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Pigeon, Duck and Chicken Ileal Loop Response to E. Coli Isolated From Pigeons, Turkeys, Ducks, and Chicken

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A TOTAL OF 257 E. COLI isolates were recovered from blood and/or internal organs of 250 pigeons, 128 turkeys and 100 ducks with septicaemic manifestations. 137 *E. coli* isolates could be serotyped. In pigeons, there were 86 *E. coli* isolates belonging to the O-groups 1,2,11,22,22,29,48,53,99,101,110,116, 119, 123, 126, 147 and 156 in turkeys they were 31 *E. coli* isoates belonging to the O-groups 1, 2, 11, 17, 29, 53 79, 86, 99 and 111 and in ducks they were 20 *E. coli* isolates belonging to the O-groups 11, 17, 22 71, 86, 99, 11, 119, 137 and 157 It was succeeded to carry out the ileal loop test for determination of the enterotoxicity of *E. coli* in pigeons, ducks and chickens using *E. coli* isolates recovered. from these birds. Chickens were also found to be suitable for testing *E. coli* isolated from pigeons, turkeys and ducks. Distention of the ileal loops was achieved by 24.6% of the tested 65 isolates. On the other hand 69.2% of the isolates caused congestion and haemorrhages when their bacteria-free filtrates were injected in 10 days old chicken embryos. The oral administration of live bacterial suspensions of 10 *E. coli* O-groups caused diarrhoes of live bacterial suspensions of 10 *E. coli* O- groups caused diarrhoea in all chickens used in the experiment. The mortality rate varied between O and 80%.

Farid *et al.* (1976) have pointed out the role of E. coli as one of the important causes of mortality in calves in Egypt.In 1977 Farid *et al.* made a survey of the various E. coli O-groups causing colisepticaemia in chickens. They reported that E. coli O-groups 1,2,8,11,22,42,53,71 and 78 are the common cause.

The present study was designed to elucidate the incidence of E. coli in pigeons, turkeys and ducks with septicaemic manifestations. The isolated strains as well as those previously isolated from chickens and apparently healthy pigeons and chickens were tested for the production of enterotoxins using ileal loops, chicken egg embryos and 6 days old chickens.

Material and methods

Isolation and identification

478 diseased pigeons, turkeys and ducks were examined. Heart blood and internal organs were cultured on MacConkey, blood and EMB-agar pla-

tes. The suspected colonies were examined biochemically (Edwards and Ewing, 1972) and serologically (tube agglutination test) using 25 E. coli O-antisera

Testing for enterotoxins

65 E. coli isolates representing the various O groups and sources were tested for enterotoxins according to the method described by Burrows and Musteikis (1966). Both live bacterial suspensions and bacteria-free fluids (Moon *et al.*, 1970) were used. Intestinal loops were made in pigeons (5 cm), ducks and chickens (10 cm). 10 days old chicken embryos were injected in the allantoic sac (6 for each strain) with 0 2 ml bacteria-free fluids of the 65 E. coli strains, to which penicillin (10000 i.u.) and streptomycin (10 mg) were added.

Bacteria-free fluids of 15 E. coli O-groups were given orally in a dose of 2 ml in 6 days old chickens (5 for each strain). The birds were put under observation for 3 weeks.

Results

Incidence of E. coli in pigeons, turkeys and ducks

A total of 257 E. coli isolates was recovered from blood and/or internal organs of 478 birds examined. Only 137 isolates could be serotyped ; 109 isolates were inagglutinable in the available antisera and 11 isolates were rough.

From the 250 pigeons examined, 86 E. coli isolates were recovered, which could be serologically grouped into 17 O- groups. 053 was the most common, followed by 02, 022, 01 and 099. The remaining O-groups constituted between 1·2 and 5·8% of the identified isolates.

From the 128 turkeys examined, 31 E. coli isolates could be typed serologically into 10 O-groups, of which 01 was the most common, followed by 053.The remaining 8 O-groups were represented by 6-9% of the isolates.

The 20 E. coli isolated from 100 ducks could be typed into 8 E. coli O-groups, whereby 011 and 0119 were the most common. (Table 1).

It is worthy to mention that E. coli 011, 017 and 099 were recovered from the 3 types of birds; O groups 48, 101, 110, 116, 123, 126, 147 and 156 were isolated only from pigeons ; 079 was recovered only from turkeys and 071 only from ducks.

