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THE OCCURRENCE OF *CLOSTRIDIUM PERFRINGENS* IN THE INTESTINE OF BROILER CHICKENS IN ASSIUT GOVERNORATE

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ABSTRACT :

A total of 90 intestinal samples of freshly dead broiler chicken were collected from different private chicken farms at Assiut Governorate. These samples suspected clinically and pathologically to suffer from the Clostridial infection. Direct microscopic examination revealed that 66.7% of samples were positive to coccidia. Clostridium perfringens was isolated from 40 samples with an incidence of 44.4%. The experimental infection in 3-days old chicken by different routes of inoculation revealed that the subcutaneous route gave a mortality rate of 90% while the oral route gave 85% mortality. The post-mortem lesions of experimental infection were similar to a great extent to those of natural infection. Reisolation of the inoculated organism from dead chicks were conducted. *In vitro* antibiotic sensitivity tests showed that the examined isolates were highly sensitive to ampicillin, ciprofloxacin, amoxycillin, colistine and lincomycin.

INTRODUCTION :

Several microbial infections are responsible for the losses of poultry industry, *Clostridium perfringens* is considered as one of the most widely spread members in the genus Clostridium affecting broiler chicken with or without complicating factors especially coccidiosis (Parish, 1961 and Gardiner, 1967).

Duben (1968) reported an outbreak of diarrhoea in poultry caused by food contaminated with *Cl.perfringens* type A, and the mortality rate was high up to 80%. Necrotic enteritis has been experimentally reproduced in chicken by giving food contaminated with *Cl.perfringens* (Long and Truscott, 1976) or by adminstering vegetable cultures of *Cl.perfringens* orally by Beriner *et al.*, 1977). Shane *et al.*, (1985) reported that a primary intestinal disease like coccidiosis play an important role in development of necrotic enteritis.

In Egypt, the effect of *Cl.perfringens* in broilers was studied by several authors. Ibrahim (1979) recovered 102 isolates of *Cl.perfringens* out of 420 samples of intestinal tract of both diseased and slaughtered chicken of different ages. Hamdy *et al.*, (1983 a) isolated *Cl.perfringens* from birds, which were not given lincomycin in drinking water and died of

necrotic enteritis. El-Ged and Hagazy (1985) reported the isolation of 160 strains of *Cl.perfringens* from the intestinal contents of dead chicken of different ages. El-Seedy (1990) diagnosed 29 isolates as *Cl.perfringens* 15.3% from chicken with necrotic enteritis. Abdel Salam and El-Sanousi (1991) studied the properties of 220 broiler strains of *Cl.perfringens* and *Cl.perfringens* like-organisms isolated from both healthy birds and birds with diarrhoea. Hussein and Mustfa (1999) isolated 30 (50%) isolates of *Cl.perfringens* out of 60 intestinal samples of broiler chicken 4-6 weeks old in Assiut Governorate. Ebtehal (2000) concluded that *Cl.perfringens* constituted 71.9% of 231 clostridial isolates from 470 broiler chicken in Assiut and El-Minia Governorates.

Lovland and Kal Dhusdal (2001) observed severe losses of broiler flocks with a high incidence of *Cl.perfringens*. Yoo Han Sang *et al*., (2001) indicated that the isolation index of *E.coli* and *Cl.perfringens* was lowered by the newly developed antibacterial (enrofloxacin sodium). Kirollos (1973) showed that neomycin sulphate in concentration of 25 ug/ml could be used for the isolation of *Cl.perfringens* type A.

The discovery of different new technique for isolation, identification of *Cl.perfringens* lead to the appearance of their role as the causative disease in broiler. For these reasons the present work was designed to cover the following items.

*Isolation and identification of *Cl.perfringens* organism from broiler chicken at Assiut Governorate.

*Experimental infections using the isolated organisms in 3-day old chicks by different routes.

**In vitro* sensitivity test of the isolated organisms against different antibiotics.

MATERIAL AND METHODS :

MATERIAL:

1- Samples:

A total of 90 intestinal samples were collected from freshly dead broiler chicken (2-7 weeks age) were obtained from private farms at Assiut Governorate. A direct microscopic examination of intestinal scraping were carried out.

2- Culture media:

a-Cooked meat medium " Mast DM 120".

b-Neomycin blood agar medium (neymycin sulphate solution was added to the media just before the addition of blood to make the final concentration of 150 μg/ml)

3- Media used for biochemical tests :

Sugar fermentation (glucose, lactose, maltose, sucrose and mannitol), gelatin medium, Glucose phosphate broth medium, peptone water, triple sugar iron agar (T.S.I.), urea agar base, semi solid agar media.

