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ISOLATION, IDENTIFICATION AND PATHOGENICITY OF SOME BACTERIAL AGENTS ISOLATED FROM OSTRICHES

(With 5 Tables)

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عزل و تصنيف و ضراوة بعض المسببات البكتيرية المعزولة من النعام

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تم تجميع ٢٤٢ عينة مختلفة من مزارع وأعمار مختلفة للنعام وتم فحصها لوجود اى عـــدوى بكتيرية بها. أسفرت النتائج عن عزل انواع مختلفة من البكتيريا وتشمل الميكروب القولونـــى وميكروب البروتيس وسودوموناس ايروجينـــوزا وباســتيوريللا هيموليتيكـــا و باســتيوريللا مالتوسيدا و الايروموناس و الشيجيللا من العينات التى تم فحصـــها و كـــانت نســب العـــزل ٤٠،٤٩% و٢٢،٧٢% و١٧،٧٦% و١٠،٧٤% و٦،٦١% و٠،٨٢% و٠,٨٢ % علـــــى التوالى. وجدت اجسام مناعية ضد السالمونيلا بللورم فى عدد ٢١ عينة من ٨٧ عينة سيرم تـم فحصها و كانت معدلاتها عالية فى الذكور و الاناث تصل الى١/٣٢٠ ماعدا عينة واحدة كانت اقل فى معدل الاجسام المناعية (١/٨٠). تم اجراء اختبارات الضراوة للعترات المعزولة فـــى الفئران والكتاكيت عمر يوم و تسجيل الاعراض ونسب الوفيات وكذلك تمت مناقشة النتائج.

SUMMARY

Two hundreds and forty two samples from ostriches at different ages were examined for the presence of bacterial infections. *E. coli, Proteus spp., Pseudomonas aeruginosa, Pasteurella haemolytica, Pasteurella multocida, Aeromonas spp.* and *Shigella spp.* were isolated from the examined samples in ratios 40.49%, 22.72%, 17.76%, 10.74%, 6.61%, 0.82% and 0.82% respectively. Antibodies against *Salmonella pullorum* were detected in 21 out of 78 serum samples collected from adult ostriches at a ratio of 24% with high titer of antibodies in 20 samples (1/320) and one sample was (1/80). Pathogenicity tests of the isolated microorganisms were studied in both mice and chickens. Mortalities and symptoms were recorded and discussed in details.

Key words: *Bacterial agents, Ostriches*

INTRODUCTION

In the last few decades, there was a distention in the number of ostrich's farms in Egypt. Ostriches were reared for many purposes including meat production, as their meat is cholesterol free, egg production, feathers and hides. Besides, their fat was used in cosmetics and ointments (Smit, 1963 and Osterhoff, 1979).

Many infectious agents were incriminated to be able to induce diseases in ostriches including virus infections (Perelman, 1991), fungi (Cho-kyoungoh *et al.*, 2001), protozoa and parasites (Christensen, 1997).

Furthermore, different bacteria can infect ostriches and produce diseased conditions. Various serotypes of *E.coli* were isolated from ostriches which was accompanied with respiratory disorders (Knobi *et al.*, 2001), leg deformities and impaction of the proventriculus (Terzich and Vanhooser, 1993), and late embryonic deaths during artificial inoculation of ostriches eggs (Khafagy and Kamel, 2001). Many salmonellae were isolated from ostriches, of which Salmonella *typhimurium* was the most prevalent isolated serotype (Vanhooser and Welsh, 1995 and More, 1996).

Moreover, septicaemic pasteurellosis was recorded in ostriches in central Saudi Arabia (El-Faki *et al.*, 2002). *Pasteurella haemolytica* infection was isolated from many diseased cases of ostriches (Youseif *et al.*, 2001).

Both Pseudomonas and Aeromonas species were isolated from ostriches and proved to produce infertility and lower egg hatchability (Deeming, 1996). Pseudomonas aeruginosa infection in adult ostriches was recorded to cause pneumonitis, fever, convulsions, respiratory distress, sever greenish diarrhea which lead to dehydration and finally death (Pandey *et al.*, 2001).

Acute clostridial hepatitis was also described in ostriches due to Closteridia sordellii infection (Poonach and Donahue, 1997). Mycoplasma and Chlamydia (Chistensen, 1996 and Andersen *et al.*, 1998), *Streptococcus faecalis*, Botulism, Tuberculosis, Actinobacillosis and Campylobacter infection were recorded and isolated from ostriches from different localities in the world (Oyarzabal et al., 1995, Bouisset, 1996, Mohan *et al.*, 1997, Shan-Songhue *et al.*, 1998, Sevckova *et al.*, 1999).

