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BACTERIOLOGICAL STUDIES ON *PASTEURELLA MULTOCIDA* IN TURKEYS IN FAYOUM GOVERNORATE

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SUMMARY

A total of 274 apparently healthy and ailing or recently dead diseased turkeys were bacteriologically examined to detect the prevalence rate of *P. multocida*. The total incidence percentage of *P. multocida* in apparently normal turkeys was 4.1% but it was 9.7% in diseased ones. Confirmation of isolates was done by morphology, growth characteristics, biochemical reactions using 20 NE/API system. Serotyping of isolates by using indirect haemagglutination and gel diffusion precipitin tests revealed that A : 1 (38.1%); A:3 (23.8%); D: 12 (14.3%); F: 4 and - : 1 (9.5%) each as A:- (4.8%) were identified. The superiority of colistin sulphate, nitrofurantoin, gentamicin, kanamycin and oxytetracycline against *P. multocida* of turkeys origin were recorded. Experimental infection of the most prevalent serovars in 6- week old Bronzy turkeys were done. The clinical signs, post - mortem examination, mean death time, mortality rate and reisolation of the organism were described in details.

INTRODUCTION

Fowl cholera associated with the bacterium Pasteurella multocida has been one of the costliest diseases in the turkey industry in Egypt (Farid et al., 1987). Fowl cholera often appears as a contagious, septicaemic disease of turkeys associated with high morbidity and mortality but chronic conditions also occur (Blackall et al., 1998). The severity of the disease and its incidence is influenced by environmental factors such as crowding, climate, nutrition and concurrent disease (Nestor et al., 1996).

Application of rapid identification system to veterinary clinical microbiologists requires accuracy in identifying these bacteria. The incidence of *P. multocida* in apparently normal turkeys varied from 3.7% (Farid et al., 1987) up to 4.7%

(Rhoades et al., 1992). Some workers have isolated P. multocida from diseased turkeys with varying percentages (Aouad , 1978, 9.64%, Caprenter et al., 1991, 10.13% and Nestor et al., 1996, 5.5%).

It was recorded that serotypes "A" and "D" of P. multocida were particularly present in turkeys by some workers such as (Snipes et al., 1990; Lee et al., 1991 and Fegan et al., 1995).

The aim of the present work was to record the prevalence rates of P.multocida isolated from apparently normal and diseased turkeys in Fayoum Governorate, complete identification and serological typing of isolates, invitro study of the response of isolates to different chemotherapeutic agents as well as studying the pathogenicity of the most prevalent serovars in experimentally infected turkeys.

MATERIAL AND METHODS

Samples were collected from the internal organs of 176 turkeys suspected to be suffering from pasteurellosis. The samples were taken from the heart blood, lungs, spleen, liver, bone marrow, ovaries and oviduct of ailing or recently dead diseased turkeys. Moreover , a total of 98 oropharynx swabs were obtained from apparently healthy live turkeys. All samples were collected from different private farms at El-Fayoum Governorate during the period from January 1998 till the end March, 1999.

Blood films were prepared from the heart blo and liver and stained with Leishman stain (Mac ie and Mac Cartney, 1996) for detection of the b polarity of the organism. All samples we streaked onto DAS selective, 5% sheep blo agar, nutrient agar and Mac Conkey bile salt la tose agar plates and then incubated at 37°C aer bically for 24-48 hours. Pure suspected colonie of P. multocida were identified morphologicall and culturally according to the criteria cited b Krieg and Holt (1984).

Biochemical identification was carried out by us ing API 20 NE (non enteric). The strip was re moved from its packing . It was placed in the tray An ampoule of 0.85% NaCl medium was opened A bacterial suspension was prepared with a tur bidity equivalent to tube 0.5 on the Mac Farlan Standard tube by picking 1-4 colonies and ho mogenized in the saline. Inoculation test was per formed by distributing the saline suspension int tubes with sterile pipette. The incubation box wa closed and incubated at 30°C for 24 hrs. The strips were read according to the interpretation ta ble provided with the kits.

Capsular typing of all isolates of P.multocida was done by using the indirect haernagglutination test after Carter and Rappay (1962). Somatic typing was performed by the gel diffusion precipitin test

according to Hofacre and Glisson (1986). P. multocida antisera were kindly obtained from Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. Pathogenicity tests for the isolates in experimentally infected white Swiss mice was carried out according to Matsumoto and Strain (1993).

