

Incidence of clostridium infection in turkey in upper Egypt

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Abstract

A total of 270 samples (200 cloacal swabs from living birds and 70 intestinal of freshly dead and sacrificed birds) were collected from different areas in EL-Menia and Sohag Governorate. The bacteriological examination revealed that 130 isolates of *Cl. perfringens* (48%) and the incidence of Clostridial infection in the intestinal samples (51.4%) that was higher than those of cloacal swabs (47%). Evaluation of *Cl. perfringens* subspecies was detected as 92 (70.8%) *Cl. perfringens* type A and 38 (28.2%) *Cl. perfringens* type D. The pathogenicity test in 4 weeks old turkey poult using *Cl. perfringens* type A and type D resulted in 40% and 10% respectively. In vitro antibiotics sensitivity test showed that the isolates were highly sensitive to Amoxacillin, Enrofloxacin, Ampicillin, Lincomycin Apramycin and Refamycin while the Streptomycin had no effect.

Key words: cloacal swabs, intestinal samples, *Cl. perfringens*, pathogenicity test.

Introduction

Recently, number of turkey farms had increased in Egypt as a result of continuous efforts of many organizations to face the increasing demand of human consumption. Most of losses among poultry flocks are related to clostridium with or without complicating factors (Vissiennon *et al.*, 2000) *Clostridium perfringens* in poultry constitutes a risk for transmission to humans through the food where it is one of the most frequently isolated bacteria pathogens in food borne disease after some other microorganisms such as *Campylobacter* and *Salmonella* (Buzby and Roberts, 1997).

Although, there are many studies have been done on *Cl. perfringens* infection in chickens but there is insufficient data about *Cl.*

perfringens infection in turkey, so the aim of the work was directed to investigate the incidence of Clostridium infection among turkey in upper Egypt. Pathogenicity test of these isolates and testing of the antibiotic sensitivity where carried out.

Material and Methods

1- Collection of the samples:

A total of 270 samples including 200 cloacal swabs of living birds and 70 intestinal samples of freshly dead or sacrificed turkeys were collected from different farms and houses in El-Menia and Sohag Governorates, Table(1). These samples were packed in box and aseptically transferred to the laboratory without delay where they were immediately examined bacteriologically.

Table (1): The number of cloacal swabs and intestinal samples collected from different areas.

Samples	Intestinal samples	Cloacal swabs	Total
El-Menia (1-7 month)	50	150	200
Sohag (2-3 month)	20	50	70
Total	70	200	270

2-Bacteriological examination:

The samples were collected aseptically from cloacal swabs and different parts of small intestine including intestinal contents or scrapings of the intestinal wall. All samples were inoculated on two tubes of cooked meat media and incubated anaerobically at 37°C for 48 hrs. One of the incubated tubes was heated in water bath at 60°C for 30 minutes and the other was left without treatment. Subcultures from each tube were made on duplicated neomycin blood agar plates and incubated anaerobically and aerobically at 37°C for 48 hrs. Only strict anaerobic isolates were examined and transferred to cooked meat media for further identification according to (Quinn *et al.*, 2002). The identification of the isolates were carried out with the help of Anerobic Department, Animal Health Research Institute, Dokki, Giza, Egypt.

3-Pathogenicity test with common isolates of *Clostridium perfringens* to the turkey poults.

Seventy one –month old poults were used to study the pathogenicity test, before infection, a random sample which included 10 birds were sacrificed for postmortem and bacteriological examination to prove that these birds were healthy and bacteria free. Other birds were divided into 3 groups, each group of 20 birds. First and second group were inoculated orally with 2 ml broth culture 1.8×10^4 *Cl. perfringens* type A and type D. Third group left as a non inoculated. All birds were kept under observation for two weeks later for the demonstration of clinical symptoms, mortality rate and post-

mortem examination. Trials for re-isolation of the inoculated agents were conducted.

4- Sensitivity test to antimicrobial agents:

In vitro antimicrobial susceptibility testing for the isolated clostridium was carried out by the plate technique described by (Finegold and Martin 1986), antibiotic sensitivity discs (Oxoid) including Amoxicillin (25ug), Ampicillin (10ug), Apramycin (25ug), Ciprofloxacin (5ug), Enrofloxacin (5ug), Doxycycline (30ug), Lincomycin (30ug), Oxytetracycline (30ug), Refamycin (25ug), and Streptomycin (10ug) were used.

Results

The clinical symptoms from the suspected farms were huddle together, loss of appetite, reluctance to move, diarrhea tinged with blood, dullness, depression, ruffled feather and variable percentage of mortalities ranged from 10-40%.

The post mortem lesions were distention of the intestine with gas, thickened mucosa and formation of thick layer of gray material over the intestine, the liver was friable with yellowish brown colour and in some cases had demarcated necrotic foci.

The relative incidence of the recovered clostridium isolates were shown in Table. (2, 3 and 4).

Table (2). Show incidence of *Cl. perfringens* in different areas.

Sources	Cloacal swab samples		Intestinal samples	
	+/No.	%	+/No.	%
El-Menia	65/150	43.3	25/50	50.0
Sohag	29/50	58.0	11/20	55.0
Total	94/200	47.0	36/70	51.4

+/No.=positive number /examined number.

Table (3). Show the number of different *Cl. perfringens* subtype.

Sampling	<i>Cl. perfringens</i> type A		<i>Cl. Perfringens</i> type D	
	+ / No.	%	+ / No.	%
Cloacal swab	67/200	33.5	28/200	14.0
Intestinal	25/70	35.7	10/70	14.3
Total	92/270	34.0	38/270	14.1

+ / No. = positive number / examined number.