Testing of E. coli for enterotoxins

1. *Testing of chicken isolates in chicken ileal loops*

Only 3 out of 8 E. coli O-groups, namely 01, 011 and 078 caused distention of the loops and increased the fluids inside each loop to 6-7 ml. Also 053 which was isolated from apparently healthy chickens caused distention of the ileal loops.

2. *Testing of pigeon isolates in pigeon ileal loops*

Of the E. coli representing the 17 O-groups of pigeon origin, only 3 serogroups, namely 01, 017 and 0126 caused dilatation of the loop, the fluid amounts ranged between 4 and 5 ml. 079 which was isolated from a healthy pigeon produced only 3 ml.

3. *Testing of turkey isolates in chicken ileal loops*

079 produced 12 ml fluid per loop, 02 and 017 produced 4 and 4·5 ml fluid respectively. The remaining 7 of the 10 isolates examined exerted no effect.

TABLE 1 **Incidence of E.coli in pigeons, turkeys and ducks with septicaemic manifestations**

	Pigeons	Turkeys	Ducks	Total
No. of cases examined	250	120	100	478
No. of pos. cases	86	31	20	137
%	34.4	24.2	20	28.7
E. coli O-group . . .				
01	10	9	—	19
02	12	3	—	15
011	2	2	4	8
017	4	3	2	9
022	12	—	3	15
029	3	2	—	5
048	4	—	—	4
053	16	5	—	21
071	—	—	2	2
079	—	1	—	1
086	—	3	1	4
099	6	1	1	8
0101	1	—	—	1
0110	2	—	—	2
0111	—	2	1	3
0116	5	—	—	5
0119	2	—	4	6
0123	2	—	—	2
0126	2	—	—	2
0137	—	—	1	1
0147	2	—	—	2
0156	—	—	—	1
0157	—	—	1	1

4. *Testing of duck isolates in duck illeal loops*

5 out of 18 E. coli O-groups caused distention of the ileal loops and increased the fluids in each loop up to 9-12·5 ml.

The use of bacteria-free fluids gave almost the same results as that of the live bacterial suspension with insignificant differences in the amounts of fluids.

5. *Testing of pigeon and duck isolates in chicken ileal loops*

From Table 3 it is clear that E. coli isolates of pigeons that gave positive reaction in pigeon ileal loops as well as duck isolates that gave positive reaction in duck ileal loops gave almost the same reaction when tested in chicken ileal loops with minor difference in the amount of fluids.

Effect of bacteria-free fluids prepared from E. coli on chicken embryos The mortality rate was 13·3%. The dead embryos showed haemorrhages and congestion of the internal organs. The results were not character istic to the O-groups and did not correlate with those of the ileal loop test.

TABLE 2. **Results of ileal loop test with live bacterial suspensions of E. coli**

E. coli O-groups	No. of tested isolates	Amounts of fluids per loop in ml			
		Pigeon isolates in pigeon loops	Chicken isolates in chicken loops	Turkey isolates in chocken loop	Duck isolates in duck loops
01	4	5	6	—	*
02	4	—	—	4	*
08	2	*	—	*	*
011	5	—	6 5	—	12.5
015	2				
017	4	4 5	*	4.5	—
022	5	—	—	*	—
029	2	—	*	—	*
048	2	—	*	*	*
053	5	—	6*	—	*
071	2	*	*	*	—
078	2	*	7	*	*
079	2	3*	*	12	*
086	4	*	—	—	—
099	2	—	*	—	12
0101	2	—	*	*	*
0110	1	—	*	*	*
0111	2	*	*	—	—
0116	1	—	*	*	*
0119	2	—	*	*	11
0123	2	—	*	*	*
0126	2	4	*	*	*
0137	1	*	*	*	9
0147	2	—	*	*	*
1056	2	—	*	*	*
0157	1	*	*	*	10

— = less than 4 ml fluids in chicken and duck loops and less than 3 in pigeon loops
* = not tested in this bird
** = isolated from an apparently healthy bird

Results of experimental infection of 6 days old chickens

The oral administration of 24 hours broth cultures of 10 E. coli that caused dilatation of ileal loops in chickens caused softening of the faeces or diarrhoea in all cases (Table 4). The mortality rate was 80% in chickens infected with 079 and 40% in chickens infected with E. coli O-groups 11, 17, and 78. E. coli was isolated from the heart blood. The oral administration of bacteria-free fluids caused, likewise softening of the faeces or diarrhoea, but only 2 chickens died after administration of 078 bacteria-free fluids.

