Animal Health Research Institute,
Mansoura Provincial Laboratory

SOME STUDIES ON CLOSTRIDIA IN THE BALADY CHICKENS IN DAKAHLIA PROVINCE

(With 4 Tables)

By

A.M. AHMED and M.M. ABD EL-LATIF

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بعض الدراسات عن الكلوستريديا في الدجاج البلدي فى محافظة الدقهلية

أحمد مصطفي أحمد، محمود محمد محمود عبد اللطيف

أجريت هذه الدراسة علي ١٥٠ عينة من أمعاء لدجاج بلدى عمر ٤ – ٨ أسابيع يعانى من إلتهابات معوية تم جمعها من مزارع مختلفة في محافظة الدقهلية. وبالفحص البكتريولوجي للعينات أمكن عزل ميكروب الكلوستريديم من ١٢٣ عينة بنسبة (٨٢%) تم تصنيفهم مورفولوجيا وبيوكيميائيا إلى كلوستريديم برفرنجينز ٨٤ (٥٦%) ، كلوستريديم كولينيم ٤٢ (٢٨%)، كلوستريديم سبوروجينز ١٥ (١٠%) وكلوستريديم سبيريفورم ٩(٦%). وبالفحص المجهري وجد أن ١٠٢عينة بنسبة ٦٨% إيجابية للكوكسيديا. بإحداث العدوى الصناعية بـ ٢٠ عترة من كلوستريديم برفرنجيز فى خنازير غينيا وجد أن ١٦ عترة (٨٠%) من النوع الممرض. وبإجراء اختبار الحساسية لعترات كلوستريديا برفرنجينز لبعض المضادات الحيوية وجد أنها أكثر حساسية لكل من أموكسيسيللين، بنسيللين، امبسيللين، ريفاميسين ولينكومايسين لكن كانت مقاومة للجنتاميسين وستربتوميسين.

SUMMARY

This study was done on 150 samples from intestinal tract taken from balady chickens aged from 4-8 weeks-old and suffering from enteritis obtained from different localities in Dakahlia province. The samples were examined bacteriologically for presence of clostridia. Out of 150 samples 123 (82%) revealed clostridial spp. which were identified morphologically and biochemically to *Cl. perfringens* 84 (56%), *Cl.colinum* 42 (28%), *Cl. sporogens* 15 (10%) and *Cl. spiroforme* 9 (6%), as some samples showed presence of mixed infection with different clostridial species. Direct microscopic examination for samples showed that 102 (68%) samples were positive to coccidiosis. Out of 20 isolated strains of *Cl. Perfringens,* 16 (80%) showed virulence factor when inoculated in guinea pigs. On the other hand, the sensitivity of

clostridial strains to some antibiotics in vitro showed more sensitive for Amoxycillin, Penicillin, Ampicillin, Rifamycin and Lincomycin but resistant to Gentamycin and Streptomycin.

Key words: *Clostridia, chicken, antimicrobial susceptability, coccidia.*

INTRODUCTION

Anaerobic bacteria are commonly inhabitant in nature and intestinal tract of animals, birds and man . Under stress and disturbed physiological conditions, they can assume a pathogenic role and can kill the host by the production of highly potent producer of exotoxins on which the pathogenicity are produced, so it is harmless bacteria but under certain circumstances it changs to be pathogenic (Cato *et al.*, 1986).

Clostridia are Gram positive, anaerobic producing endospores, rod shape and motile bacteria.

Clostridial infection appeared to be constitues one of the veterinary problems in poultry industry (El-Ged and Hagazy 1985 and Hosfshagen and Stenwig 1992). Affection by clostridia follows non specific causes such as diet high in protein and carbohydrate and infestation by coccidiosis have been incriminated as predisposing factors to an over growth by anaerobic microorganisms (Reyna *et al.*, 1983 and Kim Hong Jib *et al.*, 1996). Clostridium species are one of batcerial causes of enteritis in chickens which became more disastrous after coccidiosis resulting in necrotic and ulcerative enteritis (Shane *et al.*, 1985 and Das *et al.*, 1997). Necrotic enteritis is caused by clostridium perfringens, it is common disease of broilers accompanied with severe fetal diarrhoea due to the production of exotoxins produced by clostridial microorganisms and morbidity and mortality rates have ranged from 1 to 60% (Baba *et al.*, 1992 and Drago and Dan, 1996). Other clostridia such as *Cl.colinum* which cause ulcerative enteritis, *Cl. sporogenes* and *Cl. spiroforme* either alone or in combination with *Cl. perfringens* have also been recovered in occuring necrotic enteritis in chicks (Char *et al.*, 1986).

