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NECROTIC ENTERITIS IN BROILER CHICKENS IN ASSIUT GOVERNORATE

(With 2 Tables)

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التنكرز المعوى فى بدارى الدجاج فى محافظة أسيوط

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أستخدم في هذا البحث عدد ٦٠ عينة من أمعاء كتاكيت تسمين عمر ٤-٦ أسابيع وجمعت هذه العينات من مزارع خاصة في محافظة أسيوط لا تستخدم مضادات الكوكسيديا في العلف وهذه العينات كانت تعانى من آفات تشريحية لمرض التنكرز المعوى وبالفحص المجهرى لهذه العينات وجد أنها ايجابية للكوكسيديا فى الامعاء أو فى الأعور أوهما معا فى ٤٥ عينة (٧٥%) وبالفحص البكتريولوجى تم عزل ميكروب الكلوستريديم بيرفرنجز من ٣٠ عينة أى بنسبة ٥٠%, وباجراء اختبار الحساسية في المعمل أظهر فاعلية مركبات النيومايسين والثيومفنيكول واوكسىتتراسيكلين وابيسلين والكيتاموكس (كيتاسامين + اموكسىسيلين) في حين مركب الانروفلوكساسين غير مؤثر. وباحداث العدوى الصناعية باستخدام طريقة تنقيط مستنبت الشوربة الذى يحتوى الميكروب داخل العينة أعطت نسبة نافق ٨٠% عقب العدوىباربعة وعشرون ساعة في حين لايوجد نافق في حالة خلط المستنبت الذي يحتوى الميكروب على العليقة.

SUMMARY

Sixty intestinal samples of broiler chickens 4-6 weeks old were collected from private chicken farms in Assiut Governorate. direct microscopic examination of intestinal and cecal smeares revealed that 75 % of samples were positive to either intestinal/cecal or even mixed coccidiosis. Clostridium perfringens was isolated from 30 samples (50 %). In vitro sensitivity test indicated that Neomycin, Epicillin, Thiamphenicol, Kitamox and oxytetracycline were highly effective against tested

clostridium isolates. Experimental infection by oral route to 3 days old chicks lead to 80% mortality.

Key Words: *Necrotic Enteritis, Broiler*

INTRODUCTION

Necrotic enteritis (N.E) in domestic chickens was firstly described in England by Parish (1961). Since then it has been reported in most poultry producing countries around the world.

Necrotic enteritis has been experimentally reproduced in chickens by feeding contaminated feed with *Clostridium perfringens* Long and Truscott (1976), or by adminstering vegetable cultures of Clostridium perfringens orally by Bernier et al. (1977). Shane et al. (1985) reported that a primary intestinal disease like coccidiosis play an important rule in development of N.E.

Stephen and Lister (1997) recorded that N.E. can cause significant mortality in rapidly growing broilers. The result is not only loss of birds but also deterioration in litter quality and an adverse effect on performance.

The present work was planned to cover the following points:

- Trials for isolation of *Clostridium perfringens* from intestinal samples.
- In vitro sensitivity of isolated organisms against antibacterial drugs.
- Pathogenecity effects of isolates to baby chicks.

MATERIAL and METHODS

Direct microscopic examination:

A total of 60 intestinal samples were collected from broiler aged 4-6 weeks, obtained from private farms in Assiut did not use coccidiostat in feed. A direct microscopic examination of intestinal and cecal scraping were carried out.

Isolation and identification of *Clostridium perfringens*:

Small pieces of intestines with their contents were inoculated into cooked meat broth medium (prepared after Smith and Holdman,1968) incubated at 37°C for 24 hours. A loopful from each cultures was streaked into Neomycin blood agar plates (200 mg/ml) incubated anaerobically (using Gasbak anaerobic jar) at 37° C for 72 hours.

Suspected colonies (according to shape and haemolysis) were picked up, transfered to cooked meat broth incubated anaerobically for 48 hours at 37°C. The purified isolates were plated on reinforced clostridium agar plates, incubated anaerobically for 48 hours at 37°C and were tested biochemically according to Konemann et al. (1983).

Antibiotic sensitivity test:

Ten isolates were tested in vitro against antibacterials wich include: Thiamphenicol (Tp 30); Oxytetracycline (oT30); Neomycin (N30); Epicillin (Ep25); Amoxycillin (AML 25); Ampicillin (Amp10); Enrofloxacin (Enr 5); Kitamox (KT70) and Tetracyclin (TE 30). The isolates to be tested were inoculated into cooked meat broth, incubated at 37°C for 24 hours, then plates of reinforced clostridial agar were flooded by cultures-kept at room temperature for one hour.

The extra cultures were discarded and the antibiotic discs were distributed into the surfaces. The plates were incubated anaerobically at 37°C for 48 hours and the inhibitory zones were determined and evaluated according to Quinn et al. (1994).

Pathogenicity effect of *Clostridium perfringens*:

Thirty three days chicks divided into 3 groups each of 10 chicks were used in this experiment.