Discussion

It is interesting to note that the highest yield of *E. coli* O-groups was recorded in pigeons, where 17 different O-groups could be identified. 8 of these 17 O-groups could be recovered from ducks and turkeys. These were the O-groups 1,2,11,22,53,99 and 119. The O-groups 1,2,11,22 and 53 were isolated by Farid *et al.* (1977) from colisepticaemic chickens. 086 and 0119 were isolated by Awad *et al.* (1973) from an outbreak of colibacillosis in ducks ; 017 and 086 were isolated, likewise from ducks by Ibrahim (1977) and 0101, 0119 and 0126 were recovered from calves by Farid *et al.* (1976). The remaining O-groups, namely 048, 110, 116, 123 147 and 156 seem to be identified for the first time in Egypt. The authors failed to find any data regarding E. *E. coli* infection in pigeons in the available literature. Therefore the results obtained in this study may add significant contribution to the epidemiology of *E. coli*. On the one side, pigeons may live a semiwild life and come in contact with various animals and birds, on the other side, they may live in a domestic life in close contact with man. With regard to animals and birds we have seen that 11 out of the 17 O-groups isolated from pigeons were also recovered from turkeys, ducks, chicken and calves. On the other hand, the O-groups 86, 119 and 126 are known to be among the enteropathogenic *E. coli* that cause diarrhoea in childern (Edwards and Ewing, 1972).

Since the introduction by De and Chatterje (1953) of the ligated rabbit intestine for the determination of the enteropathogenicity of *E. coli* strains, the technique has been widely used for the study of *E. coli* associated with diarrhoea in man and animals (Taylor *et al.*, 1961 ; Gyles & Barnum, 1967 and Moon *et al.*, 1970). Of significance was the finding of Gyles and Barnum (1967) as well as Smith and Halls (1967) that strains isolated from a certain species gave positive results when tested in intestinal loops of the same species. This findings could be substantiated in the present study by the successful use of pigeon, duck and chicken ileal loops in testing E. coli strains isolated from these species. Moreover, it was proved that chickens were also suitable for testing *E. coli* isolated from pigeons, turkeys and ducks. This is of practical importance as chicken in Egypt are easier to get and are cheaper than the other birds. Iti s to be noted that the reaction in ducks was more distinct, especially with regard to the amount of fluids per loop, it needs however further study to find out whether ducks are also suitable for testing *E. coli* of other origin.

The P.M. findings in chicken embryos inoculated with bacteria-free fluids of *E. coli* broth cultures, namely congestion and haemorrhages of the internal organs, indicate the production of toxic metabolites by 69.2% of the tested strains. However, only 24.6% of the isolates caused distention of the ligated loops. It needs further study to elucidate whether the chicken embryos are more sensitive to *E. coli* enterotoxins or whether the bacteria-free fluids contain toxic substances other than enterotoxins which were responsible for the above mentioned findings.

TABE 3. **Comparison between pigeon, duck and chicken ileal loops for testing E. coli of pigeon and duck origin**

Source of E. coli	O-groups	Amounts of fluids per loop in ml		
		Pigeon loops	Duck loops	Chicken loops
Septicaemic pifeons . .	01	5.0	*	10.0
	017	4.5	*	6.0
	0126	4.0	*	5.0
Septicaemic ducks . .	011	*	12.5	10.0
	086	*	12.0	8.0
	0137	*	11.0	9.0
	015;	*	10.0	8.5
Healthy pigeons	079	3.0	*	4.0

* = not tested

TABIE 4. **Results of experimentalinfection of 6 daysold chickens withenterotoxic Ecolibroth cultures**

E. coli o-groups	No. of infected chickens	No. of chickesm died	Re-isolation from heart blood	Clinical symptom
01	5	1	+	white watery diarrh.
02	5	1	+	soft faeces
011	5	2	+	white watery diarrh
017	5	2	+	yellow watery diarrh.
079	5	4	+	white watery diarrh
078	5	2	+	,, ,, ,,
086	5	1	+	,, ,, ,,
0119	5	—	—	,, ,, ,,
0137	5	1	+	,, ,, ,,
0157	5	—	—	,, ,, ,,

