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Epidemiological prevalence of *Pasteurella multocida* in ducks

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Abstract

***Pasteurella multocida* is known to cause duck cholera leading to high morbidities and mortalities resulting in huge economic losses. Acute duck cholera is characterized by sever watery diarrhea, anorexia, respiratory manifestations and septicemia, while chronic one leads to localization of the organism in certain organs. This study was undertaken to investigate the prevalence of *Pasteurella multocida* in ducks in Egypt. Expression of some virulent-genes in the isolated *Pasteurella multocida* serotypes were examined using the polymerase chain reaction. Finally, antibiotic sensitivity testing of the identified *Pasteurella multocida* serotypes was also done. *Pasteurella multocida* could be isolated from 60% of the ducks showing respiratory manifestations and watery diarrhea. 69.8% of the isolated *Pasteurella multocida* strains followed capsular type A serotype, 20.9% of capsular type D and 9.3% as untypable. Lungs and air sacs had the highest incidence of *Pasteurella multocida* followed by liver, heart and spleen. *Pasteurella multocida* serogroups highly expressed virulence-attributes responsible for diarrhea. *Pasteurella multocida* serogroups showed marked resistance towards some used antibiotics in Egypt.**

Key words: *Pasteurella multocida,* ducks, Egypt

Introduction

Pasteurella multocida (P. multocida) causes cholera, which is a contagious and septic disease in ducks[7)]. Duck cholera is one of the most important diseases in the duck industry because prevalence of *P. multocida* carriers in healthy duck flocks is as high as 63%, and mortality may reach 50%[16)].

Several subspecies of bacteria have been proposed for *P. multocida,* and at least 16 different *P. multocida* serotypes have been recognized. Infection in poultry generally

results when *P. multocida* enters the tissues of birds through the mucous membranes of the pharynx or upper air passages. The bacterium can also enter through the membranes of the eye or through cuts and abrasions in the skin. Duck cholera is a disease that is characterized by its acute nature and causing high and rapid deaths. Few sick birds could be seen during duck cholera outbreaks. However, the number of sick birds increases when a die-off is prolonged over several weeks. Sick birds often appear lethargic or drowsy, have convulsions or throw their heads back between their wings and die. Other signs include mucous discharge from the mouth; soiling and

matting of the feathers around the vent, eyes, and bill; pasty, fawn-colored, or yellow droppings; and blood-stained droppings or nasal discharges, which also are signs of duck plague[7].

This study was undertaken to investigate the prevalence of *P. multocida* in ducks in Egypt. Expression of some virulent-genes in the isolated *P. multocida* serotypes was examined using the polymerase chain reaction. Finally, antibiogram of the identified *P. multocida* serotypes was also tested

Materials and Methods

Collection of Samples:

A total of 100 samples including 20 each of lungs, air sacs, livers, hearts and spleens were collected from 20 ducks (different breeds) suffering from diarrhea, respiratory manifestations. The collected ducks were selected randomly from cases visiting the Veterinary Animal Hospital, Faculty of Veterinary Medicine, Zagazig University, Egypt. Specimens were obtained aseptically using a sterile scalpel while taking precautions to prevent surface contamination. Following collection, the samples were transferred with undue delay to the Microbiology laboratory in Veterinary Animal Hospital, Faculty of Veterinary Medicine, Zagazig University, Egypt.

P. multocida screening:

Isolation of *P. multocida* was carried out according to the method described before13). Briefly, swabs were obtained from the collected samples and were plated on tryptic soy agar (Difco, Detroit, MI) containing 10 μg/ml NAD (Sigma, St. Louis, MO) and 5% bovine serum, MacConkey agar, and blood agar (5% fresh sheep blood). All plates were incubated at 37 °C in air for a minimum of 48 h.

Identification of isolates:

Preliminary identification of P. multocida isolates was carried out according to standard biochemical tests as described earlier[13]. The isolates were gram-negative coccobacilli and were indole, catalase and oxidase-positive. However, citrate, Methyl red (MR), Vogaes-Proskauer (VP), and gelatin liquefaction negative. They do not grow on MacConkey agar and do not show hemolysis on blood agar. Confirmation of the isolates was done by polymerase chain reaction (PCR) assay with primers specific for the amplification of the KMT1 gene, using the method before[15]. All confirmed isolates of *P. multocida* were subsequently characterized by capsular serotyping using PCR. Primers for amplification of hyaD-hyaC and DcbF genes were used for detection of capsular type A and capsular type D, respectively (Table 1). P. multocida isolates which did not yield bands on PCR when the two primers were used were classified as untyped. Following confirmation and characterization all isolates were freeze-dried and kept at -20 °C.

