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STUDIES ON NECROTIC ENTERITIS IN CHICKENS

BY

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ITRODUCTION

Necrotic enteritis is a disease frquently occurring in growing well-conditioned broiler chickens and is usually associated with high stocking density (Fowler and Hussaini, 1975). It has been established that C. perfringens plays a role in the development of the disease (Shane et al., 1985; Broussard et al., 1986; Greenham et al., 1987).

Necrotic enteritis follows non-specific causes such as diet high in carbohydrated and protein and infection with different coccidia have been inciminated as predisposing factors to an overgrowth by anaerobic microorganisms. (Reyna et al., 1983). C. perfringens type "A" has been most commonly isolated from affected birds showing necrotic enteritis (El-Sheikhly and Truscott 1976; Stutz et al., 1982 and Hofacre et al., 1986) although other C. perfringens types i.e. "C" and "F" have also been found (Bernier et al., 1974; Long, 1976; Harris and Portas, 1985). Other clostridia such as C. septicum, C. Sporogenes and C. Spiroforme either alone or in combination with C. perfringens have also been recov in spontaneously occurring necrotic enteritis in chicks (Princewill, 1985; Char et al., 1986 and Hofacre et al., 1986).

The trials described here were conducted to evaluate the incidence of anaerobic roganisms as a cause of necrotic enteritis in chickens. Investigation was done in vivo to evaluate the effect of different types of *C. Perfringens* especially in the intesting tract of broiler chicks.

MATERILA AND METHODS

A total of 190 sample for clostridial isolations were taken from each of liver and intestinal tract of all chickens dying from necrotic enteritis. Necropsy findings revealed enlarged, gas-filled small intestines with a necrotic enteritis from the duodenum to the ileum.

Pieces of the intestine and its contents were cultivated into cooked meat broth (Difco) containing 200 ug/ml neomycin sulphate for inhibition of the intestinal contaminants. After an overnight incubation, a loopful was seeded onto neomycin sulphate blood agar plates and incubated anaerobieally at 37°C for 24 hrs.

Suspected colonies were examined morphologically, shape of the colonies, double zone of haemolysis, changes in meat fragments, fermentation of sugars, nitrate reduction test, H_2S production, gelatin liquefaction and starch hydrolysis according to Cruickshank et al., (1975).

Nagler's reaction was done by using the half antitoxin method (Smith and Holdeman, 1968). Pathogenicity to guinea-pigs by experimental inoculation i/m with 0.5 ml of 24 hrs old cooked meat broth culture mixed with 0.5 ml. 5% Calcium chloride. The inoculated animals were kept under observation for 72 hrs. and post-mortem examination of dead guinea-pigs were recorded and cultures from the internal organs were made to recover the organism.

Determination of the type of toxin by using dermo-necrotic test in guinea-pigs according to Oakley and Warrach (1953) by preparing an extract from different parts of bacteriological positive infected alimentary tract contents and using specific anti-toxic sera of C. perfringens type A.B.C.D.E. and F obtained from Burroughs Wellcome, Beckenham, London, England.

Experimental production C. perfringens in chicks: Eighty-4 weeks old broiler Hubbard chickens were used in this experiment, they were all caged in four groups of 20 in separate rooms. Birds were starved for about 20 hrs and then fed on a chicken feeds inoculated with a broth culture of C. perferingens type A,C, or D to the first, second and third groups respectively. The fourth group was kept as a control.

Inoculated feed: Cooked meat medium stock culture was added as 3% inoculum to thioglycolate broth (Difco). Broth cultures were incubated at 37°C and added at 15 and 25 hrs of incubation to prepare inoculated feed twice daily. The ratio of feed to broth was 1.15. Uneaten was discarded daily. Birds were given infected feed for 5 successive days. All infected and control chickens were kept under observation for a period up to 4 weeks post-infection to observe any clinical signs. Birds which survived till the end of the experiment were slaughtered and the intestinal tracts of all birds were examined. Lesions were classified according to Prescot (1979) into four categories. Bacteriological investigations in trials to reisolate anaerobic organisms were done.

RESULTS AND DISCUSSION

Necrotic enteritis in poultry is widely distributed all over the world. The disease is mostly acute and causes severe losses for well-nourished healthy birds, therefore it can hinder poultry production. There is no doubt that anaerobic clostridial diseases cause

potential losses among chickens in Egypt. The present investigation was planned to study the incidence of the disease, isolation, identification and typing of the causative agent and experimental production of the disease.

Out of 190 intestinal and liver samples collected from broiler chickens, only 29 cases were positive for C. perfringens (15.3%) as shown in Table (1). Coccidia was frequently seen in the liver samples of bacterial positive carcases. C. perfringens was identified by the fermentative reactions in nine types of sugars. All isolates fermented glucose, maltose and lactose with the production of acid and gasses. Mannitol, glycerol and inositol were usually not affected. All isolates were catalase negative, most strains could liquify gelatin. Acid and gases in litmus milk giving a stormy fermentation within 24 hours were observed. Nitrate was reduced slightly to nitrites. There was abundant growth in cooked meat broth with excessive gasses formation, while the meat fragments were pinkish and not digested with a sour odour. All strains produced sharp beta haemolysis and incomplete zone around the suspected colonies. Nagler's reaction was detected. These seem to agree with the results obtained by Nakomura and Nishida (1974), Long et al., (1976) and Broussard et al., (1986). In the meantime, shane et al., (1985) in their studies on 200 chickens with necrotic intestinal infection, found that the incidence of C. Welchii was 19.6%. This result agrees with the observation done in the present work.