The aim of the present study was to investigate the bacterial agents present in farm ostriches in Egypt at different ages and their

environments by isolation and identification of these agents and to highlight their pathogenicity.

MATERIALS AND METHODS

Collection of Samples:

A- Samples for bacteriological examination:

Two hundred and forty-two of different samples were collected from ostriches and their environments in ostrich's farms at different localities in Egypt. Samples were collected under complete aseptic conditions and were representative for different ages. Collected samples include nasal, buccal and fecal swabs, feedstuff, water samples and organs from recently died ostriches. Postmortem examination was done for the dead birds.

B- Samples for serological examination:

Eighty–seven blood samples were collected from adult ostriches for detection of antibodies against *Salmonella pullorum* by rapid serum plate agglutination test using stained *Salmonella pullorum* antigen and tube agglutination test using white pullorum antigen. Type and number of examined samples are shown in table (1).

Table (1): Type and number of examined ostriches samples

Type of sample	No.
1-Samples for bacteriological examination :	
Buccal swabs	36
Fecal swabs	36
Nasal swabs	36
Ration	24
Water	14
Organs:	
Liver	24
Lung	24
Heart	24
Bone marrow	24
Total	242
2- Samples for serological examination:	
Blood samples	87
Total	329

Bacteriological examinations:

All samples, collected for bacteriological examinations, were inoculated in peptone buffer and incubated at 37°C for 24 hours. A loopfulls of each broth culture were inoculated onto blood agar, MacConkey agar and brilliant green agar plates. The inoculated plates were incubated at 37°C for 24-48 hours.

Examination of ration samples were carried out by adding 25 g of each ration sample to 225ml of peptone buffer and well-mixing using magnetic stirrer then incubated at 37°C for 24 hours. A loopfulls of the broth cultures were inoculated onto blood agar, MacConkey agar and brilliant green agar plates which were incubated at 37°C for 24-48 hours. Isolated colonies were identified bacteriologically using biochemical and serological identification according to Lennette, 1980 and McFaddin, 1984.

Pathogenicity tests:

(1) In white Bulb /C mice :

A total of eighty white bulb /c mice divided into eight equal groups (10 mice per group) were used for testing the pathgenicity of the isolated bacteria. Mice in group 1 were kept as negative controls while mice in groups 2-8 were infected I / P with 0.2 ml of 24 hours broth culture of each of the seven isolated bacteria and kept under observation for one week after infection. Mortality rates were recorded in different groups of infected mice with reisolation of the experimentally infected bacteria.

(2) In chicken :

One hundred and sixty, one-day old baby chicks were used for testing the pathogenicity of the isolated bacteria. Chicks were divided into eight groups (20 chicks per group). Chicks in group 1 were kept as negative non-infected control group, while chicks in groups 2,3,6,7 and 8 were infected I /P with 0.1 ml of 24 hours broth culture per chick of the isolated *Aeromonas spp.*, *E. coli*, *Proteus spp.*, *Pseudomonas aeruginosa* and *Shigella spp.* respectively. Chicks in groups 4 and 5 were reared for seventeen days then infected I/P with 0.1 ml of 24 hours broth culture per chick of *Pasteurella haemolytica* and *Pasteurella multocida* isolates. The chicks in all groups were fed on unmedicated ration and received clean water and kept under observation during the experimental period. Mortalities in infected groups were recorded for one week post-infection with reisolation of the infected bacteria.

RESULTS and DISCUSSION

In the present study, bacteriological examinations revealed that many bacterial isolates were recovered from the examined samples in which mixed infections were common. Table (2) shows types of bacteria isolated from examined ostriches samples and their incidences of isolation.

Table (2): Types and Incidence of bacteria isolated from examined ostrich's samples.

Bacterial isolate	requency of isolation	%
E. coli	98 / 242	40.49
Proteus spp.	55 / 242	22.72
Pseudomonas aeruginosa	43 / 242	17.76
Pasteurella haemolytica	26 / 242	10,74
Pasteurella multocida	16 / 242	6.61
Aeromonas spp.	2 / 242	0.82
Shigella spp.	2 / 242	0.82

The results obtained in table (2) revealed a high incidence of *E. coli* isolates (40.49%) compared with other bacterial isolates which included *Proteus spp.* (22.72%), *Pseudomonas aeruginosa* (17.76%), *Pasteurella haemolytica* (10.74%), *Pasteurella multocida* (6.61%), *Aeromonas spp.* (0.82) and *Shigella spp.* (0.82).

