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EPIDEMIOLOGICAL STUDIES ON INTESTINAL CLOSTRIDIAL INFECTION IN BROILER CHICKENS IN ASSIUT AND EL-MENIA GOVERNORATES

(With 5 Tables)

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دراسات وبائية على عدوى الكلوستريديوم المعوية فى بدارى التسمين بمحافظتى أسيوط والمنيا

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تم استقصاء مشكلة الألتهابات المعوية في الدجاج الذى تروح عمره بين اثنين وحتى سبع أسابيع من العمر. تم تقسيم الطيور تبعا لوجود الألتهابات المعوية والأصابة بالكوكسيديا الى ثلاث مجاميع. المجموعة الأولى تضم الطيور المصابة بالألتهابات المعوية والخالية من الكوكسيديا بينما ضمت المجموعة الثانية الطيور التى بها التهابات معوية ومصابة بالكوكسيديا في نفس الوقت واشتملت المجموعة الثالثة على الطيور الغير المصابة ظاهريا بالالتهابات المعوية. ارتفع معدل العزل لميكروب الكلوستريديوم من المجموعة الثانية (٦٤,٨%) والمجموعة الاولى (٥٩,٥%) بينما انخفض في المجموعة الثالثة (٢٧.٦%). شكل ميكروب الكلوستريديوم برفرنجينز نسبة (٧١,٩)% من مجموع معزولات الكلوستريديوم وتبعه الكلوستريديوم كولينم (١٤,٣%) ثم كلوستريديوم سبوروجينيز (٢١,٩%) وأخيرا كلوستريديوم سبيريفورم (٠,٩%). عند دراسة سمية الكلوستريديوم برفرنجنز المعزول اتضح ان النوع الاكثر سمية كان بمعدل أكبر في المجموعة الثانية. وعند محاولة تصنيف السموم بالحقن في خنزير غينيا الالبينو ظهر أن هناك ثلاثة أنواع (أ، ج، د) وكان النوع أ هو الأكثر تكرارا وتبعه الأنواع ج، د في كل المجموعات وخصوصا المجموعة الثانية.

SUMMARY

A problem of intestinal clostridial infection in chickens aged from 2-7 weeks was investigated in Assiut and El-Menia governorates. Birds were categorized according to enteritis and coccidiosis into three groups. Group I consists of birds with enteritis and negative coccidiosis, group II consists of birds with enteritis and positive coccidiosis, while group III

consists of birds with grossly normal intestine. The higher incidence of clostridial isolation was demonstrated in group II (64.8%) and group I (59.5%), while it was lower in group III (27.6%). *Cl. perfringens* represented 71.9% of the total clostridial isolates followed by *Cl. colinum* (14.3%), *Cl. sporogenes* (12.9%) and *Cl. spiroforme* (0.9%).On testing the toxigenicity of isolated *Cl. perfringens* it was clear that toxigenic strains were frequently higher among group II of examined birds. Typing of toxigenic strains by using dermonecrotic test in albino guinea pigs resulted in detection of types A, C and D. Type A was the most frequently detected isolates followed by types C and D in all groups specially group II.

Key words: *Clostridia, Toxins, Enteritis, Toxogenic, Coccidiosis*

INTRODUCTION

Recently clostridial infection appeared to be of high significance among poultry all-over the world, and constitutes one of the most important veterinary problems that face poultry industry in many countries including Egypt due to high economic losses (Long, 1974; Ibrahim, 1979; El-Ged and Hagazy, 1985, and Hofshagen and Stenwig, 1992). Most of losses among broiler flocks were related directly or indirectly to *Clostridium* with or without complicating factors specially coccidiosis (Kohler et al., 1974; Latinovic, 1983 and Kim HongJib et al., 1996). The present work was directed for investigation of the present types of *Clostridia species* and its severity in occurrence of intestinal affections.

MATERIALS and METHODS

Clinical examination:

A total of 470 freshly dead and sacrificed broiler chickens aged from 2-7 week-old obtained from different localities in El-Menia and Assiut governorates were subjected for clinical and post-mortem examination with special attention to gastro-intestinal tract.

