

VIRULENCE OF SOME MEMBERS OF ENTERO-BACTERIACEAE ISOLATED FROM IMPORTED POULTS

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ABSTRACT

The bacterial flora of the intestinal tract and yolk sac of day-old apparently healthy imported turkey poults was studied. The results of the bacteriological examination of 378 poult organs pools revealed 78 bacterial isolates (20.6 %). These isolates were identified biochemically into *E.coli*, *Citrobacter freundii*, *Enterobacter agglomerans*, *Proteus mirabilis*, *Shigella sonnei*, *Klebsiella pneumoniae*, *Klebsiella ozaenae* and *Serratia marcescens* in an incidence of 5.13% ,28.20% ,10.26% ,12.82% ,20.51% ,5.13% ,7.69% ,and 10.26% respectively. Pathogenicity tests revealed that *E.coli*, *Proteus mirabilis*, *Shigella sonnei* were pathogenic for 2-days-old black turkeys on intra peritoneal inoculation. Meanwhile, *Enterobacter agglomerans*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Klebsiella ozaenae* and *Serratia marcescens* were proved to be non-pathogenic for intra-peritoneal inoculation.

INTRODUCTION

Development of turkey breeding in Egypt in the last 10 years depends mainly on the importation of day-old poults. Vertically transmitted pathogens are considered one of the major causes resulting in economic losses in the field of poultry industry from production to marketing (Hewitt, 1928; Hinshaw and McNeil, 1940; Snoeyenbos, 1991 and Yoder, 1991).

Studies on dead-in-shells or newly hatched turkeys microflora are still scanty (Harrison and Hansen, 1950; Cook *et al.*, 1954; Barnes and Impey 1970; Naqi *et al.*, 1970; Bruce and Drysdale, 1983 and Sadek *et al.*, 1991).

In the present work, the bacterial flora were isolated and identified from intestine, yolk sac and liver of one-day-old, apparently healthy imported turkeys (newly hatched turkeys). The pathogenicity of these isolates for baby turkeys was undertaken to clarify their significance among turkey rearing farms in Egypt.

MATERIAL AND METHODS

1. Samples:

Samples from liver, yolk sac and intestine (duodenum and caecum) were taken from 378 imported apparently healthy day-old turkeys. These poults were submitted to Animal Health Research Institute, Dokki, Giza, Egypt for routine examination of *Salmonella pullorum* within the period from August 1998 to September 2000, organs of each 3 turkey poults were pooled and represented as one sample.

2. Experimental turkeys:

Fifty-four, two-day-old black turkey breed birds were used for pathogenicity tests.

3. Bacterial examination:

The samples were cultured in peptone broth and selenite F-broth (Oxoid) for 24 hours at 37°C then a loopfull was streaked onto MacConkey agar plates and incubated at 37 °C for another 24-48 hours. Bacterial colonies were selected for further morphological and biochemical identifications according to **Edward and Ewing, (1972)** and **Cruickshank *et al.*, (1975)**.

4. Experimental Design:

Fifty-four, 2-day old turkey poults were used in this experiment. Four of them were randomly taken and subjected to clinical, postmortem and bacteriological examinations. The examined poults proved to be apparently healthy and free from enteric bacterial infections.

The remaining 50 turkeys were divided into 10 equal groups, consisting of 5 turkeys each. All groups were intraperitoneally inoculated with 0.3 ml of 18-hours broth culture containing 2.5×10^7 CFU/ml of the isolated *E.coli* (untypable strain), *Enterobacter agglomerans*, *Citrobacter freundii*, *Proteus mirabilis*, *Shigella sonnei*, *Klebsiella ozaenae*, *Klebsiella pneumoniae*, *Serratia marcescens* for groups 1-8 (Harry and Hemsley, 1965,b). Turkey poults of groups (9) were similarly inoculated with sterile broth (0.3 ml) as a negative control, while group (10) was kept without infection as blank control. Birds of all groups were kept for 21 days under daily observation for clinical signs and mortalities. Dead as well as sacrificed turkeys at the end of

observation period were subjected to post mortum and bacteriological examinations.

RESULTS

Symptoms:

The clinical signs observed in poults after 24 hours postinoculation were, loss of appetite, ruffled feathers with dullness in-groups No.1,2,3,5,6 and 7. In addition for group No. (4) there was slight diarrhea, while there was no clinical signs observed in group No. (8). The clinical signs disappeared 48 hours post inoculation.

Postmortem lesions:

There was congestion in the internal organs and enteritis in dead turkeys. Poults in groups No. 1, 4 and 5 showed enlarged of the gall bladder, congestion of yolk sac and subcapsular hemorrhages in liver. No lesions could be detected in other groups. No lesions could be detected in survived sacrificed poults.

