

Clostridium Perfringens Associated With Sudden Death In Sheep

Kawther H Sabah

Animal Health Research Institute-Ismailia
Bacteriology Department

ABSTRACT

A total number . of 350 sheep (112 lambs and 238 adult sheep) were obtained from agricultural developing system farm at Ismaillia (not vaccinated against Clostridium perfringens). Sudden death occurred in some cases and other cases suffered from signs of enteritis with brownish diarrhea. Clinical, postmortem as well as bacteriological investigation were carried out, in addition to antimicrobial sensitivity test. The result of bacteriological examination revealed that Clostridium perfringens was recovered from lambs in mortality and morbidity cases with a percentage of 40.6% and 36.9% respectively, while reached in adult sheep to 31.5 & 29.8 in mortality and morbidity cases respectively, Clostridium perfringens type B and C were isolated from lambs in a percentage 66.6% and 33.3%, respectively and from sheep in a percentage 39.6% and 60.4 %, respectively. The *in vitro* antibacterial sensitivity testing showed that Clostridium perfringens were highly sensitive to Amoxicillin, Norfloxacin, Ampcillin and Chloramphenicol (92.3% 88.5%,83.3% and 79.4 %), while Oxytetracycline was of moderate effect (43.5 %), On other hand, Clostridium isolates were resistant to Streptomycin and Gentamycin at rate of 0.0% .

It can be concluded that, Clostridium perfringens type B and C were the causative agent of enterotoxemia in sheep, the infection was associated with high mortality and morbidity. The disease is best controlled by vaccination of the pregnant dam during the last third of pregnancy against Clostridium perfringens .

INTRODUCTION

Clostridia are obligate anaerobic bacteria and are spore-forming, Gram- positive rods (1,2).

Clostridium perfringens causes enteric diseases called enterotoxemia in sheep. This microorganism is a normal inhabitant of the intestine of most animal species, and humans, but when the intestinal environment is altered by sudden changes in diet or decrease in the intestinal motility as in case of over-eating or other factors, Clostridium perfringens proliferates producing potent toxins that act locally or absorbed into the general circulation with devastating effects on the host (3) . It is also known to be a soil saprophytic that widespread in nature (sewage, faeces, water and feeds) where animals may come in contact with the organism by ingestion (4) .

This disease is usually restricted to a single lamb, because a ewe with twins does not normally provide enough milk to allow enterotoxaemia to develop. The problem can also occur when lambs are grazing very lush

pastures. Those lambs with higher intakes are most susceptible. It may also occur when there has been a sudden introduction of, or an increase in grain or other carbohydrate-rich feed in the diet (5) .

History, clinical signs, and gross postmortem findings are useful tools for establishing a presumptive diagnosis of clostridial enterotoxemia in sheep (6).

Several cases of sudden death in lambs and adult sheep have been recorded , some cases showed brownish diarrhea before death. Signs of toxemia appear on other cases on the flock in agricultural developing system farm at Ismaillia , therefore the present study deals with a field problem showing high morbidity and mortality among lambs and adult sheep .Clinical signs, postmortem findings as well as bacteriological investigations were carried out in order to diagnose the causative agents responsible for this problem. In addition their antimicrobial sensitivity testing was carried out .

MATERIAL AND METHODS

History of The diseased sheep appeared as, depression, recumbence and moderate degree of enteritis with recurrence of unpleasant, brownish watery diarrhea, and sudden death in some cases. The sheep in the farm not vaccinated against *Clostridium perfringens*. There is no evidence of Streptomycin treatment.

The post mortem examination showed moderate to severe gastroenteritis especially in lambs. Liver was enlarged, while kidneys were swollen and congested.

1. Samples

A total of 350 sheep of different ages (112 lambs and 238 adult sheep) in the of agricultural developing system project farm were examined for the infection and prevalence of *clostridium perfringens*.

1.1. Bacteriological examinations

1.1.1. Samplin: Intestinal contents and tissue specimens from the intestine were collected under aseptic conditions, Portion of small intestine were ligated in sterile labeled plastic bags and transferred in ice box as soon as possible to the laboratory.

2. Culture media

a- Cooked meat media .

b-Neomycin blood agar media (neomycin sulphate solution was added to the media just before the additions of blood to make final concentration of 150µg/ml .

3. Antiserum for *Clostridium perfringens* toxin (type A, B, C, D and E (Burrough, s welcome, Beckenham, London, England).

4. Experimental animals

Swiss mice with an average weight 20-25 gm were used for the detection of

***Clostridium perfringens* toxins** in the intestine of infected sheep. They were kept under observation for two weeks before they were inoculated.

5. Antimicrobial sensitivity test

Different chemotherapeutic sensitivity disc, ampicillin, norfloxain, oxytetracyclin, streptomycin, chloramphenicol, gentamycin, and amoxicillin produced by Oxide LTD, London .