Detection of virulence genes:

The virulence genes of *P. multocida* isolates were detected by PCR. They included adhesins (fimA), toxin (toxA) and iron acquisition (tonB). The base sequences and the predicted sizes of the amplified products for the specific oligonucleotide primers used in detection of the genes in this study are shown in Table 1[15]. The bacterial lysates used as templates for the PCR were prepared as follows. A loopful of bacteria from a fresh overnight culture on a tryptic soy agar plate was resuspended homogeneously in 200 μl of sterile water, and the mixture was boiled at 100°C for 5 min to release the DNA and centrifuged. A 4 μl volume of the

Table1: Primer sequences, amplicon size, and annealing temperatures of different genes used in PCR in this study.

Gene	Primer sequence (5–3)	Amplicon size (bp)	Annealing (ºC)
KMT1	ATCCGCTATTTACCCAGTGG GCTGTAAACGAACTCGCCAC	460	55
hyaD-hyaC	CATTTATCCAAGCTCCACC GCCCGAGAGTTTCAATCC	760	55
DcbF	TTACAAAAGAAAGACTAGGAGCCC CATCTACCCACTCAACCATATCAG	657	55
fimA	CCATCGGATCTAAACGACCTA AGTATTAGTTCCTGCGGGTG	866	55
toxA	CTTAGATGAGCGACAAGG GAATGCCACACCTCTATAG	864	55
tonB	CGACGGTGAAACCTGAGCCA CCGAGCGATAAGCATTGACT	261	55

supernatant was used as a template for each 25 μl PCR mixture. The amplified products were analyzed in 1% agarose gels by electrophoresis, and the results were recorded with a gel documentation system. Samples were sequenced for gene verification.

Antibiogram:

Antibiotic sensitivity test was performed according to the procedures of Muller Hinton agar with 5% blood[3] by placing 20 mm antibiotic discs and measuring the diameter of zone of inhibition. The tested antibiotics were chosen based on EFSA recommendations on antimicrobials to be included in the antimicrobial resistance monitoring studies[4]. In addition, we tested some antimicrobial agents, which are commonly used in Egypt after asking some veterinarians and some owners of duck farms in Egypt. The results were interpreted as resistant, intermediate, and susceptible.

Results and Discussion

P. multocida *is a common problem in duck farms in* Egypt, which lead to significant economic losses. In this study, we could isolate P. multocida from 60% of the examined ducks suffering from respiratory manifestations, lameness, corneal turbidity and diarrhea. All *P. multocida* identification was confirmed by PCR testing of the KMT1 gene, which is a specific chromosomal region unique to *P. multocida*[15]. Similarly, *P. multocida* was previously isolated from ducks in Japan and South Korea[14,17]. Two capsular types (A and D) were detected among 39 of the 43 *P. multocida* isolates obtained as seen in figure 1. The majority (69.8%) of the isolates were of capsular type A. *P. multocida* isolates of capsular type D type was of 20.9% while 9.3% of the isolates was untypable (Fig. 1). These results go in agreement with the previous reports[9,14]

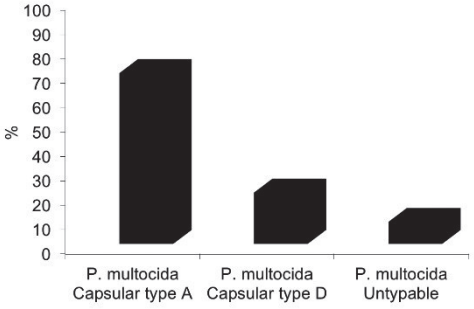

Fig. 1. Incidence (%) of different *P. multocida* serotypes isolated from naturally diseased ducks

Lungs and air sacs had the highest incidence of *P. multocida* followed by liver, heart and spleen. The incidence percentages of P. multocida in these tissues were 100%, 83.33%, 66.66%, 58.33% and 50% respectively as clear in figure 2. These results corresponds with the findings of Khamesipour et al. (2014), who could detect high incidence of *P. multocida* in the lungs of cattle. Spreading of *P. multocida* in different tissues goes in line with Hunter and Wobeser (1980), who reported that ducks that died acutely of avian cholera had lesions of a hemorrhagic septicemia with widespread vascular damage and focal necrosis in liver, spleen and other organs. Ducks that survived challenge developed chronic lesions in a variety of organs, including brain, lung, air sacs, joints, and eyes.