DISCUSSION

Examination of imported apparently healthy one-day-old turkeys should be applied to minimize the economic losses during the first day of rearing. Some studies investigated the bacterial flora isolated from dead-in-shell turkey eggs (**Bruce and Drysdale, 1983 and Sadek et al., 1991**) and others investigated the cecal or intestinal microflora of healthy turkeys (**Harrison and Hanson, 1950; Cook et al., 1954; Barnes and Impey, 1970 and Naqi et al., 1970**).

In the present work the bacterial flora of the alimentary tract and yolk sac of apparently healthy day-old imported turkeys was investigated. The obtained results are illustrated in Tables (1 and 2). Positive pool samples reached 31.7% (40 out of 126 pools) representing 378 birds. The positive pools revealed 78 bacterial isolates from liver, yolk sac and intestine (doudenun and ceacum) in the rates of 40, 20 and 18 isolates (51.3%, 25.6% and 23%), respectively. These isolates were identified biochemically and differentiated into *E.coli*, *Citrobacter freundii*, *Enterobacter agglomerans*, *Proteus mirabilis*, *Shigella sonnei*, *Klebsiella pneumoniae*, *Klebsiella azaenae* and *Serratia marcescens*.

In ratio of 5.13%, 28.20%, 10.26%, 12.82%, 20.51%, 5.13%, 7.69% and 10.26% respectively. It is worthy to mention that no *Salmonella* species isolates could be detected in all examined turkeys. This results were in complete accordance with that obtained by **Naqi et al., (1970)** who isolated several bacterial species including coliform, non-lactose fermenters *Lactobacilli*, *staphylococci* and *clostridia* that invaded the alimentary tract of apparently healthy turkeys shortly after hatching and concluded that this

bacterial flora reaching very high level of multiplication within 24-72 hours post-hatching. They mentioned that these bacterial flora considered as normal intestinal flora but the presence of coliform is considered unusual in the intestine of newly hatched turkeys.

Barnes and Impey, (1970) examined ceecal flora of 13 week-old healthy turkey and could isolate Gram-negative rods, non-sporing anaerobes, Gram positive non-sporing rods and bifidobacteria and studied their behavior to grow in a complex nutrient medium and in the presence of certain antibiotics and dyes. **Harrison and Hanson, (1950)** isolated *E.coli* out of 20 isolated bacterial species representing 12 genera from caecal feces of healthy 5-month old turkeys.

In chicken, similar results had been reported by **Harry, (1957)** and **Utomo and Poernomo (1990)** who isolated *E.coli*, *Proteus mirabilis*, *Enterobacter* species and *Klebsiella* species from liver, yolk sac, heart and spleen of day-old and 5-day-old live chicks. **Harry, (1957)** concluded that bacterial flora migrated from the alimentary tract to the yolk. Moreover, **Youseif (1995)** isolated *E.coli*, *Proteus mirabilis*, *Enterobacter* species and *Klebsiella* species from liver, gall bladder, yolk sac and intestine (duodenum and caecum) of day-old apparently healthy imported and locally produced parent chicks. On the other hand, our results does not accord with that obtained in chicken by **Utomo et al., (1990)** as well as **Mead and Adams, (1975)** who could not isolate bacteria from the yolk sac of healthy day-old chick, and alimentary tract of newly hatched chicks which was usually considered sterile.

Result of pathogenicity testing with representative strains for 2-day-old turkeys inoculated intraperitoneally was illustrated in Table (3). Our results revealed 40%, 20% and 20% mortality with congestion of all internal organs, enteritis, subcapsular hemorrhage in liver and enlarged gall bladder in groups 1,4 and 5 inoculated with *E.coli*, *Proteus mirabilis* and *Shigella sonnei* respectively.

These results are in complete accordance with that recorded by **Youseif, (1995)** who concluded that *E.coli* and *Proteus mirabilis* were pathogenic for 3-day-old chick on subcutaneous inoculation. Also, **Utomo et al., (1990)** isolated *E.coli* and *Proteus mirabilis* from abnormal yolk sac of day-old chicks. On the other hand, the present results disagree with that reported by **Reid et al., (1961)**, **Youseif et al., (1996)** and **Liu, (1989)** who recorded no mortalities with *E.coli* inoculated subcutaneously into 1 and 3-day-old chick (for first and second) and intraperitoneally into 7-day-old chick (for third). Moreover, **Ernst and Yuan (1990)** mentioned that *E.coli* was the main normal microflora in the intestine of chicks, which gave a very potent barrier against microorganisms that cause disease.