Examination of coccidia:

Pooled samples of intestinal and cecal scraping were microscopically examined for presence or abscence of coccidian oocysts.

Isolation of Clostridium:

Cultures were done from different parts of small intestine on two tubes of cooked meat media, incubated anaerobically at 37°C for 48 hrs.

One of the incubated tubes was heated in a water bath at 60°C for 30 minutes and the other was left without treatment. Subcultures from each tube were made on duplicated neomycin blood agar plates and incubated anaerobically and aerobically at 37°C for 24 hrs. Only strict anaerobic isolates were examined and transferred to cooked meat media for further identification.

Bacterial identification:

a- *Colonial and cellular morphology*: Morphological identification was done by microscopical appearance on Gram's staining, cultural characters and motility testing (Machie and McCartney, 1989).

b- *Sugar fermentation reactions*: Five sugars were tested, including glucose, lactose, maltose, sucrose and mannitol. The test was done according to Machie and McCartney (1989).

c- *Biochemical reactions*: Indole production (Spot test), gelatin liquefaction, H_2S production and urease tests were done according to Machie and MacCarteny (1989).

d- *Nagler's reaction test*: The test was carried out after Levett (1991). The half antitoxin media using *Cl. perfringens* type A antitoxin. The egg yolk agar plates, one half was covered by 2 drops of antitoxin and allowed to dry, while the other half was dropped by 2 drops of 48-hrs cooked meat media culture which was cultured on one direction toward the first half. The plates were incubated anaerobically at 37°C for 24 hrs.

Detection of toxigenic *Cl. perfringens* and types of toxins:

***I-Pathogenicity to laboratory animals*:**

Swiss Mice inoculated in tail vein with 0.3 ml of centrifuged supernatant of intestinal contents obtained from clinical cases suspected to be infected with *Cl. perfringens*. The animals were kept under observation for 72 hrs.

II- *Dermonecrotic test*:

This test was carried out for determination of the toxin types.

a- Preparation of toxins: After Bullen (1952). The recovered isolates were inoculated in peptone starch media. The pH was maintained at 6.8 for production of alpha, beta and epsilon toxins. The inoculated flasks were incubated in a water bath at 37°C for 8 hrs. Sodium hydroxide (0.1 N) and glucose (1%) were added to assist in anaerobiosis. Part of supernatant was centrifuged at 3000rpm for 30 min. The clear supernatants were divided into 5 portions (0.2 ml in test tubes). First, second and third portions were neutralized by 0.1ml of antisera

against types A, B and C respectively. Fourth portion was treated by trypsin to a final concentration of 0.05% and incubated at 37C for 1 hour and neutralized by type D antitoxin. Fifth portion was left for toxin determination in the supernatant kits of antisera were provided from Burrovgh's welcome, Beehanham, London, England.

b- Application: After Oakley and Warrack (1953). Albino guinea pigs were used. The hair of the back and sides were shaved and marked longitudinally into right and left sides. The right side was divided into four areas while left side was divided into two areas.Amount of 0.2 ml of toxins were inoculated intradermally in left side, where untrypsinized toxin was inoculated in the upper part and trypsinized toxin was applied in the lower part. The neutralized toxins were inoculated in the right side at the same time. G. pigs were kept under observation for 24-72 hrs for any dermo-necrotic reaction.

RESULTS

Clinical examination:

Out of 470 examined birds of different ages (2-7 weeks) only 285 cases showed enteritis varied from catarrhal to haemorrhagic and necrotic enteritis and distention of the intestine with gas. Table (1) shows grouping of collected samples to 3 groups according to intestinal lesions and coccidial infestation:

Group I: Birds with enteritis and negative coccidia (89 cases).
Group II: Birds with enteritis and positive coccidia (196 cases).
Group III: Birds with grossly normal intestine (185 cases).

PM examination of the internal organs of examined birds revealed one or more of these lesions: congestion and enlargement of liver and spleen, necrotic foci or yellowish brown color were observed on the liver, affections of the kidney, hydropericardium, ascites and severe emaciation.

