STUDIES ON ANAEROBIC BACTERIAL INFECTION IN OSTRICH AT ISMAILIA GOVERNORATE

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ABSTRACT

This study was carried out on an ostrich farm suffered from diarrhea, depression, and sternal recumbency at different ages in different yards. Some bird found dead and others were emergency slaughtered just before death. Complete postmortem and anaerobic bacteriological examination were done as well as antibiotic sensitivity test of the isolated bacteria and treatment regimens were applied.

A total of 92 samples of liver, heart blood, spleen, intestine, and intestinal content from 8 freshly dead and 4 emergency slaughtered birds were collected. Beside 80 cloacal swabs from (50) diseased and (30) apparently healthy ostrich of different ages. Postmortem examination revealed markedly dilated small intestine especially duodenum and jejunum with dark red to tan serosal surface. The intestinal mucosa was thickened with multifocal hemorrhage appeared from external surface. The liver necrosis and numerous yellow tan foci on the capsular surface were observed.

Bacteriological examination revealed that, 34 out of 50 (68%) from diseased, 12/12 (100%) of dead ostrich and 11/30 (36.66%) from apparently healthy ostrich were positive for anaerobic bacterial isolation. The isolated bacteria was identified biochemically, and by using pathogeincity tests to Clostridium perferingens 44/62 (70.96%), C. sordellii 20/62 (32.25%) and C. sporogens 11/62 (17.74%) from

diseased and dead birds. The incidence of anaerobic bacterial isolation were 7/20 (85%), 10/20 (50%) and 7/10 (70%) from ostrich less than 3 months, 4-12 months and over one year of age respectively. Toxigenic typing of C. perfringens indicated that, type A was the most prevalent with incidence of (75%) and type C was (25%).

Antibiotic sensitivity test showed that all Clostridium spp isolates were highly sensitive to to amoxicillin, ampicillin, penicillin, Chloramephenicol and bacitracin. Less sensitive to nitrofuran, cephalothin and Erythromycin .Resistant to streptomycin and gentamycin .

Two treatment regimens were applied based on sensitivity results. Amoxicillin as 20 mg/kg body weight in drinking water for 5 successive days was found to be more effective in improving health condition, clinical signs and control of mortality rate.

INTRODUCTION

The rapid growth in the number of ostriches placed a significant burden on the veterinary profession. Several countries are attempting to raise significant numbers of ostriches *(Struthio camelus).* Now ostriches industry is suffering greatly from pathogens especially bacterial diseases ***(Huchzermyer, 2002).***

Gastrointestinal disorders are the most frequent and economi-cally important diseases in ostrich farms ***(Harráez et al., 2005)***. Diarrhoea is the main clinical sign in ostrich chicks. Chicks develop diarrhoea after a sudden change in diet. In pathogenic cases, diarrhoea often presents as a flock problem due to bacterial, viral and protazoal causes ***(Huchzermeyer, 1997).*** It is primarily caused by bacterial infections as *Escherichia*,

Salmonella, *Pseudomonas*, *Campylobacter jejuni*, *Klebsiella*, *Clostridium perfringens*, *Clostridium sordellii and C.sporogens*. Cofactors such as dietary changes, poor management conditions, stress, or other concomitant disease contribute to the development of these conditions ***(Verwoerd, 2000).*** Enteritis is the principle cause of mortality in ostrich which are intensively reared on concrete floor. It never occur in ostrich reared on pasture, except for clostridial enteritis ***(Huchzermyer, 2002).***

Clostridial enteritis is a common disorder of ratite of all ages ***(Stewart, 1994).*** *Clostridium perfringens* is probably the most prominent cause ***(Songer, 1996).*** A diversity of syndromes are attributed to *C. perfringens* including enterotoxaemia and enteritis ***(Johansson, 2006).*** Clostridium perfringens is a normal gut inhabitant of healthy ostrich. However under acute stress or sudden change of the nutrition it can multiply out of control, produce toxins and even invade the whole body ***(Huchzerymer, 1999).*** It is classified into five types, by letters A–E. on the basis of their major toxins production α, ß, $\in$ and ι (alpha, beta, epsilon, and iota respectively) ***(Cato et al.,1986).***

The present study deals with a field problem showing diarrhea, depression, high morbidity accompanied by necrotic enteritis and sudden death. Clinical, postmortem and anaerobic bacteriological examinations were carried out, and antibiotic sensitivity tests of isolated bacteria, as well as application of treatment trials to control the disease at ostrich farms.

