avium* (Spears et al., 2003; Temple et al., 2010; Linz et al., 2016; Eldin et al., 2020). The adhesion gen, *fhaB*, is very important and its expression is regulated by *bvgA.* Flagellar genes which are responsible for the motility and the attachment of *B. avium* to the epithelium are also essential for the organism virulence (Linz et al., 2016). Eldin et al. (2020) confirmed that *B. avium* strains carried virulence-associated genes including *Bordetella* virulence gene (100%), fimbriae (71.14%), and filamentous hemagglutinin (85.68%) which are responsible for colonization of the bacterium in the respiratory tract of turkeys. It has been observed that strains of *B. avium* from cockatiels and turkeys did not show difference in virulence-associated characters such as tracheal attachment or cytotoxic effects (Grespan et al., 2012). According to the results of Knab et al. (2020), both adherence and ciliostasis assays could be used for characterization of *B. avium* virulence and any reduction of virulence could be attributed to the variations in the filamentous haemagglutinin protein. Hemagglutinin produced by *B. avium*, besides autotransporter proteins and fimbriae play the major role adhesion process (Loker et al., 2011; Stockwell et al., 2011). It has been found that *B. avium* can agglutinate guinea pig erythrocytes and the loss of hemagglutination capability results in bacterial attenuation (Temple et al., 2010).

Susceptibility

Species

A wide range of avian species develop bordetellosis, but turkey is regarded as the most susceptible host. Domestic and wild birds are susceptible to the disease (Raffel et al., 2002). In Germany, *B. avium* was isolated from domestic and wild birds such as Muscovy ducks, geese, a yellow-crested cockatoo, parrot finches, and partridges. Moreover, *B. avium* was molecularly detected in the tracheal swabs of common pheasants in Poland (Stenzel et al., 2017). Infection with *B. avium* causes locked jaw syndrome in cockatiels (Grespan et al., 2012). High titers of antibodies against *B. avium* were detected in Canada goose, but relative low titers were present in pigeons and doves (Raffel et al., 2002).

Age

All ages of birds can get *B. avium* infection, however, younger ages are more susceptible than adult (Hinz et al., 1978; Kersters et al., 1984). Two to 6-week-old turkey poults are highly susceptible to infection. Smietanka et al. (2014) found that 3-week-old turkey poults are likely to be infected sub-clinically with *B. avium*. Significant positive association between the age of birds and the titer of anti-*B. avium* antibodies was observed. Previous exposure to *B. avium* leads to development of antibodies which might be transferred to the hatching poults causing reduction of the clinical signs severity. Moreover, an increase in the titer of antibodies to *B. avium* was detected by increasing the age of turkeys from 5 to 56 days (Beach et al., 2012). Adult birds had *B. avium* infection at young age showed detectable level of antibodies in response to that infection, while young birds have had less time to become infected and develop antibodies (Raffel et al., 2002).

Infection and transmission

Horizontal transmission is important for the spread of *B. avium* infection. Transmission may occur via aerosol, water, or litter contamination, and the bacterium can remain viable and virulent in humid litter for at least 6 months (Skeeles and Arp, 1997). The bacterium also seems to live in water like other *Bordetella* species (Porter and Wardlaw, 1993). Additionally, presence of *B. avium* in solitary species such as wood thrush indicated that reservoirs may be important for the direct contact among birds in the wild (Raffel et al., 2002). Therefore, transmission of *B. avium* from free-living birds to domesticated poultry may be possible. *Bordetella avium* does not transmitted vertically from dams to their offspring (Jackwood and Saif, 2008). Exposure of birds to different management environmental stress conditions is an important predisposing factor for spreading of infection (Bartz et al., 2018). This indicates the role of biosecurity measures in the control of turkey coryza. Moreover, it has been reported that immunosuppressive viral infections could enhance the severity of *B. avium* infection (Liang et al., 2013).

The similarities between *B. avium* and *B. pertussist*; one of the causes of human's respiratory affections have been demonstrated (Gentry-Weeks et al., 1988; Spears et al., 2003). Lastly, *B. avium* has been detected in persons with cystic fibrosis (Spilker et al., 2008). Accordingly, both *B. avium* and *B. avium*-like organisms are regarded as opportunistic pathogens for humans. The gene sequence analysis of 16S rRNA of *B. avium* and *B. pertussist* revealed 98% and 100% nucleotide similarities with *B. avium* ATCC 35086 strain (Harrington et al., 2009).

