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CLOSTRIDIAL INFECTION IN CHICKENS "STUDYING THE PATHOGENICITY AND EVALUATION OF THE EFFECT OF SOME GROWTH PROMOTORS ON BROILER PERFORMANCE"

(With 7 Tables)

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عدوى الكلوستريديم فى الدجاج
" دراسة العدوى الصناعية وتقييم تأثير بعض منشطات النمو على اداء بدارى التسمين"

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أجريت العدوى الصناعية فى بدارى التسمين باستخدام أنواع الكلوستريديم الشائعة العـــزل. كانت معدلات النفوق أعلى عند أجراء العدوى باستخدام ميكروب الكلوستريديم متحـــدا مـــع الكوكسيديا عنها عند عمل العدوى بالكلوستريديم منفردا وخصوصا الكلوستريديم برفرنجينز. أزدات شدة الأعراض و الأفات التشريحية عند محاولة الاعداء باستخدام الانـــواع المفـــرزة للسموم من ميكروب الكلوستريديم برفرنجينز عنها عند العدوى بميكروبات الكلوســـتريديم برفرنجينز الغير مفرزة للسموم وكلوستريديم كولينم وكلوستريديم سبوروجينز وأصبحت أكثر حدة عند العدوى المختلطة مع الكوكسيديا. عند أضافة ميثيلين داىسلســـاليت الباسيتراســـين ،الفلافوميسين ،الانراميسين ،اللنكوميسين كل على حده الى العلائق التى تم تغذيـــة الطيـــور عليها لوحظ ان معدلات النفوق قد تقلصت عند العدوى بالانواع المفـــرزة للســـموم أو ج وكذلك الانوع الغير مفرز للسموم من ميكروب الكلوستريديم برفرنجينز، وعلـــى الناحيـــة الاخرى تحسنت الاوزان بشكل معنوى حيث كان مركب فلافوميسين هو الاكثر فاعلية عـــن باقى المركبات كما قلت الافات التشريحية بشكل واضح. أظـــهرت مركبـــات الانراميســـين واللنكوميسين انخفاض فى معدل النفوق من ٤٥% الى ٢٥% وتلاه مركبى الباسيتراســـين والفلافوميسين بمعدلى ٣٠ و ٣٥%. تأثرت جميع الميكروبات تحت الدراسة تأثرا تاما فـــى اختيار الحساسية عند أستخدام الاموكسيسيللين، بنسيللين، سبروفلوكساسين، انروفلوكساسين، فليموكوين، دوكسىسيكللين، وسلفات الكولستين بينما تأثرت بشكل متوســـط عنـــد أســـتخدام الارثروميسين والكلورامفينيكول والاوكسىتتراسيكلين وكان هناك مقاومة كاملة ضد مركبات نيوميسين، استربتوميسين والجنتاميسين.

SUMMARY

Experimental infection of broiler chickens with the most commonly isolated clostridium species was done. Mortality rates were higher in birds subjected to combined infection with clostridium and coccidia than those infected with clostridium alone especially in birds infected with *Cl perfringens*. Symptoms and lesions were more severe in case of infection with toxigenic types of *Cl perfringens* than in case of experimental infection with non-toxigenic *Cl perfringens*, *Cl colinum* and *Cl sporogenes*. The intensity became more severe after mixed infection with clostridium and coccidia. In case of birds received bacitracine methyl disalicylate (BMD), flavomycin, enramycin and lincomycin, mortality rates due to infection with toxigenic *Cl perfringens* types A&C and non-toxigenic type were reduced. A significant difference between lesions in birds receiving growth promotors mixed ration was observed. Enramycin and lincomycin reduced the mortality rates from 45% to 25% more than BMD and flvomycin (30% and 35%). On the other hand, a significant improvment in weight gain was observed especially with flavomycin followed by BMD, enramycin and lincomycin respectively. Most of the tested clostridial isolates were highly sensitive to amoxycillin, pencillin, ciprofloxacin, enrofloxacin, flumequin, doxycyclin and colistin sulphate, while they were moderately sensitive to erythromycin, chloramphenicol, oxytetracycline. Complete resistance to neomycin, streptomycin and gentamicin was noticed.