MATERIAL AND METHODS

Birds: History of diarrhea, depression, and sternal recumbency has been reported on ostrich farm with different ages at different yards. Some

birds were found dead and other were emergency slaughtered just before death. Clinical signs were recorded and postmortem examination was carried out.

Samples: liver, heart blood, spleen, intestine and intestinal content were collected from 8 freshly dead and 4 slaughtered birds. Beside 80 cloacal swabs from (50) diseased and (30) apparently healthy ostrich for anaerobic bacteriological examinations.

Bacteriological examination:

Direct microscopical examination:

Blood smears were done from heart blood and microscopically examined according **to** ***(Quinn et al., 1994)*** Also, A crushed sample of necrotic liver tissue between two slides were carried out, fixed by heating , stained by Gram stain and examined under microscope for detection of gram positive rods of clostridium spp. ***(Wages, 2003).***

1-Isolation of clostridium:

Cultures of all samples were done from each organ and swabs on two tubes of freshly prepared modified Robertson's 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 hours. A loop full from each tube was streaked into the surface of 10% sheep blood agar and neomycin sulphate sheep blood agar plates respectively. The plates were incubated anaerobically at 37°C for 48 hours using gas-packed anaerobic jar (BBL). The suspected colonies were re-inoculated into cooked meat broth and incubated an aerobically at 37°C for 48 hours for further identification.

2- **Bacterial identification:**

Suspected colonies were examined for their microscopic appearance by Gram staining, cultural characters and motility testing .Then identified by Sugar fermentation reactions including glucose, lactose, maltose, sucrose and mannitol. Biochemical tests as Indole production (Spot test), lecithinase test, lipase test, gelatin liquefaction, H_2S production and urease tests were done according to ***Mackie and McCartney (1989).***

3- Determination of typing and toxigenic isolates of C. perferingens:

a) Nagler's reaction test: ***(Levett, 1991)*:**

The plate of egg yolk medium was soaked with few drops of antiserum of type A, the second with antiserum of type B and the third acted as control and the same work was done on the other plate to type C, D, and E. after the dryness of antiserum , then adding the centrifuged supernatant (3000r.p.m) cooked meat culture. The plates were incubated anaerobically at 37℃ for 24 hr and the results were recorded. An opalescent area appeared considered as positive cases.

b) Typing of C. *perferingens* toxins:

Toxin neutralization tests: ***(Smith and Holdeman,1968).***

It was performed by adding 0.1ml of specific antisera (A, B, C ,D and E of *C. perferingens*) (Burrpugh, S Wellcome, Beckenham, London ,England from Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo, Egypt) to 3ml of the centrifuged supernatant (3000 r.p.m) cooked meat culture. Supernatant culture of only type D was treated with 0.1 trypsin for 45 minutes at 37°C. The mixture was left for 30 minutes at 37°C before its injection in mice.

4- Pathogenicity:

a- Pathogenicity tests to Swiss mice:

White mice (25 - 40 g) were injected intra peritoneally (I/p) with 0.3 ml of centrifuged supernatant of intestinal contents obtained from clinical cases suspected to be infected with *C. perferingens*. The mice were kept under observation over a period of three days for either death or disease symptoms. Reference strains of *C. perfringens* types A, B, C, D and E were included as positive controls, while the supernatant from non-inoculated cooked meat broth was also included. For *C. perfringens* type A, strains from chicken was available.

b- Dermonecrotic test in guinea pig according to *Stern and Batty (1975).*

5- Antibiotic sensitivity test:

In vitro susceptibility of isolated anaerobes to different chemotherapeutic agents was done using the disc diffusion method described by ***Koneman et al. (1988).***

6- Treatment trials:

Based on antibiotic sensitivity results and according to ratite therapeutic formulary reported by ***Tully and Shane, (1996).*** The diseased ostrichs were divided into two treatment groups as following:

1- Group 1: diseased ostrich received amoxicillin as 20mg/kg B.W in drinking water for 5 successive days

2- Group 2: diseased ostrich received chloramphenicol as 40 mg/kg B.W in drinking water for 3 successive days.

Birds **kept** under observation during treating period and for 10 day post treatment. All groups received AD_3E as 1 ml/l in drinking water for 5 days.