Pathology

Bordetella infection in poultry is characterized by sudden onset and rapid spread. The severity of clinical signs is milder in chickens than in turkey poults. Respiratory signs including sneezing, foamy nasal and ocular discharge, submaxillary edema, cough, moist tracheal rales, dyspnea, and altered vocalization could be observed in susceptible birds (Jackwood and Saif, 2013). The disease course is about 2–4 weeks (Panigrahy et al., 1981). Bordetellosis is characterized by high morbidity and low mortality rate especially in cases without complications. Turkeys had passive humoral immunity

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against *B. avium* were protected against the development of clinical signs after experimental infection (Hinz et al., 1981; Rimler and Kunkle, 1997). Postmortem lesions of *B. avium* infection include conjunctivitis, presence of exudates in the nasal cavity, trachea, and bronchi, pneumonia, and cloudiness and turbidity of air sacs (Saif et al., 1981; Arp and Cheville, 1984). Deformity of tracheal rings and damage to articular cartilages (collapsed trachea) may be related to osteotoxin activity (Stenzel et al., 2017). Complicated cases with secondary bacterial and viral infections showed visceral lesions. Tracheal sections of *B. avium* infected turkeys showed mucosal separation, lympho-plasmacytic infiltration, and loss of cilia (Van Alstine and Arp, 1988). Besides, severe pneumonia with congested blood vessels, and airsacculitis associated with edema and infiltration of lymphocytes and macrophages were also observed (Eldin et al., 2020).

Laboratory diagnosis

Isolation

Culturing of *B. avium* was previously described (Register and Jackwood, 2016). The bacterium grows aerobically on blood agar plates supplemented with 10% sheep blood for 24–48 hrs. Other concomitant fast growing bacteria such as *Escherichia coli* may associated with *B. avium* infection that can cover the growth of *B. avium* and may create some difficulties in the isolation process. Therefore, some antibiotics such as aztreonam and ampicillin could be added to the culture media to inhibit the growth of other opportunistic bacteria without affecting *B. avium* growth. It has been detected that administration of antibiotics before sampling may induce failure of *B. avium* isolation (Türkyilmaz et al., 2009).

Identification

Typical colonies of *B. avium* isolates are small or pinpoint, glistening, translucent, compact, and pearl-like with glistening surface with entire edges. Colonies of *B. avium* are 1–2 mm in diameter after 48 hs of incubation. Gram staining of suspected culture revealed Gram-negative bacilli. Biochemically, *B. avium* shows positive reactions for catalase, oxidase, and citrate, but negative reaction for lactose fermentation, urease, and nitrate reduction (Simmons et al., 1980). Moreover, on triple sugar iron agar, no acid production in the butt or the slant was determined after overnight incubation of *B. avium* isolates, however, H_2S production was detected. Biochemical tests were also used to distinguish *B. avium* from other non-fermentative bacteria or other *Bordetella* species such as *B. hinzii* (Blackall and Farrah, 1986).

Molecular characterization

Molecular assays such as *Pvu*II ribotyping and restriction endonuclease analysis using either *Hinf*I or *Dde*I could be used to distinguish *B. avium* from other species of *Bordetellae* (Sacco et al., 2000). Polymerase Chain Reaction (PCR) is also a valuable tool to detect *B. avium* infection (Ehsan et al., 2020). This test showed 100% sensitivity and 98.8% specificity for the identification of *B. avium* from different locations over 25 years (Register and Yersin, 2005). TaqMan real-time PCR detected presence of *B. avium* in tracheal swabs of pheasants at 54.54% in Poland (Stenzel et al., 2017). In the Egyptian study of Eldin et al. (2020), the overall PCR-confirmed prevalence rate of B. *avium* was 22.95% (14 out of 61). Although antibodies against *B. avium* could be serologically detected in the serum of turkeys, the PCR test may indicate negative results (Türkyilmaz et al., 2009). For the first time in Egypt, Erfan et al. (2018) reported on sequencing of *B. avium* isolates from turkeys and found that amplified 740 bp of *rec*A gene showed 100% maximum identity with German *B. avium* ATCC 35086 strain and with the American strain 197N.