Pathogenicity tests of the present work with intraperitoneally inoculation of *Enterobacter agglomerans*, *Citrobacter freundii*, *Klebsiella*

ozaenae, *Klebsiella pneumoniae* and *Serratia marcescens* for groups No. (2,3,6,7 and 8) proved that they were non-pathogenic for 2-day-old turkeys under the condition of the present experiments. These results agree with the results obtained by **Youseif, (1995)** who mentioned that *Klebsiella sp.* and *Enterobacter sp.* were non-pathogenic when given orally or subcutaneously (for *Klebsiella sp.*) and orally (for *Enterobacter sp.*) into 3-day-old chicks. On the other hand, the present result disagree with that reported by **Utmo and Poernomo, (1990)** and **Utmo et al., (1990)** who isolated and identified *Enterobacter sp.* and *Klebsiella sp.* from liver, yolk sac, heart and spleen of 5-day-old dead chicks and from abnormal yolk sac of day-old chicks.

The result of bacterial re-isolation from dead turkey as well as sacrificed turkeys is studied in Table (4). It reached 100% from dead turkeys while in sacrificed turkeys reached as 33.3%, 00.0%, 00.0%, 50.0%, 25.0%, 40.0%, 20.0% and 00.0% for groups 1 to 8 respectively. It is worthy to mention that no gross lesions were detected in the sacrificed turkeys.

The present investigation clearly shows the significance of imported turkey in the epizootology of enterobacterial pathogens including *E.coli*, *Proteus mirabilis* as well as *Shigella sonnei* which seem to be firstly isolated from day-old apparently healthy turkeys. Moreover, it could be concluded that *E.coli* and *Proteus mirabilis* isolates are considered vertically transmitted (**Awaad , 1975; Lin and Chinling, 1996 and Youseif 1989**).

REFERENCES

- Awaad, M.H.H. (1975):** Studies on *E.coli* infection in chickens Ph.D., Thesis, Dept. of Vet. Med. (Poult. Dis.) Faculty of Vet. Med., Cairo Univ.
- Barnes, E.M. and Impey, C. S. (1970):** The isolation and properties of the predominant anaerobic bacteria in the caeca of chickens and turkeys. Br. Poult. Sci., 11 : 467 – 481.
- Bruce, J. and Drysdale, E.M.(1983):** The bacterial flora of candling–reject and dead–in-shell turkey eggs. Br. Poult. Sci., 24: 391- 395.
- Cook, F. D.; Blakely, R.M.; McGregor, H. I. and Anderson, R.W. (1954):** The effect of antibiotics on the intestinal microflora of turkey poults. Poults. Sci., 33: 38 – 40.
- Cruickshank, R.; Duguid, J.P. Marmion, B.P and Swain, R.H.A (1975) :** Medical Microbiology, 12th. Ed.Churchill Livingstone, Edinburgh, London and New York.
- Edward, P. R. and Ewing, W.H. (1972):** Identification of Enterobacteriaceae. 3rd.Ed. Burgess publishing Co. Minneapolis.
- Ernst, L. B. and Yuan, C. Z. (1990):** Review of Vet. Microbiology, Blackwell Scientific Publications, INC., Boston, Oxford, London, Melbourne.
- Harrison Jr.; A. P. and Hansen, Arne, P. (1950):** The bacterial flora of the