RESULTS

Clinical signs and post-mortem examination of diseased birds:

Clinically affected birds showed depression, dehydration, reluctance to move, ruffled feather, yellowish watery to white diarrhoea which soiling around the cloacae (1), recumbency (2), either emergency slaughtered (3) or found died without previous signs. At post mortem general venous congestion specially mesentery as a result of sudden death (fig. 4). Markedly dilated small intestine especially duodenum and jejunum with dark red to tan serosal surface (Fig.5). The intestinal mucosa was thickened with multifocal hemorrhage appeared from external surface (Fig.6) with dark red to greenish pseudo-membrane. Some birds showed congestion and enlargement of liver and spleen, hydro pericardium, mild ascites, In addition to, ulceration of the intestines (Fig.7), friable small intestine (jejunum and ileum) distended with gas. The liver showed necrosis and numerous yellow tan foci on the capsular surface as well as on the cut surface (Fig. 8).

Incidence of bacterial isolation and identification:

According to the morphological characters and biochemical reactions as shown in table (1) . The allover incidence of an aerobic bacterial isolation was 57/92 (61.95%).

Table (2) showed that, 34 out of 50 (68%) from diseased and 12/12 (100%) of dead ostrich were positive for anaerobic bacterial isolation. While, in apparently health ostrich was 11/30 (36.66%). The most frequently isolated anaerobic bacteria from diseased and dead ostrich were *Clostridium perferingens* 44/62 (70.96%), *C. sordellii* 20/62 (32.25%) and *C. sporogens* 11/62 (17.74%) in table (3).

Bacterial isolation from apparently health revealed 5/30 (16.66%) *Clostridium perferingens*,3/30(10%)*C. sordelli*i,3/30(10%)*C sporogens*.

The incidence of anaerobic bacterial isolation were 7/20 (85%), 10/20 (50%) and 7/10 (70%) from diseased ostrich in different age groups under 3 months , 4-12 months and over one year respectively.

Clostridium perferingens has high incidence of isolation than any other Clostridia spp. and isolated from all dead birds 12/12 and most of diseased ones 44/62.

Table (4) showed the Incidence of toxigenic and non toxogenic types of *C. perferingens* isolated from ostrich.

Results of pathogenicity test in white mice were observed during 3 days which ends with death. All mice injected with the bacterial culture filtrate died and those injected with control broth without bacteria were alive.

The result of dermonecortic test in guinea pig revealed, 33 isolates produced irregular area of yellowish necrosis. The lesions tend to spread down words. This indicated that these 33 isolates were type A. while, Eleven isolates gave slightly greenish blue coloration after 48hr which indicated that they were related to type C.

The results of antibiogram study of the isolated *clostridium spp.* from ostrich were illustrated in table (5).

Both treated groups showed general improvement in health condition, started to eat well and diarrhea begin gradually to cease after 2 days of treatment in amoxicillin treated group and lasted for 2 days later for chloramphenicol treated groups. No more moralities were recorded.

Table (1): Morphological characters and biochemical reactions of different clostridia isolates from examined ostrich.

isolation	Morphological characters				Biochemical characters										
	Gram's stain	B Hemolysis	Motility	Spores location	lipase	Lecithinase	Indole	H2S	Urease	Hydrolysis of gelatin	Carbohydrate fermentation				
											glucose	lactose	maltose	sucrose	mannitol
C. perfringens	+ ve Bacilli	Double zone	-	-- &Rearly st	-	+	-	-	v	+	+	+	+	+	-
C. sordelli	+ ve Bacilli	+	+	c	-	+	+	-	+	+	+	-	+	-	-
C. sporogenes	+ ve Bacilli	+	+	st	+	-	-	+	-	+	+	-	+	-	-

+ = Positive - = Negative ST = Sub Terminal C = central V = variable

Table (2): Incidence of anaerobic organisms isolated from examined ostrich.

Age	No. of examined samples	Apparently health n=30				Diseased n=50				Dead or emergency slaughter n=12			
		positive		negative		positive		negative		positive		negative	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Under 3 moths	37	4/10	40	6/10	60	17/20	85	3/20	15	7.7	100	0.0	0.0
4-12 months	34	4/10	40	6/10	60	10/20	50	10/20	50	4 4	100	0.0	0 0
Over one year	21	3/10	30	7/10	70	7/10	70	3/10	30	1 1	100	0.0	0 0
Total	**92**	**11/30**	**36.66**	**19/30**	**64.34**	**34/50**	**68**	**14/50**	**32**	**12/12**	**100**	**0.0**	**0.0**

Table (3): The frequency of anaerobic organisms isolated from different ages of examined ostrich.