4-Reagents, chemicals and stains used were:

Kovac's reagent, urea, methyl red, andrade's indicator, Gram's stain, glucose 1%

5-Experimental animals:

a-Swiss mice: were used for the detection of toxin of *Cl.perfringens* in the intestine of infected broiler chicken. They were kept under observation for 2 weeks before they were inoculated.

b-Chicks: 55 three days-old chicks (balady) obtained from poultry farm of Agriculture

Collage-Assiut University were used for pathogenicity tests. Chicks were observed for 3 days and proved to be free from most pathogenic organisms by taking a random sample of 5 chicks, subjected to clinical, post-mortem as well as bacteriological examination which proved to be healthy and free from any infection, the other 50 chicks were used for the pathogenicity test.

6-Diet:

A commercial ration prepared by manufatcure of Assiut Governorate was used.

7-Gas-pak anaerobic jar "BBL-814-21":

It was used for production of anaerobiosis by using disposable hydrogen-carbon dioxide bags with socket.

8-Antimicrobial sensitivity discs: were produced by Oxoid Laboratories including: Ampcillin (10μg), Ciprofloxacin (5μg), Neomycin (30μg), Enrofloxacin (5μg), Amoxycillin (25μg), Colistine sulphate (25μg), Erythromycin (15μg), Oxytetracycline (30μg), Gentamycin (10μg), Streptomycin (10μg), Chloramephenicol (30μg), Nalidixic acid (30μg), and Lincomycin (2μg).

METHODS:

1-Isolation and identification of *Cl.perfringens* :

The intestinal tract of freshly dead broiler chicken were collected, and the intestinal caecal contents were subjected to direct microscopic examination for coccidial infestation. Small pieces of the intestines with their contents from each sample were inoculated into two sterile cooked meat media tubes. Both inoculated media were incubated anaerobically at 37°C for 48 hours. Only one of the inoculated medium was heated in water path at 60°C for 30 minutes. Subcultures from each of 48 hr cultures were made on duplicated neomycin blood agar plates. One set of the inoculated solid media was incubated anaerobically and the other aerobically at 37°C for 24 hr. Only strict anaerobic isolates were examined and identified for microscopical appearance, culture characters, motility then transfered to cooked meat medium for other biochemical tests as described by Konemann *et al.,* (1983).

2- Pathogenicity tests:

a- Pathogenicity to Swiss mice:

Swiss mice inoculated in tail vein with 0.3 ml of centrifuged supernatant intestinal contents of suspected *Cl.perfringens* cases. The animals were kept under observation for 72 hr.

b- Pathogenicity to baby chicks:

The (50) 3-days-old chicks were classified into 3 groups

Group 1 : 20 chicks were inoculated subcutaneously by 0.1 (1×10^8) of 24 hr cooked meat broth culture of the identified toxigenic *Cl.perfringens* isolates (the plate count technique, Cruickshank *et al.,* (1975) was used for the determination of the viable count of cell per ml suspension).

Group 2: 20 chicks were inoculated orally with the same previous organism and dose.

Group 3: 10 chicks were kept without inoculation as control.

All groups were kept for 21 days (period of observation) with daily examination for clinical signs. Dead and sacrified chicks survived till the end of the observation period were subjected to

P.M. as well as bacteriological examination for lesions and trials of reisolation were conducted.

3-Reisolation of inoculated organism:

4- Sensitivity test:

The isolated Clostridia were tested for sensitivity to different chemotherapeutic agents. One ml of 48 hr. broth cultures was spread on the surface of blood agar. Antibiotic sensitivity discs were placed on the surface of seeded agar. Plates were incubated anaerobically at 37°C for 24 hr. The sensitivity was judged according to the diameter of clearance zone arround the discs (Perelman *et al.,* 1991).

RESULTS :

1-Isolation and Identification of *Cl.perfringens:*

The examined intestine showed enteritis varied from catarrhal to haemorrhagic and necrotic enteritis and balloning of the intestine. Direct microscopic examination revealed that 60 (66.7%) samples were positive to intestinal caecal coccidiosis of different degrees. The suspected *Cl.perfringens* isolates produced gas and putrefied odour on cooked meat broth. The meat particles were pinkish, or grey-black without digestion. All isolates were haemolytic on neomycin blood agar plates gave double zone of haemolysis around the colony : an inner clear zone (complete haemolysis), and the auter hazy zone (incomplete haemolysis). Gram stained smears of suspected colonies revealed Gram-positive rods straight with parallel sides rounded ends, some have central or subterminal spores. According to the morphological (non motile) and biochemical studies of the suspected Clostridial organisms (sugar fermentation, Indole production, gelatin liquefaction, H_2S production, urease test, methyl red), 40 isolates were identified to be *Cl.perfringens* (a higher isolation rate was noticed in birds positive for coccidiosis). The frequency and percentage of infection are presented in table (1).