Group I: Chicks were inoculated orally by 0.5 ml of 24 hours cooked meat broth contain ***Clostridium perfringens*** isolates.

Group II: Chicks were experimentally infected by adding 0.5 ml 24 hours cooked meat broth culture to the feed of each bird, once daily for 3 days.

Group III: chicks were kept without any treatment as control.

Chicks of all groups were kept under observation, signs, lesions and mortalities were recorded. Reisolation of inoculated organism was carried out.

RESULTS

The examined intestines showed, balloning, thickening, necrosis and ulceration. Direct microscopic examination revealed that 45 (75%) samples were positive to intestinal, cecal or mixed coccidiosis.

The suspected ***Clostridium perfringens*** isolates produced gas and putrified odour on cooked meat broth. The meat particles were pinkish, or grey-black without digestion. All isolates were haemolytic on

blood agar plates, while small, circular, convex, smooth surface colonies were produced on reinforced closrtidium agar plates.

Gram positive rod stright with paralled sides rounded ends, non-motile, some have central or subterminal small, oval spores were found. Results of biochemical reactions were tabulated in Table 1. Results of sensitivity test were illustrated in Table 2.

Results of pathogenicity test:

Chicks of group I showed gradual paralysis and tremors and 80 % of chicks died after 24 hours post inoculation. Post-mortem examination revealed balloned intestines. Congested internal organs, distended gall bladder, enlargment of yolk sac.

Chicks of group II showed diarrhoea, without mortality. Slaughtered birds 5 days post inoculation revealed balloned, slightly thickned wall of intestine with semisolid content. The organism was detected in impression smears of liver from both groups of chicks and reisolation of inoculated ***Clostridium perfringens*** was successeded.

The chicks of group III were healthy without pathological lesions or mortality.

DISCUSSION

The present study try to throw some light on the role of anaerobic microorganisms which may be incriminated as a cause of diseases affecting birds.

Our samples were collected from farms did not depend on the coccidiostat as a mean for prophylaxis against coccidiosis, so a high percentage of samples 75% were positive to different types of Eimeria .

Clostridum perfringens was isolated from 30 samples (50%) out of theses examined samples 75 % were positive to coccidiosos. More or less the same results were obtained by Al-Sheikhly and Truscott (1977) and Ibrahim (1979). Moreover, the same author stated that the incidence was greatly increased in the presence of stress factors such as worm infestation or coccidiosis that provides anaerobic condition favourable for anaerobic infection. The relationship between anaerobic microflora of the intestinal tract of the chickens and coccidiosis were also reported by Medline (1986) and Baba et al. (1992).

Concerning the sensitivity test, our results showed that Neomycin; Epicillin; Thiamphinicol; Oxytetracyclin and Kitamox were highly effective while Enrofloxacin was not effective, these results agreed with

those cited by Vera et al. (1980). Medline (1988) stated that Penicilline and Cephazolin were highly active against *Clostridium perfringens*. Ibrahim (1979) reported that, Penicillin G and Oxytetracycline were highly effective while Streptomycin and Chloramphenicol were not effective.

Results of experimental infection by feeding showed that the mortality rate was high in the first trial but in the second trial chicks showed diarrhoea, without mortality . Nakamura (1922) was able to reproduce the disease in young chicks by feeding them with cultures of the anaerobic bacterium isolated from cases of chicken diarrhoea and from the soil of infected farms. Wijewanta and Seneviratna (1970) cited that, the heat-sensitivity strains of *Clostridium perfringens* type - A were capable of causing natural infection in chickens. It can be concluded that, the use of antibiotics in conjuction with anticoccidial drugs may be necessary in all cases of coccidiosis or enteritis to avoid the complications that may occur if clostridium is present.

REFERENCES

Al-Sheikhly, F. and Truscott, R. B. (1977): The pathology of necrtic enteritis of chickens following infusion of broth culturs of Clostridium perfringens into the duodenum. Avian Dis. 21: 230-340.

Baba, E.; Fulley, A.L.; Gilbert, J.M.; Thayer, S.G. and Mcdougald, L.R. (1992): Effects of E. burneti infection and dietary zinc on experimental induction of necrotic enteritis in broiler chickens. Avian dis., 36: 59-62.

Bernier, G.; Phaneuf, J.B. and Filion, R. (1977): Enterite necrotique Chez Le poulet de gril. III Etude des facteurs favorisant La multiplication de Clostridium perfringens et La transmission experimentale de La maladie. Can. J. Comp. Med., 41: 112-116.

Ibrahim, A.A. (1979): Anaerobic Microflora of the small intestine of chickens. Thesis presented to Faculty of Vet. med. Assiut University .

Konemann, E.W.; Allen, S.D. and Dowell, H.M. (1983): Color Atlas and Textbook of diagnostic microbiology. 2nd Ed. J. B. Lip. Co. New York. London.