Age / Type of samples	under 3 months				4-12 months				Over 1 year				total
	Total no. of samples	Isolated microorganisms	No.	%	Total no. of samples	Isolated microorganisms	No.	%	Total no. of samples	Isolated microorganisms	No.	%	
Diseased	20	*C. perferingens* *C .perferinges +C .sordelli* *C.. perferinges+ C. sporogenes*	7 6 4	35 30 20	20	*C. perferingens + C .sordelli* *C. perferingens+ C. sporogenes*	6 4	30 20	10	C. perferingens C. sordelli C. perferingens +C sordelli C. sporogenes	3 1 2 1	30 10 20 10	50
Dead or emergency slaughter	7	*C. perferingens* *C. perferingens + C. sordelli* *C . perferinges +C. sporogenes*	3 3 1	42.85 42.85 14.29	4	*C. perferingens* *C. perferingens +C. sordelli* *C. perferingens + C. sporogenes*	2 1 1	50 25 25	1	C .perferinges +C. sordelli	1	100	12
Apparently healthy	10	*C. perferingens* *C. sordelli* *C. sporogenes*	2 1 1	20 10 10	10	*C. perferingens* *C. sordelli* *C. sporogenes*	2 1 1	20 10 10	10	C. perferingens C. sordelli C. sporogenes	1 1 1	10 10 10	30
total	37	*C. perferingens* *C. sordelli* *C. sporogenes*	26 10 6	70.27 27.03 16.2	34	*C. perferingens* *C. sordelli* *C. sporogenes*	16 8 6	47.06 23.5 17.6	21	C. perferingens C. sordelli C sporogenes	7 5 2	33.3 23.3 9.5	92

Table (4): Incidence of toxigenic and non toxogenic types of *C. perferingens* isolated from ostrich.

Age group	No. of *C. perferingens* isolates	Typing of toxigenic organism			
		A		C	
		No.	%	No.	%
Under 3 moths	24	15	30.6	9	18.37
4-12 months	14	12	24.49	2	4.08
Over one year	6	6	12.2	--	--
Total	44	33		11	

* All C. perferingens isolates from apparently healthy ostrich were non toxigenic

Table (5): The results of antibiogram of isolated clostridium spp.

Antimicrobial disc and conc. µg	*clostridium. perferinges*						*C. sordelli N=(10)*		*C. sporogenes N=(10)*	
	type A	*N=(10)*	*type C*	*N=(10)*	*Non Toxigenic (5)*					
	No.	%	No.	%	No.	%	No.	%	No.	%
Amoxicillin (10)	9	90	9	90	10	100	8	80	8	80
Ampicillin(10)	8	80	7	70	9	90	7	70	8	80
Streptomycin(10)	0	0	0	0	0	0	2	20	0	00
Penicillin(10)	8	80	7	70	8	80	7	70	7	70
Cholormphenicol(25)	8	80	8	80	5	50	6	60	6	60
Lincomycin(15)	8	80	7	70	6	60	2	20	0	0
Tetracycline(30)	7	70	7	70	8	80	0	0	0	0
Erythromycin(15)	6	60	4	40	6	60	2	20	0	0
Bacitracin(10)	8	80	8	80	10	100	8	80	8	80
Cephalothin(30)	8	80	7	70	8	80	4	40	6	60
Gentamycin(10)	1	10	1	10	2	20	0	0	2	20
Nitrofurantoin (300)	5	50	6	60	5	50	6	60	6	60

N= number of tested isolates

LIST OF FIGURES


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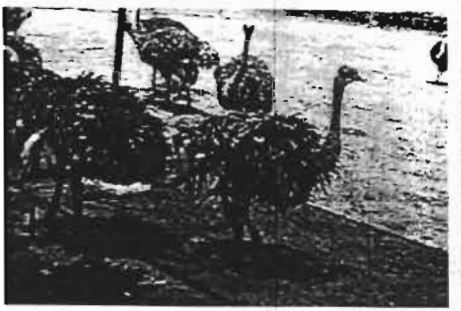