All strains of C. perfringens recovered from necrotic enteritis in chickens had emerged as the highly toxigenic types for guinea-pigs producing death within 24 hrs post infection. This finding agreed with those recorded by Princewell (1985) and Greenham et al., (1987).

Table (1) : Frequency of C. perfringens isolated from diseased dead necrotic enteritis chickens.

No. of Carcases examined	Bacteriological Findings of C. perfringens			
	Positive		Negative	
	No. of cases *	%	No of cases	%
190	29	15.3	161	84.7

* All positive strains proved to be highly toxigenic for guinea-pigs. Most positive cases represented a severe infection with coccidia.

Table (2) : Typing of the toxigenic strains of C. perfringens recovered from necrotic enteritis.

Total No. of pathogenic isolates.	Types of toxigenic isolates							
	Type A		Type C		Type D		Type F	
	No.	%	No.	%	No.	%	No.	%
29	18	62.1	7	24.1	3	10.3	1	3.4

The results obtained in Table (2), pointed out that on typing of the 29 recovered toxigenic C. perfringens 18 were type "A" (62.1%), 7 strains belonged to type "C" (24.1%), 3 strains were type "D" (10.3%) and the remaining one strain belonged to type "F" (3.4%). Therefore, C. perfringens types A,C,D, and F were isolated from necrotic enteritis of chicks in apercentage of 62.1, 24.1, 10.3 and 3.4 respectively. In comparing these results with those of other workers, one may quote the work of El-Sheikhly and Truscott (1976), Stutz et al., (1982) and Hofacre et al., (1986) who recovered type "A" from chickens showing nerotic enteritis in an incidence of 73.6%, 59.6% and 70.8% respectively. Type "A" waswidely distributed in examined chickens carcases. Therefore, the present reuslt is of great importance because type "A" of C. perfringens is also the causative agent of gas gangrene or even food poisoning in man which gives a chance for rapid transmission of the infection from birds to human beings. In the meantime, types C,D and F were also recovered in the present work in low percentages i.e. 24.1%, 10.3% and 3.4% respectively. These almost agreed with the findings recorded by Bernier et al., (1974): Long et al. (1976) and Harris and Portas (1985).

Coccidiosis is usually seen in conjunction with C. perfringens and Eimeria species was found in the liver of dead broilers showing positive bacteriological findings. This was in accordance with the findings of Maxey and Page (1977) who recorded that clinical coccidosis due to Eimeria brunetti and E. maxima was prevalent in birds dying from necrotic enteritis due to C. perfringens. It may be suggesting that coccidia played an important role in the disease syndrome.

Necrotic enteritis of chickens can be produced by feeding feed infected with various types of C. perfringens as shown in Table (3). The sudden increase in mortality in the 4-week old chickens appears to have been due to C. perfringens type "A" with a mortality rate reached

Table (3) : In vivo , the effect of types of C. perfringens in the feed of chickens on the mortality and lesions of necrotic enteritis.

Types of * C. perfringens	No. of birds infected	No. of death during observation period.	Mortality Ratio	Mortality Rate	Lesions score of survivors sacrified after 4 weeks post-infection .				
					0	1+	2+	3+	4+
A	20	8	8/20	40.0	5	0	4	1	2
C	20	3	3/20	15.0	10	1	2	4	0
D	20	3	3/20	15.0	8	2	3	3	1
Control	20	0	0/20	00.0	20	0	0	0	0

* All birds showing severe and extensive necrosis in the small intestine.

0 = No Gross changes

1+ = Thin wall or friable small intestine.

2+ = Focal necrosis or ulceration

3+ = Large batches of necrosis.

4+ = Severe extensive necrosis.

* Type F : Not Tested.

40.0% and the organism caused the necrotic enteritis leading to death of 8 chicks out of the 20-infected birds and a 60% livablity rate. On salughtering at the end of the experiment, most of the birds infected with type "A" revealed either focal necrosis or ulceration; large bactches of necrosis or severe extensive necrosis at necrospy. there is a chance that chickens were exposed to sufficient amounts of C. perfringens type "A" in the feed to result in the necrotic enteritis. These findings are similar with those of Fowler and Hussaini (1975), Maxey and Page (1977), Hofacre et al., (1986).

Mortalities associated with necrotic enteritis in chickens receiving either type "C" or "D" were observed in an incidence of 15.0% each. The incidence of mortality was greater among birds received type "A" when compared with the other types. Table (3) indicates that the severity of necrotic lesions in the birds infected with type "C" or "D" and sacrifced at 4-weeks of post-infection were much less than that produced by type "A". These findings coincide with that of Bernier et al., (1974) and Harris and Portas (1985).

In the study reported here, it was possible to produce the typical exttensive lesions of necrotic enteritis by using various types of the infused C. Perfringens. It appears probable that the major requirement is the presence of sufficient toxin to elicit the response. Finally, a correlation was found between the types of C. perfringens and necrotizing activities.

SUMMARY

A total of 190 samples for clostridial isolations were taken from the intestinal tracts of chickens dying from necrotic enteritis. Twenty nine cases were positive for C. perfringens (15.3%). All strains were highly toxigenic for guinea-pigs.

C. perfringens types A,C,D and F were isolated from necrotic enteritis of chickens in an incidence of 62.1% 24.1%, 10.3% and 3.4%.

Coccidiosis was usually observed in the liver of dead broilers showing C. perfringens infection.

Necrotic enteritis in chickens could be produced by feeding feed infected with various types of C. perfringens. A correlation was found between different types of C. perfringens and necrotizing activity.

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