Key words: *Clostridial infection, chickens, growth promotors.*

INTRODUCTION

Enteritis is considered as one of the major problems affecting severely the broiler production stocks. One of the major enteritis producing bacteria is clostridium species which become more disastrous after coccidiosis resulting in ulcerative and necrotic enteritis. These conditions were reproduced by several workers (Balauca, 1976 & Shan et al, 1985 and Das et al, 1997 a,b). The use of antibiotics as feed additives (growth promotors) in poultry ration is wide spread, which act indirectly by retaining the natural balance of the bacterial flora of the digestive tract, and preventing the bacterial detrimental toxin formation and thus facilitate the absorption of nutrients and consequently depressing the activity of clostidial species.

In formerly published work, different species of genus clostridium were isolated and classified by same authors (Ibrahim et al, 2001). In this study, further work was done to investigate the pathogenicity of isolated bacteria and evaluation of the effect of feed additives on experimental infection with the same organisms as well as testing of their antimicrobial susceptibility.

MATERIALS and METHODS

Preparation of cultures for experimental infection:

A forty eight hours culture of clostridial isolates in cooked meat medium was prepared. The culture suspensions were centrifuged at 4000 rpm for 1 hr. Purity was checked through Gram staining from the sediments. The sediment was washed three times in saline and resuspended in thioglycollate medium. Colony count technique was done according to Cruickshank et al, (1975).

Collection and sporulation of oocysts:

The intestinal and caecal contents of chickens infested with coccidia were collected, suspended with normal saline and filtered through muslin. The filterates were mixed with 2.5% aqueous solution of potassium dichromate and placed as thin layer in petri dishes followed by incubation for a week at room temperature. Daily microscopic examination was carried out to detect sporocysts and sporozoites. The mixtures were washed 3X by normal saline. The sediments containing sporulated oocysts was resuspended in saline solution. Oocysts count were determined according to Parfitt (1958).

Experimental infection:

a) *Experimental infection with isolated clostridial species only*:

This was done according to Das et al., (1997c). A total of one hundred and twenty 3-week-old chickens (Balady breed) were divided into 6 groups, each of twenty birds.

First group was infected orally with 2 ml broth culture containing $1.8x10^9$ CFU/ml of *Cl. perfringens* type A. Second group was infected orally with 2 ml broth culture containing $1.8x10^9$ CFU/ml of *Cl. perfringens* type C. Third group was infected orally with 2 ml broth culture containing $1.8x10^9$ CFU/ml of non-toxigenic *Cl. perfringens*. Fourth group was

infected orally with 2 ml broth culture containing 10^7 CFU/ml of *Cl. colinum*. Fifth group was infected orally with 2 ml broth culture containing 1.5 x 10^9 CFU/ml of *Cl. sporogens*. Sixth group was inoculated orally with 2 ml sterile broth, and kept as control. All inoculated groups received 3 successive doses at 2 days intervals and kept under observation for 4 weeks postinfection. Clinical signs, PM findings, moralities, average body weights and microbial recovery rates were recorded.

b) *Combined experimental infection with clostridium and coccidia*:

A total of hundred and twenty 2-week-old chickens inoculated orally with $5x10^4$ sporulated oocysts of coccidia. One week lattar, birds were grouped into 6 groups each of 20 birds. five groups were inoculated orally with clostridia as described in former experiment, while 6th group was left as control.

Evaluation of the effect of feed additives on clostridial infection:

A total of 240 one-day-old chicks were divided into 4 groups (60 birds each). All groups received ration mixed with feed additives along the experiment time. Group I received granulated bacitracin methycne disalicylate (BMD) 10% (Alpharma Animal Health Divission) as 440 gm/ton. Group II received Flavomycin 40 (Hoechst) as 125 gm/ton. Group III received Enramycin F40 (Takeda, LTD, Tokyo, Japan) as 125 gm/ton. Group IV received Lincomix (Upjohn Company, Kalamazoo, Mi) as 200 gm/ton. After three weeks , each group was divided into three subgroups (each of 20 birds) A,B,and C. Subgroup A were inoculated orally with 2 ml broth culture containing $1.8x10^9$ CFU/ml of toxigenic *Cl perfringens* types A and C. Subgroup B were inoculated orally with 2 ml broth culture containing $1.8x10^9$ CFU/ml of non-toxigenic *Cl perfringens* types. Subgroup C was left as control. All inoculated groups received 3 successive doses at 2 days intervals. Birds were kept separately and received the treated ration for 4 weeks. Clinical signs, PM lesions, mortalities, average body weights and microbial recovery rates were recorded.