Examination of coccidia:

Direct microscopic examinations of intestinal and caecal smears of 285 cases, revealed only 196 cases positive intestinal and caecal coccidiosis of different degrees.

Isolation of *Clostridium species* from broiler farms in different ages:

The results of isolation are shown in Table (1).

Group I revealed recovery of 53 Clostridial isolates (59.5%).
Group II revealed recovery of 127 Clostridial isolates (64.8%).
Group III revealed recovery of 51 Clostridial isolates (27.6%).

The highest isolation was recorded in bird showing enteritis and positive coccidiosis.

Bacterial identification:

According to the morphological characters and biochemical reactions, the recovered 231 isolates (49.1%) were identified as follow: 166 *Cl.perfringens*, 33 *Cl.colinum*, 30 *Cl.sporogenes*, 2 isolate *Cl.spiroforme*, as shown in Table (2).

One hundred and sixity six isolates showed physiological properties of *Cl.perfringens*. On egg yolk agar media, the colonies were surrounded by wide circular opaque zone, recognized as the lecithinase reaction and was inhibited by alpha- antitoxin serum (Nagler's reaction).

Thirty three isolates showed physiological properties of *Cl.colinum*. On egg yolk agar media, grown colonies demonstrated an absence of lipase and lecithinase production.

Thirty isolates showed physiological properties of *Cl.sporogenes*. On egg yolk agar media produce an intense restricted opacity accompanied by a fine pearly layer overlying the colonies due to lipases.

Two isolates showed physiological properties of *Cl.spiroforme*. On egg yolk media, grown colonies demonstrated an absence of lipase and lecithinase production.

Tables (2 and 3) shows results of biochemical identification of recovered isolates according to group of examined birds and their ages.

Detection of toxigenic *Cl. perfringens* and types of produced toxins:

1- *Pathogenicity to laboratory animals:*

Swiss mice dead within 3 days after inoculation of 0.3 ml of centrifuged supernatant of intestinal contents in tail vein if toxigenic *Cl.perfringens* was present. Results are shown in table (4).

2- *Dermo-necrotic test:*

One hundred sixty six *Cl. perfringens* isolates were typed by intradermal injection in the skin of guinea-pigs, the results were interpreted by the degree of the dermonecrotic reaction and its neutralization within 48 hr. post inoculation according to Stern and Batty (1975).

The results in Table (5) shows that 79 isolates produced irregular area of yellowish necrosis. The lesions tend to spread down words, this indicated that 79 isolates were type A, (24 isolates from group I, 39 isolates from group II and 16 isolates from group III).

Thirty five isolates gave after 48-hr slightly greenish blue coloration indicating that they were type C, (7 isolates from group I, 20 isolates from group II and 8 isolates from group III).

Seventeen isolates produced the lesions only after activation by trypsin, they produced circular white necrosis fully developed in 24 hrs and sometimes showed a few small areas of purplish haemorrhagic mottling, these results pointed out that the 17 isolates were type D, (8 isolates from group I, 8 isolates from group II, and 1 isolates from group III).

Thirty five isolates were non-toxigenic types, (8 isolates from group I, 24 isolates from group II, and 3 isolates from group III.).

The incidence of *Cl.perfringens* types A,C,D and non-toxigenic were 47.6%, 21.1%, 10.2% and 21.1%, respectively.

DISCUSSION

Intestinal tracts play a very important role in the bird development and protection. Digestion and absorption of the nutrient material nearly completed in the intestine. As well as the secretion of secretory IgA responsible for mucosal protection of bird during immunization process occurs by lymphoid tissues in intestinal submucosa (Hermans and Bazin, 1971). Therefore, the pathological affections of that important part may lead to hindering the formerly mentioned functions. Intestinal clostridial infections is a common problem among rapidly growing broiler chickens causing severe losses specially when complicated with coccidiosis.

In this study special attention was directed to investigate the incidence of locally distributed clostridia in several broiler farms of various ages. Four hundred and seventy examined birds of different ages 2 -7 weeks, these ages were selected as formerly reported by Narin and Bamford (1967), Cygan and Nowak (1974), Kohler et al. (1977), Perelman et al. (1991) and Kim HongJib et al. (1996).