6. Gas- pack anaerobic jar

It was used for production of anaerobic conditions bags with socket were used suitable for bacteria growth using hydrogen- carbon dioxide

1.1.1. Isolation and identification

Direct smear was obtained from intestinal contents with Gram stain to observe the clostridial organisms. The intestinal content was cultured in cooked meat media and incubated anaerobically at 37°C for 48 hours. Subcultures were made on Neomycin blood agar plates .One set of the inoculated solid media was incubated anaerobically at 37°C for 24- 48 hours and the other aerobically at 37° c for 24 hours. Only strick anaerobic isolates were examined and identified for microscopic appearance, culture characters, motility, and then transferred to cooked meat medium for other biochemical tests as previously described (7,8).

1.1.1.1. Typing of toxigenic *Clostridium perfringens* isolates

Neutralization tests in mice were performed (9) by adding 0.1ml of specific antisera (A, B, C, D, and E) of *Clostridium perfringens* to 3 ml of the centrifuged supernatant (3000 r.p.m.) cooked meat culture. The mixture was left for 30 minutes at room temperature before its injection intravenously in mice. The pattern of cross-neutralization observed in the mouse tests indicates the specific *Clostridium perfringens* type (2).

1.1.1.1.1. Sensitivity test

The isolates were tested for sensitivity to different chemotherapeutic agents (ampicillin, norfloxain, oxytetracyclin, streptomycin, Chloramphenicol, gentamycin and amoxicillin). One ml of broth culture was spread on the surface of blood agar. Plates and incubated anaerobically at 37°C for 24 hr. The degree of sensitivity was determined and interoperated (10) .

RESULTS

The results of incidence of morbidity and mortality in the affected farm as well as positive examined cases for *Clostridium perfringens*

from lambs and adult sheep are summarized in Tables 1&2 .

Bacteriological examination revealed the isolation of smooth, rounded, glistening colonies surrounded by double zone of haemolysis. Gram staining indicated gram- positive rods. All isolates were grown onto blood agar plates with no type differences found. The culture was

morphologically and biochemically identified as *Clostridium perfringens*.

Detection and typing of *Clostridium perfringens*- toxins: Mice lethality and toxin-antitoxin mice neutralization tests referred to the presence of *Clostridium perfringens* toxin type B&C in Tables 3&4 .

The *in vitro* antibiotic sensitivity test was illustrated in Table 5

Table 1. Incidence of mortality and morbidity of lambs and adult sheep

Type of sheep		Mortality		Morbidity		Total	
		No	%	No	%	No	%
Lambs	112	32/112	28.6	46/112	41.1	78/112	69.6
Adult sheep	238	54/238	22.7	104/238	43.7	158/238	66.4
Total	350	86/350	24.6	150/350	42.8	236/350	67.4

Table 2. Percentage of positive examined cases for *Clostridium perfringens* isolated from lambs and adult sheep.

Type of sheep	Mortality				Morbidity				Total positive			
	+ve		-ve		+ve		-ve		+ve		-ve	
	No	%	No	%	No	%	No	%	No	%	No	%
Lambs	13/32	40.6	19/32	59.4	17/46	36.9	29/46	63	30/78	38.5	48/78	61.5
Adult sheep	17/54	31.5	37/54	68.5	31/104	29.8	73/104	70.2	48/158	30.4	110/158	69.6
Total	30/86	34.9	56/86	65.1	48/150	32	102/150	68	78/236	33.1	158/236	66.9

Table 3. Types of *Clostridium perfringens* among lambs in the examined farm

Source of samples	No of +ve examined isolates	Type of toxin			
		B		C	
	No	%	No	%	
Mortality	13	9	69.2	4	30.8
Morbidity	17	11	64.7	6	35.3
Total	30	20	66.6	10	33.3

Table 4. Types of *Clostridium perfringens* among adult sheep in the examined farm

Source of samples	No of +ve examined isolates	Type of toxin			
		B		C	
		No	%	No	%
Mortality	17	6	35.3	11	64.7
Morbidity	31	13	41.9	18	58.1
Total	48	19	39.6	29	60.4

Table 5. Antimicrobial sensitivity test of *Clostridium perfringens* isolates

Chemotherapeutic agents	Antibiotic disc/conc. (ug)	Susceptibility of 78 of isolates of <i>Clostridium perfringens</i>					
		S		I		R	
		No.	%	No.	%	No.	%
Ampicillin	Amp-10ug	65	83.3	13	16.6	-	-
Streptomycin	S-10ug	-	-	-	-	78	100
Chloramphenicol	C-10ug	62	79.4	16	20.5	-	-
Norfloxacin	Nr-10ug	69	88.5	9	11.5	-	-
Oxytetracycline	OX-30ug	-	-	34	43.5	44	56.4
Gentamycin	Gn-10ug	-	-	-	-	78	100
Amoxicillin	AM-10ug	72	92.3	6	7.7	-	-

S= sensitive I= intermediate R= resistant

DISCUSSION

Clostridium perfringens infection is more common in sheep. It is worldwide in encountered in lambs. A diagnosis of enterotoxaemia can be considered in the case of its distribution and may occur in animals at any age. However, it is most commonly sudden, convulsive deaths in lambs fed on a carbohydrate-rich feed (11,12).