2-Results of pathogenicity to laboratory animals:

a-Swiss mice:

The inoculated mice died within 3 days after the I/V injection if toxigenic *Cl.perfringens* was present (toxigenic *Cl.perfringens* were frequently higher within specimens with enteritis and positive coccidia, on countrary such incidence of specimens with enteritis and negative coccidia was lowered). Correlation between toxigenic and non toxigenic *Cl.perfringens* are illustrated in table (2).

b-Baby chicks:

The clinical signs observed in the infected chicks of the first and second groups were depression, ruffled feathers, decreased appetite, bloody or white diarrhoea, lower body weight gain. Gradual paralysis and tremors were observed in some birds.

The P.M. lesions recorded were catarrhal to haemorrhagic with peteciation, ulcerative and necrotic enteritis. Ballooned intestines together with thickned wall of intestines were also observed. Spleen and liver showed congestion and enlargement with areas of necrosis in some chicks.

Survived chicks till the end of the observation period showed emaciation, retardation of growth with degeneration and haemorrhagic spots on the surface of the liver and kidneys. No symptoms were observed in control group. The results of the pathogenicity test in baby chicks are given in table (3).

The organism was reisolated from the intestine of experimenally infected birds on cooked meat medium and neomycin blood agar plates.

The effect of different antibiotics on the isolated *Cl.perfringens* isolates are illustrated in table (4).

III- Antimicrobial sensitivity:

Table (1): Frequency and percentage of isolated *Cl.perfringens.*

Examined specimens	No. of samples	*Cl.perfringens*	
		No.	%
Intestine of freshly dead broiler chicken	90	40	44.4

Table (2): Correlation between toxigenic and non toxigenic *Cl.perfringens*

No. of examined samples	No. of +ve *Cl.perfringens*	%	Toxigenic *Cl.perfringens*		Non toxigenic *Cl.perfringens*	
			No.	%	No.	%
90	40	44.4	30	75	10	25

Table (3): Showing the results of pathogenicity of *Cl. perfringens* in chicks

Group No.	No. of chicks	Route of infection	Dose of *Cl.perfringens* inoculum	Daily deaths past infection														Total No. of deaths	No. of survivors	M.R
				1	2	3	4	5	6	7	8	9	10	11	12	....	21			
1	20	S/C	0.1 ml ($1X10^8$) cfu	12	2					1	1				2			18	2	90%
2	20	oral		7	3			2			1	2	1	1				17	3	85%
3	10	Control	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	10	0.0%

M.R. = Mortality Rate

Table (4): Results of sensitivity of *Cl.perfringens* isolates

Antimicrobial agents	Sensitivity of *Cl.perfringens* isolates
Ampcillin	+++
Ciprofloxacin	+++
Amoxycillin	+++
Colistine	+++
Lincomycin	+++
Enrofloxacin	++
Chloramephenicol	++
Erythromycin	++
Oxytetracycline	++
Nalidixic acid	++
Neomycin	R
Gentamycin	R
Streptomycin	R

+++ = Highly sensitive ++ = Moderate sensitive R = Resistant

DISCUSSION :

Intestinal Clostridial infection is a common problem among rapidly growing broiler chicken causing severe losses especially when complicated with coccidiosis.

The present work aimed to study the role played by *Cl.perfringens* in broiler chicken. As evident from our results the bacteriological examination of the intestines of freshly dead broiler chicken revealed that the organism was recovered from (44.4%). Out of these examined samples (66.6%) were positive to Eimeria infestation. Similar results are observed by good number of workers (Duben, 1968; Al-Sheikly and Truscott, 1977; Ibrahim, 1979; El-Ged and Hagazy, 1985; Hussein and Mustfa, 1999 and Ebtehal, 2000). Ibrahim (1979) stated that the incidence was greatly increased in the presence of stress factors such as worm infestation or coccidiosis, that provided anaerobic condition favourable for multiplication of *Cl.perfringens* infection. The relationship between anaerobic microflora of the intestinal tract of chicken and coccidiosis were also reported by Medline (1986) and Baba *et al.,* (1992).

The post mortem lesions of examined samples from which *Cl.perfringens* was isolated showing congested intestine, haemorrhagic and necrotic enteritis, ballooning intestine filled with blood were observed, Exactly the same observations were recorded by Awad *et al.,* (1976); Hussein and Mustfa (1999); Ebtehal (2000) and Kaldhusdal, *et al.,* (2001).