Enzyme Linked Immuno-sorbent Assay

It has been shown that Enzyme Linked Immuno-sorbent Assay (ELISA) is considered as the most sensitive test for serological screening of *B. avium* infection in turkeys and chickens (Tsai and Saif, 1991). This test can give an indication for the previous or recent exposure of the flock to *B. avium* infection. The prevalence rate of *B. avium* among turkey flocks in Egypt was 72.13% (44 out of 61) using ELISA (Eldin et al., 2020). In Poland, Smialek et al. (2015) found anti-*B. avium* immunoglobulin Y in young and old ages turkeys. A positive correlation between the increase in the antibody titers against *B. avium* and the age of the birds was detected (Stenzel et al., 2017). Past exposure of turkeys to *B. avium* infection resulted in development of passive humoral immunity and protection of birds from the development of signs after experimental infection (Rimler and Kunkle, 1997). Additionally, Beach et al. (2012) found an increase in the titer of *B. avium* antibodies by increasing the ages of turkeys. Positive significant correlation has been reported between the age of diseased turkeys and the titer of antibodies against the bacterium (Eldin et al., 2020).

Prevention and control measures

Biosecurity measures

Application of strict biosecurity measures in turkey flocks is the must for prevention of bordetellosis. Thorough cleaning and disinfection of farms are crucial as the bacterium can persist for months in damp litter (Van Alstine, 1987). Thus, clean up measures are required to remove *B. avium* from contaminated premises, besides hygienic disposal of litter, and disinfection of all surfaces, feeders, and drinking water systems.

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Treatment

Antibiotic treatment of *B. avium* shows variable success; it is likely that antibiotics may treat the secondary bacterial infections instead of *B. avium*. Very early study of Glunder et al. (1979) showed that water treatment of *B. avium* infected turkeys with sulphaquinoxalin/trimethoprim or tetracycline for 5 days was effective, but relapse occurred after the discontinuation of the treatment and the carrier birds were not eliminated. Thus, treatment of *B. avium* with various drugs was often ineffective. Isolates of *B. avium* showed variations in their susceptibilities to various antimicrobials in different countries (Beach et al., 2012; Grespan et al., 2012). Formerly, strains of *B. avium* showed tetracycline resistance (Cutter and Luginbuhl, 1991). Further, in Minnesota in 1998–1999, 4 isolates of *B. avium* were susceptible to ampicillin and tetracycline, while they were resistant to erythromycin (Malik et al., 2005). Moreover, 17 *B. avium* strains collected over thirty years from turkey flocks in USA revealed sensitivity to gentamicin, cefoperazone, cefepime, ceftazidime, piperacillin, and amikacin, and resistance to chloramphenicol, ampicillin, cipfloxacin, sulfa-trimethoprim, and oxytetracycline (Beach et al., 2012). In Hungary, turkey isolates of *B. avium* showed complete resistance to ceftiofur and lincomycin, and moderate resistance to chloramphenicol and nalidixic acid, but complete sensitivity to sulfa-trimethoprim, polymyxin B, and gentamicin (Szabó et al., 2015). Among 50 *B. avium* isolates, the range of resistance against cephalosporin, penicillin, erythromycin, and enrofloxacin were more than 50% (Nhung et al., 2017). Ehsan et al. (2020) detected that *B. avium* isolates in Iran were partially sensitive to ampicillin. The author's referred ampicillin-resistance of *B. avium* isolates to the lack of penicillin-binding protein 3 (PBP3) gene. In an Egyptian study of Erfan et al. (2018), the results of the antibiogram showed that *B. avium* isolates were susceptible to norfloxacin, ciprofloxacin, cefotaxime, florfenicol, and gentamicin, while isolates were resistant to ampicillin, erythromycin, oxytetracycline, sulphamethxazole/trimethoprime, and lincomycin exhibited the highest resistance rates. Moreover, Egyptian isolates of *B. avium* were resistant to penicillin, ceftiofur, nalidixic acid, and lincomycin, but sensitive to gentamicin and neomycin (Eldin et al., 2020). These differences in sensitivities or resistances among *B. avium* isolates might be owing to the uncontrolled application of antimicrobials and presence other concurrent infections and environmental factors.