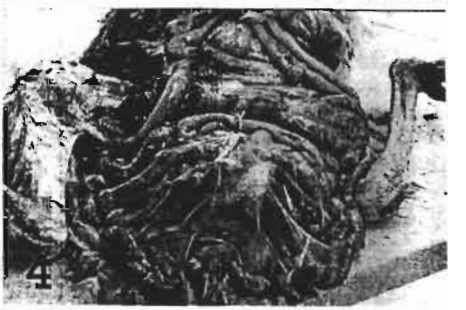
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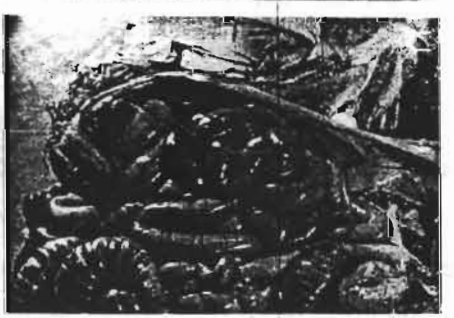

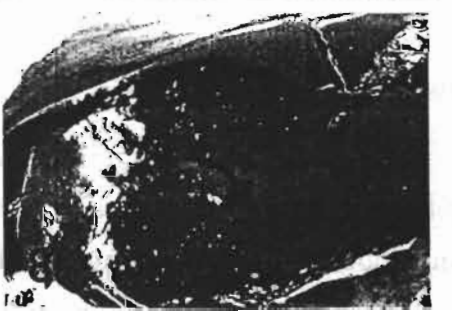

DISCUSSION

Diarrhoea and Necrotic enteritis (NE) remain important diseases in poultry ***(Olkowski et al., 2008).*** They are major causes of mortality in intensively reared ostrich chicks. Necrotic enteritis never occurs in ostrich chicks reared on pasture and influenced by a multiplicity of factors such as intestinal factors, nutrition and environmental factors. ***Huchzermyer (2002).***

The clinical signs of diseased ostrich chicks were manifested in the form of poor growth included dehydration, emaciation, abnormal appetite, anorexia and yellowish watery to white diarrhoea. At post mortem markedly dilated small intestine especially duodenum and jejunum with dark red to tan serosal surface. The intestinal mucosa was thickened with multifocal hemorrhage appeared from external surface. Liver necrosis and numerous yellow tan foci on the capsular surface as well as on the cut surface. Similar clinical signs and post mortem lesions were recorded by ***Tully, (1998), Kwon et al. (2004), Asaoka et al.(2004) and Huchzermeyer, (2002).***

Clostridia are commonly found in the environment, occurring in soil, sewage and water, as well as in the intestines of both man and animals. Members of the genus *Clostridium* are widely recognized as enteric pathogens for man, domestic animals and wildlife ***(Songer, 1996).***

The results of bacterial examination revealed that 34 out of 50 (68%) from diseased and 12/12 (100%) of dead ostrich were positive for anaerobic bacterial isolation. Higher incidence of isolation was recorded in diseased ostrich than apparently healthy ones. The most frequently

isolated anaerobic bacteria from diseased and dead ostrich were *Clostridium perferingens* 44/62 (70.96%), *C. sordellii* 20/62 (%) and *C. sporogens* 11/62 (17.74%). This result agreed with ***Affaf and Basma (2000) and Shivaprassad, (2003).***

The results indicated that, *Clostridium perferingens* have high incidence of isolation than any other *Clostridia spp.* among diseased and dead birds and considered the main causative agent of enteritis in the ostrich farms. This observation coincided with that reported by ***Huchzermeyer (1994) and Van Immerseel et al.(2004)*** and ***Johansson, (2006).***

Also *Clostridium sordellii* has been reported to be responsible for mortalities in 3-4 months ostrich chicks ***(Poonacha and Donahue, 1997).***