From table (2) it seems clear that incidence of toxigenic *Cl.perfringens* were frequently higher within specimens positive for *Cl.perfringens* infection than non toxigenic one of such specimens. These results seemed to agree with the observations done by El-Ged and Hegazy (1985); Kim Hong Jib *et al.,* (1996) and Ebtehal (2000).

The experimental infection of 3-day old chicks by s/c and oral route with culture of toxigenic *Cl.perfringens* revealed that s/c route was slightly more effective than the oral route producing a mortality rate of 90% in contrast with 85% of the oral route. The pathogenic effect of *Cl.perfringens* in chicks was previously reported by Ibrahim (1979); George *et al.,* (1982) and Hussein and Mustfa (1999).

However, the mortality rate recorded as a result of the experimental infection in the present study is much higher than that reported by George *et al.,* (1982) who found that mortality rate among chicks which were inoculated in the crop with *Cl.perfringens* was 37.5%. Besides, Kageyama *et al.,* (1987) and Fukata *et al.,*(1988) found that 37.5% of chicken died after receiving an oral inoculation of a broth culture of *Cl.perfringens*, while Alwan and Swierczewska (1995) and Baba *et al.,* (1997) recorded 45% mortality in chicks given orally freshly prepared *Cl.perfringens*. On the other hand Duben (1968); Hussein and Mustfa (1999) and Ebtehal (2000) reported that *Cl.perfringens* given orally to chicks caused 80%, 50% and 80% mortality, respectively. Nevertheless, the present results come in disagreement with those of Long (1974), Awad *et al.,* (1976) who could not reproduce the disease directly or indirectly with *Cl.perfringens* or its toxins. Reisolation of the organism from dead and sacrified inoculated chicks proved that the inoculated isolates were responsible for the pathogenic effect mentioned before.

***In vitro* sensitivity testing of the isolates to 13 antimicrobial agents revealed that they were highly sensitive to ampicillin, ciprofloxacin,**

amoxycillin, colistine, lincomycin and moderately sensitive to enerofloxacin, chloramephenicol, erythromycin, oxytetracy-cline, nalidixic acid, while neomycin, gentamycin, streptomycin had no effect at all. In this respect, the results agree to some extent with those reported by Long and Truscott (1976). Ibrahim (1979);Hamdy *et al.,* (1983 a, b); Kondo (1988); Das *et al.,*(1997 a,b); Ebtehal (2000); Yoo Han Sang (2001) but disagree with those reported by Watkins *et al.,*(1997) who found that lincomycine appeared to be resistant against most strains of *Cl.perfringens*, while Hussein and Mostfa (1999) stated that neomycin was highly effective but enerofloxacin was not effective.

Finally, it may be concluded from the present investigation that *Cl.perfringens* infection in broiler chicken is of especial significance since it causes economic losses among baby and broiler chicks especially if it is complicated with coccidia. Therefore, the use of antibiotics shoud go hand in hand with anticoccidial drugs in all cases of coccidiosis or enteritis.

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مدى تواجد ميكروب الكلوستريديم بيرفيرنجينز فى أمعاء بدارى التسمين فى محافظة أسيوط

الدكتور/ عبد التواب محمد عبد الجواد ، الدكتور/ حسين على عبد القادر

معهد بحوث صحة الحيوان – أسيوط

فى هذا البحث تم فحص عدد ٩٠ عينة من أمعاء بدارى التسمين النافقة حديثاً جمعت من مزارع خاصة فى محافظة أسيوط ، وهذه العينات كانت تعانى من آفات تشريحية لعدوى الكلوستريديم ، وبالفحص المجهرى لهذه العينات وجد أنها ايجابية للكوكسيديا فى ٦٠ عينة بنسبة ٦٦,٧% ، وبالفحص البكتريولوجى تم عزل ميكروب الكلوستريديم بيرفرنجينز من ٤٠ عينة بنسبة ٤٤,٤% ، وباجراء العدوى الصناعية بهذا الميكروب فى الكتكوت عمر ثلاثة أيام وصلت نسبة النفوق إلى ٩٠% فى الكتاكيت التى حقنت تحت الجلد فى حين كانت ٨٥% فى مجموعة الكتاكيت التى حقنت عن طريق الفم .

وقد كانت الآفات التشريحية تشبه إلى حد كبير تلك المسجلة فى العدوى الطبيعية. هذا وقد تم عزل الميكروب مرة أخرى من الكتاكيت النافقة. وباجراء اختبار الحساسية فى المعمل للعترات المعزولة وجد أنها عالية الحساسية لكل من الأمبيسلين ، السبروفلوكاسين ، اموكسيسلين ، الكولستين ، اللينكومايسين.