References

Awad, F.I. l Bassiouni, A.A.and Awaad, M.H. (1973) Escherichia coli septicaemia in ducklings, *Vet Ital* **24**, 389

Barrows, W. and Musteikis, G. (1965) Cholera infection and toxin in the rabbit ileal loop, J. *Infect. Dis.* **116**, 183

De,S.N. and Chatterje, D.N. (1953) An experimental study of the mechanism of action of Vibrio cholerae on the intestinal mucous membranes .J. *Path. Bact.* **16**, 559

Edwards, P.R. and Ewing, W.H. (1972) "*Identification of Enterobacteriaceae*", Burgess Publishing Company, Minneapolis

Farid, A , Ibrahim, M.S. and Refai, M. (1976) Studies colibacillosis in calves in Egypt. Incidence of E. coli serogroups among live colves, Zbl. Vet. Med, B. 23, 44

Farid, A., Refai, M., Bl-Shinawy, M. and Nasser, A. (1977) Escherichia coli infection of Chickens in Egypt, *Wiener Tierarzzl. Mschr.* **64**, 233.

Gyles, C.L. and Ba num, D.A. (1969) A heat-labile enterotoxin from strains of Escherichia coli enterspatho, genic for pigs, J. Infect. Dis. 120, 419

Ibrahim, A.A. (1977) Colisepticaemia in ducklings, *Thesis, Faculty of Veterinary* Medicine *Assiut University, Egypt.*

Moon, H.A., Whip, S.C.; Engstrom, G.W.tand Baetz, A.L. (1970) Response of the rabbit ileal loop to celle-free products from Eszherichia coli enteropathogenic for swire, J *Infect. Dis.* 121, 182.

Smith, H.W. and Halls, S. (1967) Observations by the ligated intestinal segments and oral inoculation me-thods on Escherichia coli infection in pigs, calves, lambs and rabbits. II, Studies on Escherichia coli cnerotoxin. *J. Path. Bact.* **93**, 499,

Taylos,tJ.;Wikins, M.P.tand Payne, J.M. (191) Relation of rabbit gut reactions to enteropathogenic Escherichia coli, *Brit. J. Exp. Path.* 42, 43.

تأثير الميكروب القولونى المعزول من الحمام والرومى والبط والدجاج على الأمعاء المربوطه فى الحمام والرومى والبط

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تم عزل ٢٥٧ عترة من الميكروب القولونى من الدم والأحشاء الداخلية لـ ٢٥٠ حمامة ١٢٨ رومى و ١٠٠ بطة مصابة بالتسمم الدموى • تم تصنيف ١٣٧ عترة من الميكروب القولونى من الحمام وكانت المجموعات المصنعة كالتالى :

1, 02, 011, 022, 029, 048, 053, 099, 0101, 0110, 116, 0119, 0123, 0126, 0126, 0147, 0156,

وكذلك تم تصنيف ٣١ عترة من الرومى تنتمى الى المجموعات التالية :

01, 02, 011, 017, 029, 053, 079, 086, 099, 0111

أما فى البط فقد تم تصنيف ٢٠ عترة تنتمى الى المجموعات التالية :

011, 017, 022, 021, 086, 099, 0111, 0119, 0157, and 0157,

أجرى اختبار خلقات الأمعاء المربوطة لمعرفة العترات المحتوية على الانتيروتوكسين من بين العترات المعزولة من الحمام ، البط والدجاج وجد أن أمعاء الدجاج مناسبة لاختبار العترات المعزولة من الحمام والرومى والبط • سببت ٦ر٢٤٪ من ٦٥ عترة امتلاء فى حلقات الامعاء المربوطة • وكذلك وجد أن ٢ر٦٩٪ من العترات سببت احتقان ونزيف عند حقن السوائل الخالية من البكتريا فى أجنة الدجاج عمر ١٠ أيام •

أجريت العدوى الصناعية عن طريق الفم بمعلقة البكتريا الحى لـ ١٠ مجموعات من الميكروبات القولونى سببت كلها اسهال وكانت نسبة الوفيات تتراوح بين ٥ ـ ٨٠٪ •