- cecal feces of healthy turkeys. *J. Bact.*, 59: 197 – 210.
- Harry, E.G. (1957):** The effect on embryonic and chick mortality of yolk contamination with bacteria from hen. *Vet. Rec.*, 69 : 1433-1439
- Harry, E.G. and Hemsley, L. A. (1965 b):** The relationship between environmental contamination with septicaemia strains of *Escherichia coli* and their incidence in chickens. *Vet. Rec.*, 77 (9): 241-245.
- Hewitt, E.A. (1928):** Bacillary white diarrhoea in baby turkeys. *Cornell Vet.*, 18: 272 – 276.
- Hinshaw, W. R. and McNeil, E. (1940):** Eradication of pullorum disease from turkey Flocks. *Proc. 44th Annu Meet US Livestock Saint Assoc.*, pp.178 - 194.
- Holt J.G; Krieg N.R; Sneath P.H.A; Staley J. T. and Williams S.T.(1996):** Bergey's Manual of determinative bacteriology. 9th ed. William & Wilkins, 428 East Preston St., Baltimore, Maryland, 21202; USA.
- Lin, J. A. and ChinLing, S. (1996):** Studies on egg transmission of colibacillosis in chicks. *Taiwan J. Vet. Med. Anim. Husband.*, 66 (3): 199 – 205.
- Liu, S. S. (1989):** Isolation, Identification, Pathogenicity and drug sensitivity of *Citrobacter* spp. from bone marrow of clinically affected or dead birds. *Taiwan, J. Vet. Med. Anim. Husband.*, 54 : 51-60.
- Mead, G. C. and Adams, B.W. (1975):** Some observation on the caecal microflora of the chick during the first two weeks of life. *Br.Poult. Sci.*, 16 : 169-176.
- Naqi, S.A.; Lewis, D. H. and Hall, C.F. (1970):** The intestinal microflora of turkeys. *Avian Dis.*, 14: 620-625.
- Reid, W.M.; Maag, T. A.; Boyd, F. M.; Klecknor, A. L. and Schmittle, S.C. (1961):** Embryo and baby chick mortality and morbidity induced by a strain of *Escherichia coli*. *Poult. Sci.*, 40 (6): 1497-1502.
- Sadek, A., Hassanein, K. M.; Abdel Fattah, A. M. and Soliman, A.M. (1991):** Bacterial agents affecting the hatchability rate of turkey embryos. *Assiut Vet. Med.J.*, 25 (50): 56-63.
- Snoeyenbos, G. H. (1991):** Pullorum disease. Cited in *Dis. of poult.* 9th ed. Pp.77 by Calnek, B.W.; John Barnes, H.J.; Beard, C.W.; Reid, W.M. and Yoder, Jr. H.W. Iowa State Univ. Press, Ames, Iowa 50010.
- Utomo, B. N. and Poernomo, S. (1990):** Pseudomonas in improved day-old chicks. *Penyakit Hewan*, 22 (40): 97-101.
- Utomo, B. N.; Poernomo, S. and Iskander, (1990):** Bacteria isolated from chicken yolk sac infection at the Research Institute for Vet. Sci., *Penyakit Hewan*, 22 (40): 102-105.
- Yoder, Jr. H.W. (1991):** *Mycoplasma gallisepticum* infection. Cited in *Dis. of*

Poult. 9th ed. pp. 202, by Calnek, B.W.; John Barnes, H. J. ; Beard, C.W. ; Reid, W.M. and Yoder , Jr.H.W. Iowa State Univ. Press, Ames, Iowa 50010.

Youseif, H. M. Z. (1989): Further studies on Proteus infection in poultry. Ph.D. Thesis, Dept. Vet. Med. (Poult. Dis.) , Fac. of Vet. Med., Cairo Univ.

Youseif, H.M.Z. (1995): Incidence of enterobacterial Pathogens isolated from imported and locally produced one-day-old parent chicks. J. Egypt Vet. Med. Ass., 55 (6): 1189-1199.

Youseif, H.M.Z.; Assia El - Sawy and Aisha - Ragab, A. M. (1996): Incidence of bacteria in imported grandparent chicken eggs with special reference to their pathogenicity to baby chicks. J. Egypt Vet. Med. Ass., 56 (3): 349-366.

Table (1) :- Incidence of positive pools of day-old turkeys poults examined for enterobacterial isolates.

species	No.of examined turkey poults	No.of lots	No.of pools samples	positive pool samples		total No of organ pools	Total positive organ pools	
				No	%		No	%
one-day turkey	378	63	126	40	31.7	378	78	20.6

Table (2) :- Incidence of enterobacterial species isolated from different organs of day-old turkey poults .

Group No.	bacterial species	organ isolates			total	
		liver	y.sac	Intestine	No	%
1	<i>E-coli</i> (untypable)	4	-----	-----	4	5.13
2	<i>Enterobacter agglomerans</i> *	4	2	2	8	10.26
3	<i>Citrobacter freundii</i>	12	2	8	22	28.20
4	<i>Proteus mirabilis</i>	8	2	-----	10	12.82
5	<i>Shigella sonnei</i>	6	8	2	16	20.51
6	<i>Klebsiella ozaenae</i> **	-----	2	4	6	7.69
7	<i>Klebsiella pneumoniae</i> ***	-----	2	2	4	5.13
8	<i>Serratia marcescens</i>	6	2	-----	8	10.26
Total No.		40	20	18	78	100
%		51.3	25.6	23.1	100	

The recent names according to Halt et al.(1996).

(*) : *Pantoea agglomerans* .

(**) : *Klebsiella pneumoniae* subspecies *ozaenae* .

(***) : *Klebsiella pneumoniae* subspecies *pneumoniae* .

Table (3):- Pathogenicity of bacterial isolates for 2-day-old turkey poultis inoculated I/P with a dose 2.5×10^7 CFU/ml .

