Studies on Pasteurellosis in birds


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Abstract

Two hundred pooled samples including (liver, heart and spleen) were collected from examined 454 dead birds that were suspected to be infected with fowl cholera. These birds were collected from 132 poultry farms from different governorates. Eight isolates could be biochemically identified as Pasteurella spp. Using PCR, all isolates were identified as Pasteurella multocida (P. multocida) capsular type A with overall incidence of 1.76%. The incidence of avian pasteurellosis in Sharkya (4%) was higher than that in Qaliubia (1.38%). The incidence of isolation of P. multocida from examined chickens was 2.2 (8 out of 360 chickens) while it could not be isolated from duck and turkey samples. Pasteurella multocida could be isolated only from layers chickens.

Key words: Pasteurella multocida in birds, PCR capsular typing, fowl cholera, Avian pasteurellosis, Histopathological changes of Pasteurella.

Introduction

Avian pasteurellosis has been reported as an important disease in domestic poultry for more than 200 years that causes devastating economic losses to poultry industry worldwide (Aye et al., 2001). P. multocida is a Gram negative bacterium infects a wide range of birds causing fowl cholera in poultry (Glisson et al., 2003) which is generally caused by serotype A: 1, A: 3 or A: 4.

Diagnosis of fowl cholera is based on clinical signs, pathological findings and isolation and identification of P. multocida (Rimler and Glisson, 1997).

Conventional methods of characterizing isolates of P. multocida are often time consuming and don’t type all strains. Recently, DNA-based identification and typing systems are emerging as reliable alternatives, providing rapid identification of pathogens. (Blackall and Miflin 2000).

The polymerase chain reaction (PCR) has the potential to detect low numbers of a target organism in heavily contaminated samples. Several PCR tests have been described for detection or identification of P. multocida species (Kasten et al., 1997; Townsend et al., 1998 and Miflin and Blackall, 2001).

The present study aimed to investigate the prevalence of P. multocida among chicken, duck and turkey samples in Egypt and the pathogenicity of the isolated organism in 12 week old layers.

Material and methods

Samples
A total of 200 freshly dead birds of different ages suspected to be infected with fowl cholera were collected from 132 different poultry farms (120 chicken farms, 7 duck farms and 5 turkey farms) from different localities at Qalubia, Sharkya, Minofia, Assuit and Gharbeya Governorates, Egypt. (table-1 and 2).

Heart, liver and spleen were pooled from each bird. Heart blood smears, tissue impression smears from liver were prepared and stained with Leishman stain. Heart blood and tissues were subjected to bacteriological examination for isolation of P. multocida.

Bacterial isolation and identification

The heart blood and tissue samples were inoculated into brain heart broth (Oxoid) and incubated at 37°C for 18 hrs. Then subcultured on blood agar, MacConkey agar (Quinn et al., 1994) and DAS media.
colonies were subjected to biochemical tests for identification of P. multocida (Cruijshank et al., 1975; Quinn et al., 1994 and Holt et al., 1994).

Pathogenicity in mice

Pure isolates were tested for pathogenicity to white mice. According to Balakrishnan, and Parimal (2012) as following 0.2ml of brain heart broth culture (108 C.F.U/ml) were inoculated intraperitoneally in mice and observed for 48 hr. Dead mice were subjected to post mortem examination and reisolation of the inoculated organism. Dead mice without signs and lesions were proved to their positive mortality.

Polymerase chain reaction (PCR) for identification of P. multocida

DNA was extracted from the overnight culture of Pasteurella isolates using QIAamp DNA Mini Kit Catalogue no.51304. P. multocida polymerase reaction (PM-PCR) was carried out using species specific primers KMT ISP6 and KMT 177 designed by Townsend et al., (1998) to amplify KMT1 gene. The analysis of PCR product was carried out in 1.5 % agarose stained with ethidium bromide (10mg/ml). 100bp DNA ladder and appropriate controls were incorporated to rule out false positive and false negative results. The gel was viewed under UV transillumination.

Pathogenicity of the isolated P. multocida in chickens

Freshly prepared culture from P. multocida strain (108cfu/0.5ml) (Petersen et al., 2001) was inoculated intratracheally into 12 week old layer type chickens (within 30s). The experimental birds were kept under observation for 14 days for clinical signs and/or mortality. At the end of observation all survived birds were sacrificed for lesions, re-isolation and/or histopathological examination.

Results and Discussion

P. multocida has been consistently found in the upper respiratory tract, spleen, lungs, blood and liver of infected birds (Rhoades, 1964; Hunter and Wobeser, 1980). All isolates were non hemolytic and had bipolarity.

The results of the present study revealed the isolation of 8 (2.2%). P. multocida isolates out of 200 samples, collected from freshly dead chickens, ducks and turkeys from different localities at Qalioubia, Sharkya, Minofia, Assuit and Gharbeya provinces in Egypt.