This work was done to illustrate the prevealance of clostridial microorganisms in balady chickens in Dakahlia province, determine the pathogenicity effects of isolates and testing the susceptability of the isolates to some antibacterial durgs.

MATERIALS and METHODS

Samples:

A total of 150 freshly dead and sacrificed balady chickens aged from 4-8 weeks old were obtained from private farms in Dakahlia province. The samples includes different parts from intestines were subjected for coccidial and clostridial examination.

Examination of coccidia:

A direct microscopic examination of intestinal and cecal scraping were carried out for presence or absence of coccidia oocysts.

Isolation of clostridium:

From each sample about 5 gm of intestine with their contents were inoculated into two tubes of freshly prepared cooked meat medium. One tube was heated at 80 °C for 10 minutes to eliminate the non spore forming aerobes while the other was left without heating. Both tubes were incubated anaerobically at 37 °C for 48 hrs. A loopful from each tubes was streaked into the surface of 10% sheep blood agar plates and incubated anaerobically at 37 °C for 48 hours using gas- pack anaerobic jar (BBL).

Suspected colonies were reinoculated into cooked meat broth and incubated anaerobically at 37 °C for 48 hours to have a pure culture of isolate for further indentification.

Identification of the isolates:

Identification was applied according to the technique recommended by Konemann *et al.* (1988), Machie & Mc Cartney (1989) and Levett (1991) using the following tests:

1- Gram stained smears from suspected colonies.
2- Cultural characters (size and shape of the colonies, type of haemolysis and changes in meat particles).
3- Motility.
4- Sugar fermentation.
5- Indole production (spot test).
6- Gelatin liquefaction.
7- H_2S production.
8- Nagler's reaction test.
9- Urease test.

Antimicrobial susceptability test:

The isolated clostridia were tested for sensitivity to (11) chemotherapeutic agents (Amoxycillin, Ampicillin, Penicillin, Rifamycin, Lincospection, Streptomycin, Oxytetracycline, Chloramphenicol, Erythromycin, Gentamycin and Enrofloxacin). One

ml of 48hr broth cultures was spread over the surface of blood agar. Chemotherapeutic sensitivity discs were placed aseptically on the surface of blood agar. The plates were inverted and incubated anaerobically at 37°c for 24 hrs., the inhibitory zones were determined and evaluated according to Quinn *et al.* (1994).

Pathogenicity test according to Wilson and Miles (1955):

Pathogenesis was performed on 20 well identified *Clostridium perfringens* isolates using guinea pig with an average weight of 300-500 gm, each inoculated intramuscularly in the thigh by 0.2 ml of an 18 hours glucose broth culture of the isolates, the inoculated animals were kept under observation for 72hrs. Post mortem examination of the dead animals was carried out and cultures from local effusion (subcutanous tissues around the local lesion) on cooked meat broth and horse blood agar were made to recover the organisms.

RESULTS and DISCUSSION

Results recorded in Table (1) point out that clostridial spp. could be isolated from 123 out of 150 chicken samples with an incidence percentage of 82%, these findings are less higher than that reported by Awad *et al.*, (1976), while coccidian oocysts were found in 102 out of the same samples with an incidence percentage of 68% which are lower than that previously reported by Hussein and Mustafa (1999). These results clarified that most of enteritis cases usually associated with clostridial infection, also results found in Table (1) showed that 58% of the samples were suffering from mixed infection of clostridium and coccidia which indicate presence of coccidia exaggerate the role of clostridia in problem of enteritis Baba *et al.*, (1992).

The highly percentage of infection with clostridia which may associated with contamination of the chicken during transport of chick's from hatchery to the farm or from inside and outside chicken house before and during grow out (food, workers, letter, water lins in farm and air dust), also the mice, wild birds, crawling insects may play a role in contamination, lastly the age of chicken from which taken samples may affect an percentage of clostridial presence Craven *et al.*, (2001).