Antibiotic Resistance Profiles :

Resistance profiles were determined by the disc diffusion method (Mackie and Mac Cartney, 1996). Antibiotics tested and the size of zone of inhibition (mm) used to define resistance included: colistin sulphate (10 ug), cephaloridin (30 ug), erythromycin (15 ug), nitrofurantoin 300ug) ; gentamicin (10 ug), kanamycin (30 ug), oxytetracycline (30 ug), penicillin - G (10 U) and sulphamethoxypyridine (300 ug). Also, it was determined whether there were significant differences in the scores of each serotype of P. multocida.

Pathogenicity of different P.multocida Serovars for turkeys :

A total of 40 Bronzy turkeys six weeks old were used in this experiment. They had been reared in closed confinement. Swabs rubbed over the surface of oropharynx of turkeys before experimental infection were negative for P. multocida, and no antibodies were detected in their sera. Twenty four hours pure culture representings each of P. multocida serovars A:1; A:3 and D:12 were suspended separately in sterile saline. Viable organisms per ml. was obtained by using Mac Farland opacity tube No. 1 containing 7×10^8 .

All turkeys were classified into four groups, each of 10 birds. The last group was kept as a control and inoculated through the nasal cleft with sterile saline. The 1st group was inoculated through nasal cleft with A:1; The 2nd group through nasal cleft with A: 3 and the 3rd group with D:12 strain. Turkeys were observed for 3 weeks post infection to record any clinical symptoms or deaths. All turkeys that died or survived till the end of the experiment were necropsied and specimens from the lungs, liver, heart blood, sternal bursa; articulations and air spaces in the head were cultured for P. multocida. The mean death time was calculated as described by Hanson and Brandly (1955) according to the following formula :

$$MDT = \frac{\text{(No dead at x hr) x (xhr) + (No dead at Y hr) x (Yhr) .....}}{\text{Total No. of dead turkeys}}$$

RESULTS AND DISCUSSION

The most important enzootic bacterial disease that threatens the turkey industry in Egypt is the fowl cholera which causes great economic losses in the field of turkey breeding . This is due to the rapidity of spread of the disease and deaths shown by many of P.multocida serovars.

Table 1: Prevalence rates of *P. multocida* isolated from examined turkeys.

Source of samples	No. of examined cases	No. of positive cases	Prevalence rate
Apparently normal turkeys	98	4	4.1%
Diseased ailing and dead turkeys	176	17	9.7%
Overall total	274	21	7.7%

Concerning 98 apparently normal oropharynx turkey samples examined bacteriology, 4 isolates of P. multocida could be retrieved with an incidence of 4.1 % as shown in (Table 1) . This agrees with the previous findings of Farid et al., (1987); Prantner et al., (1990) and Rhoades et al., (1992) who found that the prevalence rates of *P.multocida* among healthy turkeys were : 3.7%; 4.4% and 4.71% respectively.

The recovery of *P.multocida* from the oropharynx of apparently healthy turkeys provides an evidence of the importance of the role played by carrier turkeys in transmitting the infection to healthy ones.

From the results illustrated in (table 1), it can be concluded that out of 176 diseased dead turkeys examined, 17 cases revealed *P. multocida* with an overall incidence rate of 9.7%. Nearly similar findings have been recorded by Caprenter et al., (1991) who stated that the incidence of *P. multocida* in diseased turkeys was 10.13%. On the other hand, Nestor et al., (1996) found *P. multocida* isolation rate was only 5.5% which is lower when compared with the present data .

Regarding the colonial appearance of isolated P multocida from turkeys in Fayoum Governora as shown in (table 2), it is interesting to note tha out of 21 isolates from apparently normal and dis eased turkeys, 76.2% showed irrediscent colonies followed by 14.3% of mucoid form but intermedi ate and rough colonies were the lowest (4.8% each) . It is of interest to note that all types of co lonial variants were recognized from apparently normal and diseased turkeys.

Biochemical properties of the isolates were deter mined by the API 20 NE tests system. All isolates exhibited positive results to nitrate, indole, oxi dase and fermentation of glucose but they gave negative reactions in urease , citrate, phenyl ace tate and fermentation of mannose, gluconate, cap pate, adipate, malate, esculin and not liquefied gelatin. These results are fully in agreement with that postulated by Nancy and Michael (1980) and Mackie and MacCartney (1996).

As shown in table (3), the vast majority of capsular types from all isolates were type A. Capsular type "A" accounted for 66.7% of isolates from turkeys. The preponderance of capsular type A is in accordance with data of Snipes et al., (1990) and Fegan et al., (1995). In the present work, capsular typing of the 21 turkeys field isolates, indicated that 66.7% were belonging to capsular type "A"; 14.3% were capsular type "D", 9.5% were capsular type "F" and 9.5% of the strains were untypable. Farid et al., (1987) classified turkey field isolates and found that many of these

Table 2: Confirmation of P. multocida strains isolated from turkeys by stained films, colonial morphology, growth characteristics and biochemical reactions using 20 NE API system.