Sensitivity to antimicrobial agents:

The clostridial types used in this work were tested for their *in vitro* sensitivity to different antimicrobial agents using

blood agar plates under anaerobic conditions (Gas-Pack Anaerobic Jar, Baker Platinum LTD, London). The sensitivity was judged according to diameter and clearance of inhibition zone (Perelman et al, 1991).

RESULTS

Experimental infection:

Results are recorded in tables 1&2. Deaths occurred at first and second week postinfection. Mortality rates were 40%, 50%, 25%, 5% and 5% in chicks infected with *Cl. perfringens* toxigenic types A and C, non-toxigenic type, *Cl colinum* and *Cl sporogens* respectively, while in case of combined infection with clostridium and coccidia, mortality rates were much higher and recorded as 70%, 75%, 40%, 10% and 5% respectively. Clinical signs recorded as depression, ruffled feathers, decreased appetite and bloody or whitish diarrhea. Observed PM lesions were catarrhal to hemorrhagic,ulccerative and necrotic enteritis, together with mucosal thickening of small intestine (dudenum, jujunum, ileum and cecum). spleen and liver may be congested and enlarged with necrosis. Symptoms and lesions were more prominent in birds infected with toxigenic types of *Cl perfringens* with or without coccidia, while other types demonstrated mild signs and lesions. Clostridia were reisolated from intestinal tract of experimentally infected birds on cooked meat media and blood agar plates.

Evaluation of feed additives effect on chicks infected with clostridium:

Results are shown in Tables (3,4,5 and 6). All feed additives used in this study were effective in minimizing the mortality due to infection with toxigenic and non-toxigenic *Cl perfringens*. The average mortality rate due to infection with toxigenic *Cl perfringens* types A&C is 45%, while in case of non-toxigenic type is 25%. In case of birds received BMD, flavomycin, enramycin and lincomix, mortality rates due to infection with toxigenic *Cl perfringens* types A&C and non-toxigenic type were decreased from 45% & 25% to 30% & 5%, 35% & 5%, 25% & 0% and 25% & 5% respectively. A significant difference between lesions in birds receiving feed additives mixed ration and those received normal ration was

noticed on experimental infection, where intensity of lesion became much less severe. On the other hand, a significant improvement in weight gain was observed especially with flavomycin followed by BMD, enramycin and lincomix respectively.

Susceptibility of clostridial isolates to antimicrobial agents:

Results are illustrated in Table 7. Most of the tested clostridial isolates were highly sensitive to amoxycillin, pencillin, ciprofloxacin, enrofloxacin, flumequin, doxycycline and colistin sulphate. on the other hand, the isolates were moderately sensitive to erythromycin, chloramphenicol, oxytetracycline while it was resistant to neomycin, streptomycin and gentamicin.

DISCUSSION

Mortality rates were higher in birds subjected to combined infection with Clostridium and coccidia than those infected with Clostridium alone especially in birds infected with *Cl perfringens*. The observed clinical signs were manifested by whitish or bloody diarrhea and decreased body weight. On PM examination, different degrees of enteritis were demonstrated together with mucosal thickening and liver and spleen affections. Symptoms and lesions were more severe in case of toxigenic types of *Cl perfringens* than in case of experimental infection with non-toxigenic *Cl perfringens*, *Cl colinum* and *Cl sporogenes*, and getting more exaggerated after mixed infection with clostridium and coccidia. Similar results were described by Balauca et al (1976), Shane et al (1985), Fukata et al (1988) and Das et al (1997 b).