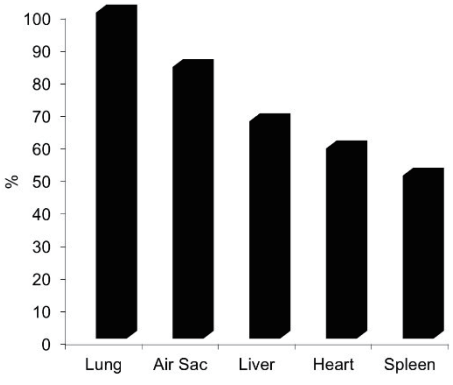

Fig. 2. Incidence (%) of *P. multocida* in different tissues of naturally diseased ducks

Virulence factors play a key role in disease production by bacterial pathogens. Their functions include competence, adherence, synthesis, and export of capsules; and evasion of host immune responses[12]. In the present study, all identified *P. multocida* isolates from naturally infected ducks harbored at least one virulence gene as displayed in fig. 3. Figure 3 shows the distribution of virulence genes by capsular serotypes. The tested isolates strongly expressed fimA gene, which is responsible for the adhesions. Fimbria (fim A) gene plays a key role of fixing bacterial pathogens on the surface of the epithelial cells of hosts[5]. Presence of adhesins on the bacterial surface is usually linked to virulence, as these proteins are known to play a crucial role in facilitating host invasion and colonization[10]. It is noteworthy that the dermonecrotoxin encoding toxA gene was not detected among the isolates (Fig. 3). Some other researchers indicated that this particular gene is more frequently expressed by strains of serogroup D[6]. The observation in the current study could be attributed to the small sample size of capsular type D isolates. Unlikely, tonB, the gene responsible for iron acquisition was strongly expressed in all tested isolates (Fig. 3). The obtained results in this study corresponds with the results recorded before [9,17].

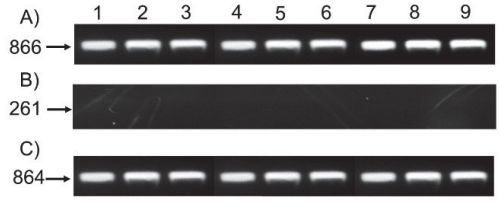

Fig. 3. DNA expression of different virulece genes in *P. multocida* isolates.
A)fimA, B)toxA, C)tonB
Lanes 1-3: *P. multocida* capsular type A.
Lanes 4-6: *P. multocida* capsular type D.
Lanes 7-9: *P. multocida* untypable strains.

Infections with *P. multocida* are commonly managed by broad spectrum antimicrobials[2]. Studies have however reported occurrence of resistance to a large number of antimicrobial agents among *P. multocida* isolates[1]. In the current study, antimicrobial resistance profiles of *P. multocida* isolates are displayed in Table 2. All the isolates were susceptible to chloramphenicol, ciprofloxacin, enrofloxacin and oxytetracycline. Resistant to ampicillin, and slightly resistant to streptomycin and erythromycin. Similar observations for ciprofloxacin, enrofloxacin and tetracycline [6,11] have also been reported.

Table2: Antibiogram of the isolated *P. multocida* strains

Antibiotics	Resistant isolates	Intermediate isolates	Susceptible isolates
Ampicillin	25 (58.14%)	0 (0%)	18 (41.86)
Chloramphenicol	0 (0%)	0 (0%)	43 (100%)
Ciprofloxacin	0 (0%)	0 (0%)	43 (100%)
Enrofloxacin	0 (0%)	0 (0%)	43 (100%)
Gentamicin	3 (6.98%)	0 (0%)	40 (93.02%)
Oxytetracycline	0 (0%)	0 (0%)	43 (100%)
Streptomycin	5 (11.62%)	3 (6.98%)	35 (81.40)
Erythromycin	8 (18.60%)	5 (11.63%)	30 (69.67%)

In summary, the current study declared high incidence of P. multocida infection among ducks in Sharkia governorate, Egypt. *P. multocida* isolates harbored virulence associated genes and marked resistance to some of the used antibiotics in Egypt. Thus, strict precautions and preventive measures should be taken to avoid transmission of the disease among duck farms.

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