Long, J.R. and Truscott, R.B. (1976): Necrotic enteritis in broiler chickens. III. Reproductions of the disease.Can. J. Comp. Med. 40: 53-59.

Medline, R. (1986): Necrotic enteritis in cage-reared comercial layer pullets. Avian - dis. Sep. 30: 617-619.

Medine, R. (1988): In vitro lecithinase activity and sensitivity to 22 antimicrobial agents of Clostridium perfringens isolated from necrotic enteritis of broiler chickens. Faculty of agriculture, Miyazaki University, Japan. Res. Vet. Sci. 45: 337-340.

Nakamura, N. (1922): Investigation on a diarrhoea in young chickens in Japan. J. Jap. Vet. Sci., 1: 93-99.

Parish, W. E. (1961): Necrotic enteritis in the fowl (Gallus gallus domesticus). I - histopathology of the disease and isolation of a strain of Clostridium welchii. J. Comp. Pathol., 71: 377 - 393.

Quinn, P.J.; Carter, M.E.; Markey and Carter, G.R. (1994): Clinical Veterinary Microvbiology. Published by Wolfe publishing, an imprint of mosby book europe limited printed in Spain by Grafos, S.A. Arte Sobre Papel.

Shane, S.M.; Gylmah, K.S.; Harrington, K.S. and Snider, T.G. III (1985): Etiology and pathogenesis of necrotic enteritis. Vet. Res. Commun. 9: 269-287.

Smith, L.D. and Holdman, L.V. (1968): The pathogenic anaerobic bacteria. P.P. 201 -205 1st Ed. Charles, C. Thomas, Springfield, Illinois, U.S.A.

Stephen, A. and Lister, M. (1997): Necrotic enteritis. J. Poultry International. Jouly, 1997.

Vera, L. S.; Diane, M.C. and Sydney, M.F. (1980): Wadsworth anaerobic Bacteriology manual. 3rd Ed. - page 68. The C.V. Mosby company. St. Louis. Toronto - London.

Wijewanta, E. A. and Seneviratna, P. (1970): Bacteriological studies of fatal *Clostridium perfringens* type-A infection in chickens. University of Ceylon, Vet. Sci., 7: 654-661.

Table (1) show the biochemical reaction of *Closrtidim perfringens* isolates

No. of sample	Glucose	Lactose	Maltose	Sucrose	Manitol	Gelatin	litmus milk	H_2S
1	+	+	+	+	-	+	+	-
2	+	+	+	+	-	+	+	-
3	+	+	+	+	-	+	+	-
4	+	+	+	+	-	+	+	-
5	+	+	+	+	-	+	+	-
6	+	+	+	+	-	+	+	-
6	+	+	+	+	-	+	+	-
7	+	+	+	weak +	-	+	weak +	-
8	+	+	+	+	-	+	+	-
9	+	+	+	+	-	+	+	-
10	+	+	+	+	-	+	+	-
11	+	+	+	+	-	+	+	-
12	+	+	+	+	-	+	+	-
13	weak +	+	+	+	-	+	+	-
14	+	+	+	+	-	+	+	-
15	+	+	+	+	-	+	+	-
16	+	+	+	+	-	+	+	-
17	+	+	+	+	-	+	+	-
18	+	+	+	+	-	+	weak +	-
19	+	+	+	+	-	+	+	-
20	+	+	+	+	-	+	+	-
21	+	+	+	+	-	+	+	-
22	+	+	+	+	-	+	+	-
23	+	+	+	+	-	+	+	-
24	+	+	+	+	-	+	+	-
25	+	+	+	+	-	+	+	-
26	+	+	+	+	-	+	+	-
27	weak +	+	+	+	-	+	+	-
28	+	+	+	+	-	+	+	-
29	+	+	+	+	-	+	+	-
30	+	+	+	+	-	+	weak +	-

Table (2) show in vitro sensitivity test of Clostridium perfringens isolates to different antibiotics

Antibiotices	Number of sample									
	2	3	7	9	10	15	19	23	27	30
Thiamphenicol (Tp 30)	+	+++	+++	-	-	+++	++	+++	-	+++
Oxytetracycline (oT 30)	+++	-	+++	-	++	+++	-	++	+++	+++
Neomycin (N 30)	+++	+++	+++	-	-	+++	-	++	+	+++
Epicillin (EP 25)	-	-	++	++	-	-	-	+	+++	+++
Amoxycillin (AML 25)	-	-	-	++	++	-	-	++	++	++
Ampecillin (AMP 10)	-	-	+	+	+	-	-	+	+	+
Enrofloxacin (Enr 5)	-	-	++	-	-	-	-	+	-	-
Kitamox (KT 70)	-	-	+++	-	-	-	-	++	+++	-
Tetracyclin (TE 30)	+++	-	-	++	-	-	+	-	-	+++

(-) = Resistant

\+ = weak

++ = Moderatly susceptible

+++ = strong susceptible