Mortality rates were 40%, 50%, 25%, 5% and 5% in chicks infected with *Cl. perfringens* toxigenic types A and C, non-toxigenic type, *Cl colinum* and *Cl sporogens* respectively, while in case of combined infection with clostridium and coccidia, mortality rates were much higher and recorded as 70%, 75%, 40%, 10% and 5% respectively. The formerly mentioned results were confirmed by Parish (1961), Kattich et al (1966), Hein and Timmus (1972), Bradley and Radharishnan (1973), Kondo et al (1988) and Baba et al (1997). Contrasting to our results, Long (1974) and Awad et al (1976) could not reproduce

the disease directly or indirectly with *Cl perfringens* or its toxins.

Regarding to our results, birds which have been received BMD (440 gm/ton), flavomycin (125 gm/ton), enramycin (125 gm/ton) and lincomix (200 gm/ton), showed lower mortality rates when experimentally infected with toxigenic *Cl perfringens* types A&C and non-toxigenic type. Mortality rates were decreased from 45% & 25% to 30% & 5%, 35% & 5%, 25% & 0% and 25% & 5% respectively. Stutz et al (1983) and Das et al (1997c) found that bacitracin showed 100% efficacy against *Cl perfringens* isolates *in vitro*, while Williams (1972) reported that bacitracin was active against *Cl perfringens* both *in vitro* and *in vivo*. Our results disagree with Benno et al, (1988) and Devriese et al, (1993) who detected aquired resistance against bacitracin by some isolates.

Regarding to the effect of lincomix, several authors reported on its good activity on clostridium, body weight, reducing morbidity and mortality rates, and clostridial shedding by infected birds (Hamdy et al, 1983 a&b; Secasiu, 1995 and Shen Jian Zhong et al, 1997). On the other side Watkins et al (1997) found that lincomycin appeared to be resisted by most strains of *Cl perfringens*.

In case of enramycin and flavomycin, our results agree with those recorded by Benno et al., (1988), Sheldon and Essary (1982), Palic et al (1998) regarding to improvement of body gain, suppressing the shedding and reducing the mortality rate. Devriese et al., (1993) reported on flavomycin resistance by *Cl perfringens*.

The recorded susceptibility and resistance patterns of *in vitro* sensitivity test in present work had been fully described by several workers (Trishkins and Rokhmanina, 1973; Ibrahim, 1979; Kondo et al., 1988 and Secasiu, 1995).

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Table (1): Results of experimental infection of chicks with commonly isolated clostridial species.

Group no	Inoculated organisms	Dose (ml.)	No. of inoculated birds	Route of inoculation	Weekly deaths post infection				Total no. of deaths	Mortality rate %	Mean body weight gm
					1st	2nd	3rd	4th			
1	*Cl. perfringens* type A	1.8×10^9	20	Oral	6	2	0	0	8	40	240
2	*Cl. perfringens* type C	1.8×10^9	20	Oral	7	3	0	0	10	50	238
3	Non-toxigenic *Cl. perfringens*	1.8×10^9	20	Oral	2	3	0	0	5	25	271
4	*Cl. colinum*	10^7	20	Oral	1	0	0	0	1	5	279
5	*Cl. sporogens*	1.5×10^9	20	Oral	1	0	0	0	1	5	275
6	Control	Sterile broth	20	Oral	0	0	0	0	0	0	281

Table (2): Results of combined experimental infection of chicks[a] with coccidia and commonly isolated clostridial species

Group no.	Inoculated organisms	Dose (ml.)	No. of inoculated birds	Route of inoculation	Weekly deaths post infection				Total no. of deaths	Mortality rate %	Mean body weight gm
					1st	2nd	3rd	4th			
1	*Cl. perfringens* type A	1.8×10^9	20	Oral	9	5	0	0	14	70	229
2	*Cl. perfringens* type C	1.6×10^9	20	Oral	11	4	0	0	15	75	230
3	Non-toxigenic *Cl. Perfringens*	1.8×10^9	20	Oral	5	3	0	0	8	40	242
4	*Cl. colinum*	10^7	20	Oral	1	1	0	0	2	10	253
5	*Cl. sporogens*	1.5×10^9	20	Oral	1	0	0	0	1	5	258
6	Control	Sterile broth	20	Oral	0	0	0	0	0	0	272

N.B. [a] Previously infected with 5×10^4 sporulated oocysts of Eimeria at 14-day-old.