Criteria		No. of positivity/ No. of strains	% of positivity
Stained Gram's method (Gram-Negative)		21/21	100%
Type of Colonial appearance	Irrediscent	16/21	76.2%
	Mucoid	3/21	14.3%
	Intermediate	1/21	4.8%
	Rough	1/21	4.8%
Growth characterisitics	In broth	21/21	100.0%
	DAS media	21/21	100.0%
	Haemolysis onto blood agar	0/21	0.0%
	Growth onto MacConkey agar	0/21	0.0%
Tested used	Substrate		
NO_3 (nitrate test)	Pot. Nitrate	21/21	100.0%
TRP (Indole test)	Tryptophane	21/21	100.0%
URE (Urease test)	Urea	0/21	0.0%
CIT (Citrate test)	Citrate	0/21	0.0%
PAC	Phenyl acetate	0/21	0.0%
OX (oxidase test)	Tetramethyl P-phenyline diamine	21/21	100.0%
NAG	N-acetyl glucosamine	4/21	19.0%
GLU	Glucose	21/21	100.0%
ARE	Arabinose	10/21	47.6%
MNE	Mannose	0/21	0.0%
MAN	Mannitol	20/21	95.2%
MAL	Maltose	19/21	90.5%
GNT	Gluconate	0/21	0.0%
CAP	Carpate	0/21	0.0%
ADI	Adipate	0/21	0.0%
MLT	Malate	0/21	0.0%
ESC	Esculin	0/21	0.0%
GEL	Gelatin	0/21	0.0%

isolates were Carter type "A" with high incidence of type "D" . Also , Abd El-Dayem (1990) isolated 8 strains of *P. multocida* from infected and apparently healthy turkeys and found that type "A" was the most prevalent isolates. The results recorded in table (3) describes the somatic typing of *P. multocida* by Hofacre and Glisson (1986) and revealed that 47.8% of these strains were type "1", 23.8% as type "3"; 14.3% as type "12", 9.5% as type "4" and 4.8% were untypable. Generally, the serotypes 1, 3 , 12 and 4 were the principle serotypes obtained from turkeys. Also serological identification revealed that 38.1% of the isolates were type A:1; 23.8% were type A:3 ; 14.3% as type D : 12; 9.5% as type F : 4 , 9.5% as type -: 1 and 4.8% as type A :- Similar serotyping were recorded by Hofacre and Glisson (1986); Farid et al., (1987); Snipes et al., (1990) and Fegan et al (1995).

One of the steps in the treatment of fowl chole in turkeys , is the use of appropriate antibiot agents. The type of antibiotic used in the trea ment should better be selected on the basis of di diffusion technique as shown in table (4) whic revealed that A : 1, A : 3, D: 12, F : 4, A: - and - 1 serovars of P. multocida were highly sensitiv to colistin sulphate; nitrofurantoin; gentamici kanamycin and oxytetracycline . Resistance wa shown only to cephaloridin, erythromycin, peni cillin - G and sulphamethoxypyridine. All teste serotypes showed no significant differences i

Table 3: Coorrelation between capsular and somatic typing of *P. multocida* isolated from turkeys.

Source of sample	No. of strains	Capsular Typing	Somatic Typing					
			1	3	4	6	12	Untypable
Apparently normal turkeys	4	A	2(9.5)	1(4.8)	-	-	-	1(4.8)
		D	-	-	-	-	-	-
		F	-	-	-	-	-	-
		Untypable	-	-	-	-	-	-
Ailing and Dead diseased turkeys	17	A	6(28.6)	4(19.0)	-	-	-	-
		D	-	-	-	-	3(14.3)	-
		F	-	-	2(9.5)	-	-	-
		Untypable	2(9.5)	-	-	-	-	-
Total capsular: Somatic typing	A:1 = 38.1% A:3 = 23.8% D:12 = 14.3% F:4 = 9.5%		A :- = 4.8% - : 1 = 9.5%					

Table 4: The results of antibiogram of *P. multocida* serovars isolated from turkeys