The presence of such organisms in these bird species reflects the distribution of the disease (Avian cholera) in these governorates. The isolation of P. multocida from poultry population in Egypt was reported earlier by Abd El-Dayem (1990); Ibrahim (1991): Gergis et al. (1992); Bebars (2000); Hassan et al. (2001); El-Shamy (2008) and Hekal (2009). The low isolation percentages in this study may be due to the fact that most of our samples were not taken from birds in the acute stage of the infection which agrees with the findings of Mraz et al., (1980), who found a higher prevalence of P. multocida in convalescent chicken flocks than in disease free flocks, or could be attributed to the uncontrolled use of antibiotics in nearly all farms.

For isolation of P. multocida from freshly dead birds on DAS media (DAS 1958), blood agar, brain heart infusion agar and MacConkey agar. (Carter, 1967 and Cruickshank et al., 1975).

Table (1): The prevalence of avian Pasteurellosis in different localities in Egypt.

<table>
<thead>
<tr>
<th>Governorates</th>
<th>No. of examined farms</th>
<th>No. of examined suspected birds</th>
<th>Pasteurella positive isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qalioubia</td>
<td>75</td>
<td>250</td>
<td>4 1.8%</td>
</tr>
<tr>
<td>Sharkya</td>
<td>34</td>
<td>150</td>
<td>4 4%</td>
</tr>
<tr>
<td>Minofia</td>
<td>14</td>
<td>40</td>
<td>9 0.5%</td>
</tr>
<tr>
<td>Assuit</td>
<td>5</td>
<td>22</td>
<td>0 0%</td>
</tr>
<tr>
<td>Gharbeya</td>
<td>3</td>
<td>13</td>
<td>0 0%</td>
</tr>
<tr>
<td>Total</td>
<td>122</td>
<td>454</td>
<td>8 1.705%</td>
</tr>
</tbody>
</table>
The prevalence of avian Pasteurellosis according to different avian species.

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of examined farms</th>
<th>No. of examined birds</th>
<th>Incidence of avian pasteurellosis in different species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>120</td>
<td>360</td>
<td>8</td>
</tr>
<tr>
<td>Duck</td>
<td>15</td>
<td>21</td>
<td>9</td>
</tr>
<tr>
<td>Turkey</td>
<td>5</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>132</td>
<td>454</td>
<td>8</td>
</tr>
</tbody>
</table>

The mice pathogenicity test is often used to detect *P. multocida* in samples contaminated with other microorganisms (Quan et al., 1986). White mice injected i/p with *Pasteurella multocida* strain succumbed within 24hrs (Kasten 1997). However, virulence for mice has been reported to be variable (Curtis et al., 1980). 8 strains isolated from chickens were highly pathogenic for mice. All mice died within 18-24 hours of inoculation. These results agreed with the findings of Jaya Kumar, (1998) and Balakrishnan and Parimol (2012) who recorded that *Pasteurella multocida* isolated from cases of fowl cholera were highly virulent for mice.

*P. multocida* species specific polymerase specific PCR (PM-PCR) assay developed by Townsend et al., (1998) was used in this study to identify *P. multocida* isolates by amplifying the gene encoded by clone KMT1 of *P. multocida*. The primer pair KMT1SP6-KMT1T7 amplified a product of 455 bp from all tested isolates. (Fig 1)

[Fig (1): PCR amplified products of *P. multocida* isolates
Lane 1: 100bp DNA ladder, Lane POS control positive,
Lane Neg control negative and lane 1-5 the isolates.]

Purushothaman et al.,(2008) detected *P. multocida* by the use of PCR technique. A nucleic acid based diagnostic test has been found to be more sensitive and reliable than the conventional method. The main advantages of the nucleic acid based tests are that they reduce the time consumption and allow detection of the organism's genome even if it is in minute quantities, thus increasing sensitivity and specificity of the test (Innis et al., 1999). PCR is one such test that can be used for the identification of organisms at any level, viz: strain, species, genus or all members of a domain, just by using a specific primer sequence. (Bhimani et al., 2014).

Within this investigation, *P. multocida* isolates were characterized serologically by the capsule serogroups with molecular serotyping. Carter and his colleagues (Carter and Rappay 1963 and Carter and Chengappa 1981) identified 5 serotypes (A, B, D, E, and F) apparently on the basis of differences in the capsular substances. These results agreed with those reported by Karmyet al. (1983), Abd El-Motelib and Salem (1986), Akella et al. (1986) and Kuczkonwiski et al. (2006). Also Shivachandra et al. (2006) isolated 72 strains of *P. multocida* from chickens, ducks, turkeys, geese and quails typed them in “A” and “D” serogroups. All isolates were *P. multocida* serogroup A (Fig. 4). *P. multocida* isolates obtained from chickens mostly belong to serotype A and cause avian pasteurellosis, with incidences of high mortality and morbidity in infected farms, causing significant economic losses all over the world (Rhoades and Rimler, 1989).