These results agreed with that of Gross (1991) and Jordan and Pattison (1996) who reported that the most commonly implicated *E. coli* serotypes being responsible for colibacillosis are O1:K1, O2:K1 and O78:K80. They were also in agreement with Terzich and Vanhooser (1993) who mentioned that *E. coli* and / or *Klebsiella pneumoniae* were isolated from 40-100% of the examined ostrich's samples. Our findings are supporting the previously published results by Ley *et al.* (2001) who detected *E. coli* O157: H7 in 91% of examined dressed ostriches samples.

Table (3): E.coli serotypes isolated from ostriches

Serotype	Incidence of isolation	%
Untyped	27 / 98	27.55
O78:K80	25 / 98	25.51
O9 :K 59	18 / 98	18.36
O2:K56	15 / 98	15.31
$O2:K_{unknown}$	11 / 98	11.22

Serotyping of the isolated *E. coli* revealed that they were belonged to O78: K80 (25.51%), O2; K56 (15.31%), O2:$K_{unknown}$ (11.22%) and O9: K59 (18.36%) while 27.22% of the isolated E. coli were untyped (table 3). These results are matching those mentioned by Kamel *et al.*, 2001 and Knobi *et al.*, 2001 who isolated and identified serologically almost the same E. coli serotypes from ostriches.

Isolates of *Pasteurella multocida* organisms were obtained from bone marrows of dead ostriches while that of *Pasteurella haemolytica* were isolated from water samples, rations and lung abscesses. Aeromonas organisms were also isolated from lung abscesses while *E. coli*, Proteus, Pseudomonas and Shigella isolates were obtained from internal organs and bone marrows.

The presence of bacterial infections in ostriches could affect the quality of ostriches products and may be of a great hazard to human health as most of the isolated microorganisms were incriminated to cause intestinal disturbances, sever enteritis, bacterial dysentery, food poisoning and urinary tract infections in man (Akhtar *et al.*, 1982, Varnam and Evans, 1991 and Marriott, 1997). From other aspect, These results agreed with Deeming, 1996, who isolated Aeromonas, *E. coli* and Pseudomonas from contaminated spoiled ostriches eggs and he proved that they have bad effect on both fertility and hatchability of ostriches' eggs. It was suggested that in adult ostriches the incidence of bacterial infections might produce microbial spoilage of the contaminated produced eggs, which in turn may result in embryonic deaths.

Serological examination of the collected serum samples showed the presence of antibodies against *Salmonella pullorum* in twenty-one out of eighty-seven examined serums samples (24%). The positive samples had a high antibodies titers in twenty samples (12 females and 8 males) which reached 1/320 in all samples while that of the remaining positive one was 1/80 which was a female sample. The high antibody titers were indicating the occurrence of Salmonella infection in the serologically positive cases. These results were in disagreement with that of Ley *et al.*, 2000 who could not detect antibodies against *Salmonella pullorum*, *Salmonella gallinarum* and *Salmonella typhimurium*, while Cadman *et al.* (1994) could detect antibodies against *Salmonella enteritidis* using ELISA technique. These results were in agreement to a great extent with that obtained by Gopo and Banda (1997) who reported Salmonella infection from ostrich's samples. They also agreed with Ley *et al.*, 2001,

who detected Salmonella and Campylobacter in examined ostrich's carcasses.

Table (4): Pathogenicity of isolated bacteria in Balb / C mice.

Group No.	Bacterial isolate	No. of Infected mice	Route of infection	Mortality rates	%
1	Negative control	10	I / P	0 / 10	0%
2	*Aeromonas spp.*	10	I / P	0 / 10	0%
3	*E. coli*	10	I / P	6 / 10	60%
4	*Pasteurella haemolytica*	10	I / P	6 / 10	60%
5	*Pasteurella multocida*	10	I / P	8 / 10	80%
6	*Proteus spp.*	10	I / P	4 / 10	40%
7	*Pseudomonas aeruginosa*	10	I / P	0 / 10	100%
8	*Shigella spp.*	10	I / P	4 / 10	40%

Table (5): Pathogenicity of isolated bacteria in chicken.