There was a great variation between the incidence of clostridial isolates and the general status of examined intestines. Such incidence was higher among birds with enteritis and positive coccidia (64.8%) and birds with enteritis and negative coccidia (59.5%). On the contrary it was lower among birds with grossly normal intestine (27.6%). Moreover, the overall incidence of clostridial isolates among all examined birds was (49.1%). These findings closely resembled the results obtained by Hussein (1972) and Awad et al. (1976) who

observed that the incidence of intestinal clostridial infection among normal and dead chickens was 48.4%.

Cl.perfringens constitutes 71.9% of the total clostridial isolates from examined birds, this organism appeared to be the most prevalent isolate and these results agreed with the observations done by Hussein (1972), Kohler et al. (1974), Awad et al. (1976). Ibrahim (1979), Shane et al. (1984), El-Ged and Hagazy (1985), Benno et al. (1988), Prukner - Radovcic and Milakovic - Novak (1991), Tschirdewahn et al. (1992) and Hussein and Mustfa (1999).

It seems clear that incidence of toxigenic *Cl.perfringens* was frequently higher within birds with enteritis and positive coccidia and then within birds with enteritis and negative coccidia, on the contrary such incidence among birds with grossly normal intestine was lower. These results are in agreement with the observation done by Kohler et al. (1974), Long (1974), Ibrahim (1979), Latinovic (1983), El-Ged and Hagazy (1985), Kim HongJib et al. (1996).

Typing the different isolates of toxigenic *Cl.perfringens* indicated that out of 39 isolates proved to be toxigenic isolated from birds with enteritis and negative coccidia, 24 were type A, 7 were type C and 8 were type D. Typing of the 67 recovered toxigenic *Cl.perfringens* isolates from birds with enteritis and positive coccidia, 39 were type A, 20 were type C, and 8 were type D, and typing of 25 recovered toxigenic *Cl.perfringens* isolates from birds with grossly normal intestine, 16 were type A, 8 were type C and only one type D. This indicated that type A is widely distributed in all groups of birds, these findings come in agreement with results recorded by long (1974), Awad et al. (1976), Ibrahim (1979), Latinovic (1983), El-Ged and Hagazy (1985), El-Seedy (1990), Hofshagen and Stenwig (1992).

The recovery of *Cl.colinum* (14.3%) and *Cl.spiroforme* (0.9%) from broiler chickens aged 2-7 weeks in this study was the first record in Egypt and our results come in agreement with Peckham (1960), Kaneuchi et al. (1979) who found *Cl. spiroforme* in the faeces of healthy chickens, Berkhoff (1985), Kondo et al. (1988), Perelman et al. (1991), Jia ShiYu et al. (1999).

Our results revealed recovery of *Cl.sporogenes* (12.9 %) from broiler chickens aged 2-7 weeks and these results were parallel to that recorded by many authors whom previously isolated these organism, Hussein (1972), Awad et al. (1976), Ibrahim (1979), El-Ged and Hagazy (1985).

This work clarified that most cases of enteritis usually associated with clostridial infection specially *Cl. perfringens*. Moreover, presence of coccidia exaggerate this problem and resulting in severe enteropathy. Isolation of clostridium from grossly normal intestine indicated its wide existence among poultry in high percentage and under devitalizing conditions it can flair up and causing severe enteritis and subsequent economic losses.

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Table (1): Incidence of clostridial isolation from intestine of the examined birds.

Age of exam. Birds	Group I Birds with enteritis and negative coccidia			Group II Birds with enteritis and positive coccidia			Group III Birds with grossly normal intestine		
	No. of exam. birds	No. of clostridial isolates	%	No. of exam. birds	No. of clostridial isolates	%	No. of exam. birds	No. of clostridial isolates	%
2- weeks	10	5	50.0	5	3	60.0	53	6	11.3
3- weeks	27	15	55.5	28	18	64.3	46	12	26.0
4-5 weeks	17	10	58.8	65	42	64.6	44	14	31.8
6-7 weeks	35	23	65.7	98	64	65.3	42	19	45.2
Total	89	53	59.5	196	127	64.8	185	51	27.6

% according to number of the examined cases.