Table (3): Results of experimental infection of chicks received Bacitracine Methylene Disalicylate (BMD).

Group no	Inoculated organisms	Dose (ml.)	No. of inoculated birds	Route of inoculation	Weekly deaths post infection				Total no of deaths	Mortality rate %	Mean body weight gm
					1st	2nd	3rd	4th			
1	*Cl. perfringens* type A, C	1.8×10^9	20	Oral	5	1	0	0	6	30	297
2	Non-toxigenic *Cl. perfringens*	1.8×10^9	20	Oral	1	0	0	0	1	5	308
3	Control	Sterile broth	20	Oral	0	0	0	0	0	0	315

Table (4): Results of experimental infection of chicks received Flavomycin.

Group no	Inoculated organisms	Dose (ml.)	No. of inoculated birds	Route of inoculation	Weekly deaths post infection				Total no of deaths	Mortality rate %	Mean body weight gm
					1st	2nd	3rd	4th			
1	*Cl. perfringens* type A, C	1.8×10^9	20	Oral	5	2	0	0	7	35	302
2	Non-toxigenic *Cl. perfringens*	1.8×10^9	20	Oral	1	0	0	0	1	5	317
3	Control	Sterile broth	20	Oral	0	0	0	0	0	0	320

Table (5): Results of experimental infection of chicks received Enramycin.

Group no.	Inoculated organisms	Dose (ml.)	No. of inoculated birds	Route of inoculation	Weekly deaths post infection				Total no of deaths	Mortality rate %	Mean body weight gm
					1st	2nd	3rd	4th			
1	*Cl. perfringens* type A, C	1.8×10^9	20	Oral	4	1	0	0	5	25	295
2	Non-toxigenic *Cl. perfringens*	1.8×10^9	20	Oral	0	0	0	0	0	0	312
3	Control	Sterile broth	20	Oral	0	0	0	0	0	0	315

Table (6): Results of experimental infection of chicks received lincomix.

Group no.	Inoculated organisms	Dose (ml.)	No. of inoculated birds	Route of inoculation	Weekly deaths post infection				Total no. of deaths	Mortality rate %	Mean body weight gm
					1st	2nd	3rd	4th			
1	*Cl. perfringens* type A, C	1.8×10^9	20	Oral	3	2	0	0	5	25	291
2	Non-toxigenic *Cl. perfringens*	1.8×10^9	20	Oral	0	1	0	0	1	5	307
3	Control	Sterile broth	20	Oral	0	0	0	0	0	0	315

Table 7: Results of *in vitro*-antimicrobial susceptibility test of isolated clostridium species.

Antimicrobial	Concentrations	*Isolated clostridial species*						
		Cl. perfringens Type A (5)*	*Cl. perfringens* Type C (5)	*Cl. perfringens* Type D (3)	Non-toxigenic *Cl. perfringens* (5)	*Cl. colinum* (5)	*Cl. sporogenes* (5)	*Cl. spiroforme* (2)
Erythromycin	15 µg	+ +	+	+	+ +	+ +	+	+ +
Colistin sulphate	25 µg	+ +	+	+ + +	+ +	+ +	+ +	+ +
Neomycin	30 µg	-	-	-	-	-	+	+
Amoxycillin	25 µg	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +
Oxytetracycline	30 µg	+ +	+	+	+ +	-	-	+ +
Ampicillin	10 µg	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+ + +
Streptomycin	10 µg	-	-	-	-	+	-	-
Ciprofloxicin	5 µg	+ + +	+ + +	+ + +	+ +	+ +	+ +	+ + +
Doxycycline	30 µg	+ +	+ + +	+ +	+ + +	+ +	+ +	+ +
Enrofloxacin	5 µg	+ + +	+ +	+ +	+ + +	+ +	+ + +	+ + +
Flumequin	15 µg	+ +	+ +	+ +	+ +	+ + +	+ +	+ + +
Chloramphenicol	30 µg	+ +	-	+	+	+ +	-	+
Gentamycin	10 µg	-	-	-	-	-	-	-

-. resistant +. weak + +. moderately susceptible + + +. strong susceptible

* Figures in parenthesis indicate number of examined strains.