On the basis of biochemical tests 150 isolates from the samples were identified as *Clostridium* spp. as presented in Table (2). Results given in Table (3) reveals the types of anaerobic isolates, the results declared that *Cl. perfringens* were the most predominant identified microorganisms which constitutes 56% of the total clostridial isolates,

this results nearly agreed with that recorded by El Ged and Hagazy (1985), Benno *et al.*, (1988), Prukner and Milkovic (1991) and Tschirdewahn *et al.*, (1992). Other clostridial spp. were identified as *Cl. colinum*,*Cl. sporogens* and *Cl. spiroforme* with a percentage of 28%, 10% and 6% respectively. These clostridial spp. were also isolated by Ibrahim *et al.*, (2001) who recoverd the *Cl. colinum* (14.3%), *Cl. sporogenes* (12.9%) and *Cl. spiroforme* (0.9%). The pathogenicity of *Cl. perfringens* in this study revealed that 16 (80%) out of 20 isolates were toxigenic types for guinea pig causing death within 24 hours post infection, while 4 (20%) of isolates were non-toxigenic types. The obtained results are lower than those reported by El-Seedy, (1990) who mentioned that all isolates were toxigenic for guinea pig.

Table (4) showed that Amoxycillin, Penicillin, Ampicillin, Rifamycin, and Lincomycine, were highly effective on clostridia, while Oxytetracycline and Erythromycin had moderate effectiveness, on the other hand clostridia less sensitive for Chloramphenicol and Enrofloxacin and its showed resistance for Streptomycin and Gentamycin. These results concide with that recorded by Gazdzinski and Julian (1992) who found that *Cl. perfringens* isolates were sensitive to Ampicillin, Penicillin and Lincomycin but resistant to Gentamycin. Hussein and Mustafa (1999) and Yanny and Shalaby, (2000) reported that *Cl. perfringens* isolates were highly susceptible to Chloramphenicol, Penicillin and Tetracyclines.

From the results of our study, we can concluded that the clostridia perfringens is the most pathogenic strain in bolads chickens causing necrotic enteritis either alone or with other Cl. species. Clostridial infection become highly pathogenic in presence of infestation with coccidiosis, treatment of clostridia in chickens not depend on using of antibiotics only but need to good hygienic and management measures also keep the coccidia under control.

Table 1: Incidence of clostridial microorganisms and coccidia from examined samples

No. of examined samples	Positive samples for clostridia and coccidia		Positive samples for clostridia		Positive samples for coccidia		Negative samples for clostridia and coccidia	
	No.	%	No.	%	No.	%	No.	%
150	87	58	36	24	15	10	12	8

Table 2: Biochemical identification of the suspected clostridium isolates

No. of reacted isolates	Biochemical reactions										
							Acid production from				
	Indole	Lecithinase	Lipase	H_2S	Urease	Hydrolysis of gelatin	Glucose	Lactose	Maltose	Sucrose	Mannitol
84	-	+	-	-	V	+	+	+	+	+	-
42	-	-	-	-	-	-	+	-	W	+	V
15	-	-	+	+	-	+	+	-	+	-	-
9	-	-	-	-	v	-	+	+	-	+	-

+, positive
-, negative
v, variable
w, weak

Table 3: Frequency distribution of isolated clostridia species from examined samples (150)

Bacteria	Number	%
Clostridium perfringens	84	56
Clostridium colinum	42	28
Clostridium sporogens	15	10
Clostridium spiroforme	9	6

Table 4: Sensitivity test of some Clostridium perfringens isolates (20) from examined samples to different antibiotics.

Antimicrobial disc	Disc concentration	Sensitivity
Amoxycillin	25µg	+++
Penicillin	10µg	+++
Ampicillin	10µg	+++
Rifamycin	30µg	+++
Lincomycin	15µg	+++
Streptomycin	10µg	-
Oxytetracycline	30µg	++
Erythromycin	15µg	++
Chlormphenicol	30µg	+
Gentamycin	10µg	-
Enrofloxacin	5µg	+

+++ = Highly effective
++ = Moderate effective
+ = Less sensitive
- = Resistance

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