Table (2): Biochemical identification of the suspected clostridial isolates.

Morphological characters				Biochemical reactions									No. of reacted isolates
								Acid production from:					
Gram's stain	B. haemolysis	Motility	Spores location	Indole	Lecithinase	Lipase	H_2S	Urease	Hydrolysis of gelatin	Glucose	Lactose	Maltose	Sucrose	Mannitol	
+ ve bacilli	+	-	St	-	+	-	-	v	+	+	+	+	+	-	166
+ ve bacilli	-	+	St	-	-	-	-	-	-	+	-	w	+	v	33
+ ve bacilli	+	+	St	-	-	+	+	-	+	+	-	+	-	-	30
+ ve spiral	-	-	T / St	-	-	-	-	v	-	+	+	-	+	-	2

+; positive
-; negative
v; variable
w; weak
St; subterminal
C; central
T/St, terminal to subterminal

Table (3): Results of biochemical identification of recovered isolates according to age and general status of birds.

Age of exam. birds	Group I				Group II				Group III			
	No. exam. isolates	Cl. perfringens	Cl. sporogenes	Cl. spiroforme	No. exam. isolates	Cl. perfringens	Cl. sporogenes	Cl. spiroforme	No. exam. isolates	Cl. perfringens	Cl. sporogenes	Cl. spiroforme
2- weeks	5	5	-	-	3	2	-	-	6	5	1	-
3- weeks	15	14	-	-	18	13	2	-	12	9	2	-
4-5 weeks	10	7	2	-	42	27	7	-	14	5	4	1
6-7 weeks	23	21	-	-	64	49	8	1	19	9	7	-
Total	53	47	2	-	127	91	17	1	51	28	14	1

Table (4): Correlation between toxigenic and non-toxigenic Clostridium perfringens and their relation to age and general status of birds.

Age of birds	No. of exam. birds	Cl. perfringens isolates		Toxigenic Cl. perfringens						Non. toxigenic Cl. perfringens					
				Group I		Group II		Group III		Group I		Group II		Group III	
		No.	%	No.	%*	No.	%*	No.	%*	No.	%*	No.	%*	No.	%*
2- weeks	68	12	17.6	3	25.0	2	16.7	4	33.3	2	16.7	-	-	1	8.3
3- weeks	101	36	35.6	12	33.3	9	25.0	8	22.2	2	5.6	4	11.1	1	2.8
4-5 weeks	126	39	30.9	7	17.9	19	48.7	5	12.8	-	-	8	20.5	-	-
6-7 weeks	175	79	45.1	17	21.5	37	46.8	8	10.1	4	5.0	12	15.2	1	1.3
Total	470	166	35.3	39	23.5	67	40.4	25	15.0	8	4.8	24	14.5	3	1.8

% according to number of the examined cases.

%* according to number of the positive cases for Cl. perfringens.

Table (5): Results of serotyping of toxigenic Clostridium perfringens.

Age of birds	Group I					Group II					Group III				
	No. exam. isolates	Cl. perfringens A	Cl. perfringens C	Cl. perfringens D	Non-toxi. Cl. perfringens	No. exam. isolates	Cl. perfringens A	Cl. perfringens C	Cl. perfringens D	Non-toxi. Cl. perfringens	No. exam. isolates	Cl. perfringens A	Cl. perfringens C	Cl. perfringens D	Non-toxi. Cl. perfringens
2- weeks	5	3	-	-	2	2	2	-	-	-	5	2	2	-	1
3- weeks	14	6	2	4	2	13	6	2	1	4	9	4	3	1	1
4-5 weeks	7	4	3	-	-	27	10	8	1	8	5	4	1	-	-
6-7 weeks	21	11	2	4	4	49	21	10	6	12	9	6	2	-	1
Total	47	24	7	8	8	91	39	20	8	24	28	16	8	1	3