Dealing with the incidence of anaerobic bacterial isolation among different age groups. The result revealed that, *Clostridium perferingens* was noticed with high incidence in young diseased ostrich less than 3 months of age, which indicated that, this age group more susceptible to infection than other age groups. Similar observations were reported by ***Shivaprasad,(1993)., Drouol et al. (1995)., Huchzermeyer, (1997) and Affaf and Basma,(2000). Kwon et al. (1994)*** referred the high incidence of *Clostridium perferingens* isolation in young ostrich between 3-80 days to the increased feed consumption associated with the high growth rate during this period which may lead to either intestinal stasis and in turn increased clostridial growth or greater amount of clostridia would be ingested with contaminated feed .Also another possible cause would be that associated with the transmission of infection from brooder to grower house . Where, this nascent age group close to hatching period could harbour the infection from hatchery or brooder indeed, *Clostridium*

perferingens was founded in egg shell fragment, chicken fluff and proper pad in hatchery ***(Craven et al., 2001).*** In the same context, improper cleaning of young birds yards may be anther reasonable cause. ***Ruempler, 1978*** reported that, massive infection with *Clostridium* in young rhea had been fed faeces of adult bird. It is also shown that intestinal droppings of wild birds contain high numbers of *C. perfringens* and that free-living birds can suffer from necrotic enteritis ***(Craven et al., 2000; Asaoka et al., 2004).***

Agab et al. (2008) reported that the mortality rate was 46.3% for the whole ostrich chicks population during 4 production seasons. Enteritis was 15% as a cause of mortality in chicks under 3 months of age while, it was 4.2% in grower 4-14 months. *Clostridium perferingens* has been encountered.

The low incidence of *C. perfringens* isolation in ostrich 4-12 month and over one year of age compared to less than 3 months of age, may be due to well developed immunity in this age consequently, and decreased risk of infection. ***Huschzermeyer (1997)*** who suggested in that, during this age, most birds develop immunity to the enterotoxins elaborated by clostridia due to changes of bird's behaviors such as decreased eating which in turn, reduce the rest of exposure to clostridial microorganisms. Also, these findings supported by ***Afaf & Basma (2000)***who mentioned that the age group (4-12 month) showed lower incidence of anaerobic isolation compared to group (2-4 month).

Typing of the different isolates of toxogenic *C. perfringens* indicated that out of 44 isolates proved to be toxigenic, 33 were type A (75%) and 11 were type C (25%). These results nearly agreed with ***Affaf & Basma (2000)*** and ***Kwon et al.(2004)*** which reported that the most

toxigenic *C. perfringens* in ostrich type A, which was more prevalent than type C. Also, ***Hofshagen et al. (1992) ,Shivaprasad (2003)*** and ***Van Immerseel et al. (2004)*** found the most predominant type of *C. perfringens* in poultry is type A and followed by type C. Also the result showed that All *C. perferingens* isolates from apparently healthy ostrich were non toxigenic. Where, C. perfringens is a common intestinal inhabitant, but what can be questioned is the significance without further typing of the strains for toxin production ***(Craven et al. , 2000).*** The presence of *C. perfringens* in the intestinal tract of chickens, does not lead to the development of necrotic enteritis***(LaRagione and Woodward, 2003).*** One or several predisposing factors may be required to elicit the clinical signs and lesions of necrotic enteritis.

The result of antibiotic susceptibility of isolated anaerobic bacteria to different chemotherapeutic agents showed that, variable degrees of sensitivity according to each isolate type but in brief they conventionally were highly susceptible to amoxicillin, ampicillin, penicillin, Chloramphenicol and bacitracin. Less sensitive to nitrofuran, cephalothin and Erythromycin .While resistant to streptomycin and gentamycin These results agreed with ***Affaf & Basma (2000). Abd-EL-Twab, 2002 and Abdel-Rhman et al., 2007.***

Concerning with field treatment trials, although both treatment groups had an improvement in diseased ostrich health, amoxicillin which is semi synthetic penicillin showed fast and effective response in controlling the diseases condition than chloramphenicol. This result agreed with ***Button, (1995) and Huchzermeyer, (1998)*** who mentioned that clostridial infection in ostrich treated by dosing synthetic or semi synthetic penicillins.

Finally, It is clear from the present findings that anaerobic bacterial infection were the main cause of diarrhoea, enteritis and losses in the ostrich farm at Ismailia. This conclusion is reached from isolation of *C. perfringens* with high incidence followed by *C. sordelli and C. sporogenes* from diseased and freshly died birds in all age groups. These organisms were normal inhabitant in soil so; continuous disinfectant and hygienic measures should regularly apply in ostrich farm. Moreover, Amoxicillin administration as 20 mg/kg B.W in drinking water for 5 successive days was found to be more effective in improving health condition, clinical signs and control of mortality rate.