Table (4) Show the results of pathogenicity test in one –month old poultts with different *Cl. perfringens* (A and D).

Groups	Inoculated organ-ism subtype	No. of infected chicks	Dose	Route	M.R in 1 st week	M.R in 2 nd week	M.R%
1	typeA	20	1.8×10^4	orally	0	8	40%
2	TypeD	20	1.8×10^4	orally	0	2	10%
3	control	20	---	---	0	0	0

Table (5) Show the results of in-vitro antibiotic sensitivity test

Antimicrobial agents	<i>Cl. perfringens</i> type A	<i>Cl. perfringens</i> type D
Amoxicillin	+++	+++
Ampicillin	+++	+++
Apramycin	+++	+++
Ciprofloxacin	++	++
Doxycycline	++	++
Enrofloxacin	+++	++
Lincomycin	+++	+++
Oxytetracycline	++	+
Rifamycin	+++	+++
Streptomycin	–	–

+++ = highly sensitive

+ = weakly sensitive

++ = moderately sensitive

-- = Resistant

Discussion

Although, Clostridial infection was well known since 19th century as investigated by **Welch and Nuttall (1892)** but still constitutes as one of the most important problems facing veterinarian in the field of poultry industry resulting in severe economic losses among many countries including Egypt.

Regarding upper Egypt there is a little work on the Clostridial infection in turkeys, so this study is directed to investigate the incidence of Clostridial infection among turkeys in different

ages, identification of the most common species and study the sensitivity of strains of different chemotherapeutic agents in vitro as well as pathogenicity of these strains to one-month old turkey poultts.

As shown in Table (2) the bacteriological examination of 270 (200 cloacal swabs and 70 intestinal samples) showed that 94 *Cl. perfringens* (47.0%) were isolated from the cloacal swabs while 36 *Cl. perfringens* (51.4%) from intestinal samples. These results were to some extent similar to those recorded by

(Hussein 1972), and (Ibrahim 1979) they isolated Clostridial species from cloacal swabs with an incidence of 24%, 43% and 19.3% respectively. While they isolated Clostridial species from intestinal samples with an incidence of 71%, 99% and 38% respectively.

Regarding to typing of *Cl.perfringens* that shown in table (3) 92 isolates of *Cl.perfringens* type A (34.0%). Our results agreed with the results obtained by (Das *et al* ; 1997b,c) and (Siragusa *et al.*, 2006) who recorded that *Cl.perfringens* type A was the most common type among poultry.

The pathogenicity of *Cl.perfringens* type A and type D for turkey poults (4 weeks old) revealed that there is a variation in the virulence between these strains where the mortality rate was 40% and 10% in the birds infected with *Cl.perfringens* type A and D respectively (Table 4). These results in accordance to (Hofshagen and Stenwig 1992) who recorded that *Cl.perfringens* type A produced a large amount of toxin in comparison to the another types of Clostridial species. Also (Long *et al.*, 1974), (Awad *et al.*, 1976) and (Das *et al.*, 1997b) recorded mortalities with a rate of 50%, 38.4% and 45% respectively when infected birds with *Cl.perfringens* type A. On other hand, the most clinical symptoms observed were depression, ruffled feathers, greenish diarrhea, lameness and gradually paralysis. Our results agreed with (McClane *et al.*, 2006). Post mortem lesions were catarrhal, hemorrhagic to fibrinonecrotic enteritis with dilatation of the intestine.

As shown in Table (5) the results of antimicrobial sensitivity for *Cl.perfringens* type A and D revealed that/ the *Cl.perfringens* type A was highly sensitive to Amoxicillin, Ampicillin, Apramycin, Enrofloxacin, Lincomycin and Rifamycin. These results of effective antibiotics against clostridial species proved that these groups will cover gram positive bacteria. These agreed with the results obtained by Hussein and Mustafa (1999) and Johansson *et al.* (2004).

From this study we concluded that, isolation of Clostridium from grossly normal intestine indicated its wide range among poultry in high percentage and under devitalizing conditions it can flair up and causing severe enteritis and subsequent economic losses among turkey

farms.

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مدى تواجد الإصابة بالكلوستريديم في الرومي في صعيد مصر
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الملخص العربى

تم فحص عدد ٢٧٠ عينة (عبارة عن ٢٠٠ مسحة مجمع من الطيور الحية و ٧٠ عينة من أمعاء طيور مذبوحة و حديثة النفوق) وجميعها من مزارع خاصة من محافظة المنيا وكذلك من التربية المنزلية بمحافظة سوهاج ، وقد أظهر الفحص البكتريولوجي عن عزل ١٣٠ معزولة من الكلوستريديم بيرفيرنجس (٤٨%) وكانت الإصابة بالميكروب أعلى في الطيور النافقة عن مسحات المجمع وتصنيف المعزولات كانت عبارة عن ٩٢ كلوستريديم بيرفيرنجس (أ) (٧٠.٨%) و ٣٨ من النوع (د) (٢٨.٢% . و بأجراء العدوى الصناعية لمعزولات من الكلوستريديم بيرفيرنجس (أ) و (د) في كتاكيت رومي عمر ٤ أسابيع عن طريق الحقن في الفم كانت نسبة النفوق ٤٠% و ١٠% علي الترتيب . و قد أثبت اختبار الحساسية أن العترات المعزولة كانت حساسة بدرجة كبيرة لكل من الأموكسيسيلين و الأمبسلين والأبراميسين اللينكوميسين وكذلك الريفاميسين في حين أن الأستربتوميسين لم يكن له تأثير علي العترات المعزولة .