Group No.	Bacterial isolate	No. of Infected birds	Age of infection (days)	Route of infection	Mortality rate	%
1	Negative control	20	One-day old	I /P	0 / 20	0%
2	*Aeromonas spp.*	20	One-day old	I / P	18 / 20	0%
3	*E. coli*	20	One-day old	I / P	18 / 20	0%
4	*Pasteurella haemolytica*	20	17 days old	I / P	20 / 20	100%
5	*Pasteurella multocida*	20	17 days old	I / P	16 / 20	80%
6	*Proteus spp.*	20	One-day old	I / P	14 / 20	70%
7	*Pseudomonas aeruginosa*	20	One-day old	I / P	20 / 20	100%
8	*Shigella spp.*	20	One-day old	I / P	0 / 20	0%

The results of pathogenicity tests of the isolated bacterial agents in balb / c mice, one-day old and seventeen days old chicks (tables 4 and 5) revealed that *Pseudomonas aeruginosa* isolate was highly pathogenic to both balb / c mice and one-day old chicks (100% mortalities for each) and the infection was accompanied with the production of nervous

manifestation, respiratory disorders and death within 24 hours while the pathogenicity testing of *E. coli* isolate showed 60% mortalities in balb /c mice and 90% mortalities in day-old chicks, and isolate of *Shigella spp.* were caused 40% mortalities in balb /c mice but no mortalities occurred in day-old chicks.

These results were in agreement with that obtained by Youseif (1995) who found that *E.coli* strains isolated from local and imported day old chicks were highly pathogenic to three day-old baby chicks(100% mortalities) after subcutaneouse route of inoculation. They also agreed in a great extent with that obtained by Istania (1993) who isolated *Pseudomonas spp.*, *Proteus spp.*, and *E. coli* from cases of post-hatching mortalities in kedu chicks. They also agreed with Al-Sadi *et al.* (2000) who isolated *E. coli*, *Pseudomonas spp.*, *Shigella spp.*, *Proteus spp.* and *Salmonella spp.* from dead-in-shell embryos in three local hatcheries.

The isolates of *Proteus spp.* were highly pathogenic for day-old chicks (70% mortalities) and less pathogenic for balb/ c mice (40% mortalities). These results were in agreement with that obtained by Youseif (1995) who found that *Proteus mirabilis* strains isolated from foreign and local breeds of day old parent chicks revealed 85.7% and 100% mortalities in day old baby chicks respectively after subcutaneouse inoculation. The results also in agreement with Youseif (1985) and Lin and Chin Ling (1996) who described *Proteus spp.* among the most common important bacterial infections that causes economic losses in chicken embryos.

In contrast, *Aeromonas spp.* was highly pathogenic (90 % mortalities) in infected day-old chicks but was unable to produce mortalities on balb / c mice. These results agreed with those by Shane and Gifford (1985) who showed that 2- and 4-days old chickens and poults were highly susceptible to exposure with *Aeromonas hydrophila* introduced by yolk sac, intra-cerebral or intra-muscular route.

Pasteurella multocida isolate was highly pathogenic for both balb /c mice and seventeen days old chicks (80% mortalities in each group). These results agreed with Kapetanov *et al.* (2000) who detected outbreaks caused by *Pasteurella multocida* in breeder flocks of 16000 and 20000 birds at 32 weeks of age on 2 farms in Yugoslavia. They also agreed with Engestrom *et al.* (2001) who described outbreaks of fowl cholera in four broiler breeder flocks in the country of Vestfold.

Finally, *Pasteurella haemolytica* isolate was highly pathogenic for seventeen days old chicks (100% mortalities) and less pathogenic for balb / c mice (60% mortalities). These results disagreed with Youseif *et*

al. (2000) who recorded that isolates of *Pasteurella haemolytica* which obtained from naturally infected ostriches were non pathogenic for chickens, and in contrast, these results agreed with Birbir *et al.* (1995) and Abdel-Aziz (2000) who isolated *Pasteurella haemolytica* from diseased conditions and considered the organism as pathogenic for chicken. On the other hand, Abdel-Aziz (2000) could not observe any mortalities among *Pasteurella haemolytica* I/P inoculated mice while Youseif *et al.* (2000) revealed 100% mortalities in infected mice with sever septicaemic picture.

Thus, it was clear that most of bacterial agents isolated from ostriches and their environments were pathogenic for both chicken and mice which indicated that they constitute a great threaten for both poultry and human health.

Consequently, from all the above mentioned results, great attention should be given to bacterial infections in ostriches farms. Good biosecurity with adequate management could reduce the bacterial infections in ostrich's farms which could produce health hazard importance to human consumers. Separate quarantine areas should be present in all farms to avoid soil contamination by newly introduced infected birds. Also, ostrich's farms should be far from poultry farms to avoid transmission of bacterial infections from ostriches, which proved to be highly pathogenic for chickens.

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