Group No.	Bacterial species	Source of bacterial isolate	No.of inoculated Turkeys	Mortality per week.			Total mortality.		Total survival.	
				1st	2nd	3rd	No	%	No	%
1	<i>E.coli</i>	Liver	5	2	—	—	2	40	3	60
2	<i>Enterobacter agglomerans</i>	Y.sac	5	—	—	—	—	—	5	100
3	<i>Citrobacter freundii</i>	Y.sac	5	—	—	—	—	—	5	100
4	<i>Proteus mirabilis</i>	Y.sac.	5	1	—	—	1	20	4	80
5	<i>Shigella sonnei</i>	Y.sac	5	1	—	—	1	20	4	80
6	<i>Klebsiella ozaenae</i>	Y.sac	5	—	—	—	—	—	5	100
7	<i>Klebsiella pneumoniae</i>	Y.sac	5	—	—	—	—	—	5	100
8	<i>Serratia marcescens</i>	Y.sac	5	—	—	—	—	—	5	100
9	Control (negative)	Y.sac	5	—	—	—	—	—	5	100
10	Control (blank)	Y.sac	5	—	—	—	—	—	5	100

Table (4) :- Reisolation of bacteria from dead or sacrificed turkeys .

Gr. No.	Bacterial Isolates	Organs of Reisolation.													
		Heart		Liver		Yolk sac*		Intestine*		Spleen		Lung		B.M.	
		D	S	D	S	D	S	D	S	D	S	D	S	D	S
1	<i>E-coli</i>	2/2	0/3	2/2	1/3	1/2	—	2/2	3/3	0/2	0/3	1/2	1/3	0/2	0/3
2	<i>Enterobacter agglomerans</i>	—	0/5	—	0/5	—	0/5	—	0/5	—	0/5	—	0/5	—	0/5
3	<i>Citrobacter freundii</i>	—	0/5	—	0/5	—	0/5	—	0/5	—	0/5	—	0/5	—	0/5
4	<i>Proteus mirabilis</i>	1/1	1/4	1/1	2/4	1/1	—	1/1	—	0/1	0/4	0/1	0/4	0/1	1/4
5	<i>Shigella sonnei</i>	1/1	1/4	1/1	1/4	1/1	—	1/1	—	0/1	0/4	0/1	0/4	0/1	0/4
6	<i>Klebsiella ozaenae</i>	—	2/5	—	2/5	—	—	—	—	—	0/5	—	2/5	—	0/5
7	<i>Klebsiella pneumoniae</i>	—	1/5	—	1/5	—	—	—	—	—	0/5	—	1/5	—	0/5
8	<i>Serratia marcescens</i>	—	0/5	—	0/5	—	—	—	—	—	0/5	—	1/5	—	0/5
9	Control(negative)	—	0/5	—	0/5	—	—	—	—	—	0/5	—	0/5	—	0/5
10	control(blank)	—	0/5	—	0/5	—	—	—	—	—	0/5	—	0/5	—	0/5

B.M : Bone marrow.

D : Dead turkeys.

S : Sacrificed turkey.

(*) : This organs were subjected to reisolation for 1st week of age.

الملخص العربي ضراوة بعض البكتيريا المعوية المعزولة من الكتاكيت الرومي المستوردة

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تمت دراسة البكتيريا المعوية الموجودة بالقناة الهضمية و كيس المح للكتاكيت الرومي المستوردة عمر يوم و السليمة ظاهريا وقد تم فحص حوالي ٣٧٨ كتكوت بكتريولوجيا و تم عزل ٧٨ عترة بنسبة ٢٠,٦%. تم تصنيف هذه العترات إلى الاشيريشيا كولاي و ستروباكترا فروندي و الانتيروباكترا أجلومرانس و بروتيس ميرابيليس و شيجيلا سوني و كليبيلا نيموني و كليبيلا أوزيني و سيراتيا ميراسنز بمعدل ١٠,٢٦%؟ ٢٨,٢%؟ ١٠,٢٦%؟ ١٢,٨٢%؟ ٢٠,٥١%؟ ٥,١٣%؟ ٧,٦٩%؟ ١٠,٢٦%؟ بالترتيب. وقد أوضح اختبار الضراوة على أن كل من الاشيريشيا كولاي و بروتيس ميرابيليس و شيجيلا سوني ذات ضراوة مرتفعه عند الحقن في الغشاء البريتوني لكتاكيت الرومي عمر ٢ يوم بينما باقي البكتيريا غير ضارية عند الحقن في الغشاء البريتوني .