Townsend et al., (2001) and Mohamed and Moeman (2012) reported that PCR and multiplex PCR for capsular type detection were found to be a rapid and sensitive
The pathogenicity of the isolated 
P. multocida strains was tested in 12 week old 
chickens. An inoculation dose of 
approximately 2x10^8 C.F.U./0.5mL of 
the isolates P. multocida was used. The post 
mortem lesions were recorded. Moderate 
clinical signs and no mortalities could 
be observed in experimental chickens infected 
with the isolated organisms although the same 
inoculated killed mice within 24 hrs.

Mariana and Hirat (2000) isolated five 
Pasteurella organisms pathogenic for mice.

Glisson et al (2003) reported that 
pathogenicity or virulence of P. multocida in 
relation to fowl cholera is complex and 
variable depending on the strain, host species 
and variations within the strain or the host 
and condition of contact between the two. 
Mature chickens are more susceptible than 
young ones and turkeys are much more 
susceptible than chickens to infection with P. multocida 
(Heddleston, 1962).

Intratracheal inoculation of 
approximately 10^4 C.F.U. of the strain 
isolated from an outbreak of fowl cholera in 
wild birds including eiders, cormorants, 
coots, -catchers and guilis, was highly 
pathogenic for turkeys (100% mortality), 
Partridges (91% mortality) and pheasants 
(58% mortality), while chickens were found 
not to be much more resistant (no mortality). The 
finding is in accordance with previous 
observations showing that turkeys of all ages 
were highly susceptible to P. multocida 
infections, while chickens under 16 week of 
age were resistant (Rimler and Glisson 1997) .
Six - to fourteen weeks-old chickens, however 
have subsequently been found susceptible to intra 
tracheal inoculation with P. multocida (Scott et al., 1999 and Wilkie et al., 2000).

The routes by which P. multocida gains 
entry to the body during outbreaks of avian 
cholera are presently unknown, but there is a 
prevailing belief that P. multocida is a 
respiratory pathogen (Simensen and Olson 1980 and Gustafson et al., 1998). For this 
reason, an intra-tracheal challenge model 
was used in the present investigation. The 
inoculation dose of approximately 
10^4 C.F.U./0.5mL is considered to be a low 
dose (Matsumoto et al., 1991).

Intratracheal inoculation of chickens 
aged six-to fourteen weeks old showed that 
10^7 to 10^8 C.F.U. were required to achieve 
greater than 75% mortality (Scott et al., 1999). A universally accepted model using a 
standardized i.t. dose in chickens and other 
avian species of the same age would be 
useful for comparative studies of virulence 
Mbuthia et al., (2008) studied the clinical 
signs after experimental infection of chickens 
with P. multocida in relation to age group. They found that no birds died during 
the experiments, although all chickens except 
two (16 weeks old) expressed clinically signs 
of fowl cholera at some points during 14 day 
observation.

References

Abd El-Dayem, S. Nagah (1990): Pasteurella 
infection in poultry in Kaloubiaprovince. 
Zag. Univ. (Benza branch).

Abd El-Matehi, T.Y. and Salem, B. (1986): 
Characterization of Pasteurellastrains isolated 
J., 16:32-38

Akeila, M.; Ezzat, M. and El-Nimr, M.M. 
(1986): Drug sensitivity of Pasteurella 
multocida isolated from carrier birds. Alex. J. 

Aye, P.P., Angrick, E.J., Morishita, T.Y. and 
Harr, B.S., 2001: Prevalence and 
characteristics of Pasteurella multocida in 
commercial turkeys. Avian Dia.,45(1):182- 
190.

Blackall, P.L. and Millin, J.K., (2000): 
Identification and typing of Pasteurella 
multocida: a review. Avian Pathol. 29: 271- 
287.

Baldrias, L.; Frost, A.G. and Boyle , 
organisms from the tonsillar region of dogs 
and cats. J. of small Animal practice, 29,63- 
66.

Bebars A. S (2000): Characterization of 
Pasteurella multocida of duck origin by using 
protein profile analysis and DNA 
finger printing. M.V.Sc. thesis (Bacteriology, 
Immunology, Mycology) Fac.Vet.Med. Cairo 
Univ.

Isolation, Identification and Antibiogram of 
Pasteurella multocida isolates of avian origin 


after intratracheal inoculation into turkeys. Poul. Sci. 70, 2259-2266.


دراسات على الباستيريليا في الدجاج

المتوفي

تم تجميع 200 عينة جمعوا من البستيريليا في الدجاج. تم تجميع العمالات عند الرغب في العينات من المرضى وتم تح💁‍♀️ل العينات للمراقبة. تم الحصول على 8 معايير ومعايير التفاعلات الكيميائية المحلية للكائنات. ومثلت الباستيريليا متعددة بين KMT186 و KMT177. كشف التسجيل الجيني أن جميع العينات ملائمات ناقصة في نوع أ. 61.7 %

تم استخدام معايير الباستيريليا متعددة لتحسين الفحص ل 200 طائر ثبت أن نسبة على الباستيريليا متعددة من ناجمة 1.83 % أكبر نسبة على الباستيريليا متعددة 2.2 %. تم التحكم في الحصول على تعريفات الباستيريليا متعددة من البستيريليا ومصادر الدجاج التي تظهر على أنها من هذا النوع.