In a study of Ehsan et al. (2020), in Iran, the author referred the partial ampicillin sensitivity to the absence of penicillin-binding protein 3 gene in *B. avium* strains. Some Egyptian *in vitro* studies revealed that isolates of *B. avium* were resistant to ampicillin, lincomycin, erythromycin, sulphamethxazole/trimethoprime, and oxytetracycline, but they were sensitive to ciprofloxacin, norfloxacin, gentamicin, florfenicol, and cefotaxime (Erfan et al., 2018). Nearly similar Egyptian study of Eldin et al. (2020) demonstrated that *B. avium* were resistant to penicillin, lincomycin, ceftiofur, nalidixic acid, while they were susceptible to gentamicin and neomycin.

Vaccination

Vaccination of turkeys is another approach for prevention of bordetellosis. Commercially available vaccines for bordetellosis may have marginal efficacy, possibly due to vaccine delivery or strain specificity. As a result of *B. avium* persistence in the premises of the infected farm, vaccinated poults would experience an anamnestic response due to continuous exposure to infection.

Living vaccines against bordetellosis have been used in many countries especially in the early life of turkey. Therefore, detection of maternal derived antibodies is important prior to vaccination (Smialek et al., 2015). Oral vaccination with a temperature-sensitive mutant strains of *Alcaligenes faecalis* could protect turkey flocks and produced humoral antibodies in vaccinated turkeys without development of alcaligenes rhinotracheitis outbreaks (Jensen and Marshall, 1981). Moreover, Burke and Jensen (1981) demonstrated that vaccination of 6-week-old turkeys with a temperature-sensitive mutant of *Alcaligenes faecalis* via drinking water in doses of 90 million bacteria resulted in development of a high degree of protection against challenge with the same bacterium.

Twice vaccinations of turkey poults with *B. avium* inactivated oil-adjuvant vaccine at 4 and 27 days of age induced significant immune response to the challenge at 41 days of age and the vaccinated birds were able to eliminate the bacteria faster than non-vaccinated ones (Glunder et al., 1980). Jackwood and Saif (1980) did not find maternal immunity against the infection in offspring's after vaccination of turkey parent hens with formalin inactivated bacteria or with an aluminium hydroxide adjuvant bacterin. Whereas, the study of Hinz et al. (1981) showed that subcutaneous vaccination of 24 and 28-week-old turkey breeder flocks with heat-inactivated and Freund's adjuvant *B. avium* bacterin could protect the progeny against infection within the first 10 to 17 days after hatching. Moreover, agglutinating antibodies were transmitted via the yolk and detected in the progeny. Johnson et al. (1980) found that convalescent turkey flocks with a history of previous exposure to *Bordetella* infection showed usual delay in the onset as well as the severity of the disease. Progeny from vaccinated breeder turkey hens with double doses of oil emulsion adjuvanted *Alcaligenes faecalis* bacterin showed considerable resistance to infection, improved livability and growth, and delayed onset of infection with less severe clinical picture even after infection (Barnes and Hofstad, 1983). Subcutaneous vaccination of day-old turkey poults with formaldehyde and aluminum hydroxide *B. avium* inactivated bacterin induced antibody titers twice the levels that were found in non-exposed flocks. Moreover, Akeila and Saif (1988) studied the effect of oil-adjuvant pili in protection against *B. avium* infection in turkey poults in comparison with other types of bacterins. The authors postulated that *B. avium* pili are important immunogens in terms of reduction of the disease severity and isolation of the bacterium from the respiratory tract.

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CONCLUSION

Avian bordetellosis represents a significant problem especially for turkey industry. Research work regarding the surveillance studies of *B. avium*, especially in some developing countries, is much needed. In addition, increasing awareness about the rational usage of antimicrobials will decrease the possibilities of *B. avium* treatment failure, and consequently reduce related financial losses. Trials for preparation of local or autogenous vaccine against *B. avium* is crucial as a preventive measure. Besides, application of strict biosecurity measures can reduce the incidence of bordetellosis among turkey flocks.

DECLARATIONS

Corresponding author

E-mail: wafaa.soliman@cu.edu.eg

Author's contribution

Abd El-Ghany WA has collected and drafted the manuscript, formatted it, and approved the final manuscript.

Conflict of interests

The author has not declared any conflict of interest.

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