Antimicrobial Agemt	Conc.	A : 1 (8)		A : 3 (5)		D : 12 (3)		F : 4 (2)		A : - (1)		-: 1 (2)	
		No. Sensitive	%	No. Sensitive	%	No. Sensitive	%	No. Sensitive	%	No. Sensitive	%	No. Sensitive	%
Colistin Sulphate	10ug	8	100.0	4	80.0	3	100.0	2	100.0	1	100.0	2	100.0
Cephaloridin	30ug	2	25.0	1	20.0	1	33.3	0	0.0	0	0.0	0	0.0
Erythromycin	15ug	1	12.5	1	20.0	0	0.0	0	0.0	0	0.0	0	0.0
Nitrofurantoin	300ug	8	100.0	4	80.0	3	100.0	2	100.0	1	100.0	2	100.0
Gentamicin	10ug	7	87.5	5	100.0	3	100.0	2	100.0	1	100.0	2	100.0
Kanamycin	30ug	8	100.0	4	80.0	2	66.7	2	100.0	1	100.0	2	100.0
Oxytetracyline	30ug	7	87.5	4	80.0	3	100.0	2	100.0	1	100.0	2	100.0
Penicillin	10u	1	12.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Sulphamethoxy pyridine	300ug	0	0.0	1	20.0	0	0.0	0	0.0	0	0.0	0	0.0

Number between brackets represents the number of tested strains

Table 5: Results of experimental infection in 6 weeks old Bronzy turkeys infected with different serovars of *P. multocida*.

P multocida serovar	No. of turkeys infected	No. of deaths per hours								No.of dead turkeys	Lesion score	Mean Death time	No. Survivors/ No. exposed	Mortality rate	Reisolation rate
		18	24	48	72	96	120	144	168	192					
A:1	10	-	1	-	2	1	-	1	1	1	7	+++	109.7hrs	3/10	70.0	100.0%
A:3	10	1	-	-	1	1	1	1	-	-	5	+++	90.0hr	5/10	50.0	100.0%
A:12	10	1	-	1	1	1	-	-	-	-	4	++	58.5hr	6/10	40.0	80.0%
Control	10	-	-	-	-	-	-	-	-	-	0	---	0.0	10/10	0.0	0.0%

their sensitivity to various chemotherapeutic agents. No serotypes specific sensitivity to antibiotics was detected. The superiority of the aforementioned drugs for the invitro sensitivity against *P. multocida* of turkey origins are also recorded by Jacques et al., (1991) and Bada et al., (1992).

Experimental infection using P. multocida serovars A : 1 , A : 3 and D : 12 was studied in 6-week old Bronzy turkeys inoculated by nasal oral technique. Turkeys inoculated with strain A : 1 developed ,peaked at 72-96 hours, severe progressive bacteraemia that began at 24 hours post - inoculation and with mortality rate reaching 70.0%. The mean death time was 109.7 hours for turkeys inoculated by serotype A:1 . The clinical signs observed in experimentally infected turkeys were : depression, dullness, sleepy appearance dropping of the wings, closed eyes. Post-mortem findings varied according to the duration of the disease. Turkeys which died up to 72 hours (3 turkeys) after infection revealed typical form of septicaemia including haemorrhages of the subcutaneous blood vessels, congestion of the lungs, liver , spleen and kidneys and congestion and petechial haemorrhages on the coronary fats. Turkeys which died after 96 hours post - infection showed fibrinous pericarditis, perihepatitis, peritonitis and lungs appeared to have areas of congestion. Reisolation rate from all dead turkeys was 100.0% and lesion score was (+++) . These results agrees with Lee et al., (1991) and Rhoades et al., (1992).

Regarding the pathogenicity and virulence of *P. multocida* isolates serovars A: 3 and D : 12, it can be noticed from (Table 5) that A: 3 was moderate in its virulence to turkeys with mortality rate 50.0% and a mean death time reaching 90.0 hours and reisolation rate from heart blood, liver, spleen and lungs of dead turkeys had reached 100% with lesion scores (+++) . Death from serovar A : 3 usually occurs from 18 hours up to 144 hours post - infection . The clinical signs and post - mortem findings among turkeys dead from A : 3 infection showed nearly similar results as that recorded for turkeys infected with serovar A : 1 . On the other hand ; the incubation period among turkeys infected with D : 12 varied from 18-96 hours post-infection . Mortality rate lowered to 40.0% with reisolation rate reaching 80.0% from dead infected turkeys and lesion score of (++). The clinical signs and post-mortem findings observed were similar to that described in turkeys infected with serovar A:1. These results seemed to agree with that recorded by Ab El-Dayem (1990) and Lee et al., (1991).

It can be concluded that turkeys carry the *P. multocida* organism in their oropharynx without showing clinical signs and they may serve as a vector to infect the flock of susceptible turkeys and other birds. Accurate vaccinatin programme is essential in order to safe guard turkeys from such dangerous of *P. multocida* infection, which represent a continuous hazard to turkey industry

in El-Fayoum Governorate. Education programmes for owners is very important to follow sanitary conditions, avoid overcrowdness and the use of the best vaccination programmes.

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