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دراسات على العدوى بالميكروبات اللاهوائية في النعام بمحافظة الإسماعيلية

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أجريت هذه الدراسة على مزرعة نعام تعانى طيورها من الإسهال والكآبة، وانبطاح على عظمة القص في الأعمار المختلفة وفي مختلف الحظائر. كما وجدت بعض الطيور نافقة وأخرى ذبحت اضطراريا. وقد تم إجراء الصفة التشريحية الكاملة وتم إجراء فحص البكتريولوجي للميكروبات اللاهوائية بالإضافة إلى إجراء اختبار حساسية بالمضادات الحيوية للبكتيريا المعزولة مع إجراء العلاج الحقلي المناسب.

تم جمع عدد 92 عينة وهى عبارة عن عينات من الكبد، دم القلب، الأمعاء،الطحال ومحتويات الأمعاء من 8 طيور نافقة حديثا و4 طيور مذبوحة اضطراريا بجانب عدد80 مسحة من المستقيم (50) مسحة من طيور مريضة وعدد (30) مسحة من نعام صحيح ظاهريا وذلك من الأعمار المختلفة.

وكشفت نتيجة الفحص التشريحي عن وجود اتسع وانتفاخ ظاهر للأمعاء الدقيقة خصوصاً الإثنى عشري ومنطقة الصائم علاوة على وجود بقع داكنة الاحمرار وأخرى مائلة للسواد وان الغشاء المخاطي المعوي ثُخّنَ بالنزف المتعدّد والتى تظهر بوضوح على السطح الخارجى للأمعاء. كما لوحظ وجود نخر فى الكبدَ مع بؤرَ عديدة سمراءَ صفراءَ على السطحِ الخارجيِ.

وقد كشفت نتائج الفحص البكتريولوجي عن عزل الميكروبات اللاهوائية من النعام المريض بنسبة 68 % (34 /50) و من 12/12 بنسبة (100 %) من النعام النافق و 30/11 بنسبة (36.66 %) من النعام السليم ظاهريا.

وقد صنفت البكتيريا المَعْزُولة باستخدام الاختبارت البيوكيميائيةً وباستعمال اختبارات الضراوة إلى الكلوستردیوم بيرفيرنجن (المطثية الحاطمة) بمعدل 62/44 بنسبة70.96% والكلوستردیوم سورديلى (المطثية السورديلية) بمعدل 62/20 بنسبة 32.25% والكلوستردیوم سبوروجينز (المطثية المبوغة) بمعدل 62/11 بنسبة بلغت 17.74% مِنْ الطيورِ المريضةِ والميتةِ.

وقد أظهرت النتائج اختلاف معدل عزل الميكروبات اللاهوائية باختلاف أعمار الطيور المصاب وقد بلغت 20/7 (85 %)، 20/10 (50 %) و10/7 (70 %) مِنْ النعام اقل من 3 شهورِ، 4-12 شهور فى العمر وأكثر من سَنَةٍ واحدة مِنْ العُمرِ على التوالي. وان النوع (أ) من ميكروب الكلوستردیوم بيرفيرنجن كَانَ الأكثر عزلا بنسبة بلغتِ (75 %) والنوع سي بنسبة (25 %).

واظهر إختبارَ الحسّاسيةِ بالمضادات الحيوية المختلفة بأنّ ميكرب الكلوستردیوم حسّاس جداً تجاه الاموكسيسلين و البنسلين و الامبسلين والكلورامفينكول والباستراسين وكانت أقل حسّاسية إلى السيفالوسين والنيتروفيوران والاريثروميسين و مقاومة إلى الاستربتوميسين والنيوميسين.

وعند إجراء العلاج الحقلى مستندا على نَتائِجِ اختبار الحسّاسيةِ.وجد ان إعطاء الاموكسيسلين بمعدل20 ملجم / كيلوجرام من الوزن الحى في الماء الصالح للشربِ لمدة5 أيامٍ متعاقبةٍ كان أكثرَ فعّالية في تحسن الصحةِ العامة للطيورِ، والأعراض مع تقليل معدلِ النفوق عن استخدام الكلورامفينكول بمعدل 40 ملجم / كيلوجرام مِن الوزن الحي في الماء الصالح للشربِ لمدة 3 أيامٍ متعاقبةٍ.