In the present study, clinical signs of affected sheep include increased respiratory efforts, recumbency and convulsions, while it has been recorded (12) that the clinical signs are generally absent and sudden death occur without clinical signs.

Gross postmortem changes were observed included pulmonary edema; excess pericardial,

peritoneal and pleural fluid. Liquid small intestinal contents and subcapsular petechiae on kidneys were recorded. Similar observation was recorded in one day old lambs (13).

In the present study mortality rate reached 86/350 (24.6%) represent 32/112 (28.6%) & 54/238 (22.7) in lambs and sheep respectively, similar results were recorded in previous studies in Egypt (14,15) and from (16) reported that the average case of fatality rate in their study was 40% (51/128)

Bacteriological examination revealed the isolation of anaerobic gram positive bacilli which identify biochemically as *Clostridium perfringens* as their incidence reached to 40.6% & 36.9% in lambs mortality and morbidity respectively and reached in adult sheep to 31.5

& 29.8 in mortality and morbidity respectively, this result partially similar to that reported in Egypt (17).

The most accepted criterion in establishing a definitive diagnosis of enterotoxaemia is detection of *Clostridium perfringens* toxins in intestinal contents (3).

In the present study, *Clostridium perfringens* type B & C were recognized from both mortality and morbidity samples by percentage reached to 66.6% & 33.3% in lambs as type B & C respectively and reached to 39.3% & 60.4% in adult sheep as type B & C respectively. (3) *Clostridium perfringens* type B and C were isolated and some types of *Clostridium perfringens* (e.g., B and C) can be of diagnostic value, but other types (e.g., A) are so commonly found in the intestine of normal animals which indicates that isolation is value less from a diagnostic point of view. High prevalence of *Clostridium perfringens* type A followed by types B, C and D were previously recorded by several authors (18,19).

While (20) reported that, one *Clostridium perfringens* strain produced beta toxin (type C) and 21 strains produced large amounts of alpha-toxin.

Infection with *Clostridium perfringens* types B and C causes severe enteritis, dysentery, toxemia, and high mortality in young lambs, calves, pigs, and foals. Types B and C both produce the highly necrotizing and lethal β toxin that is responsible for severe intestinal damage (21).

Major exotoxins produced by *C. perfringens* were types B and D, in the intestinal content of affected animals in both field and experimental infection (22).

Due to the short course of the disease, treatment is generally not possible or practical. Valuable animals can be treated with anti-toxin and antibiotics, but the rapid and sudden onset of the disease generally means that the first thing that is noticed is a dead animal (12).

Treatment is usually ineffective because of the severity of the disease, but if available, specific hyperimmune serum is indicated, and

oral administration of antibiotics may be helpful (21).

The *in vitro* sensitivity testing showed that *Clostridium perfringens* was highly sensitive to Amoxicillin, Norfloxacin, Ampicillin and Chloramphenicol (92.3% 88.5%, 83.3% and 79.4 %) while Oxytetracycline was of moderate effect (43.5 %) ,on other hand *clostridium* isolates were resistant to Streptomycin and Gentamycin at rate of (0.0%). Several previous studies cited similar information (23-25). *Clostridium perfringens* related livestock infections have been reported in most parts of the world. It is often not feasible to perform it on domestic animals. The most practical way to handle *perfringens*-related illnesses in animals is to prevent them in the first place (26).

It could be concluded that, enterotoxaemia caused by *Clostridium perfringens* type B and C can be considered as disease of sheep depending principally on the predisposing factors that stimulate the organism, which located normally in the intestine of sheep to proliferate and produce its toxins. Young animals should be weaned slowly in addition to gradual diet replacement. Management practices aimed at reducing digestive upsets will also help control the disease. Restricting access to grain, concentrates will reduce the disease level. Sudden changes to diet should be avoided. Diets high in grain should be supplemented with adequate roughage. The disease is best controlled by vaccination of the pregnant dam during the last third of pregnancy.

REFERENCES

1. Buxton D and Donachie W (1991): *Clostridial diseases in* :W.B. Martin and L.D.Altken (eds.). Diseases of sheep. 2nd ed. (Blak well Scientific Publication,(Oxford) pp.104-114.
2. Quinn P J, Markey B K, Carter M E, Donnelly W J C and Leonard F C (2002): "Veterinary Microbiological and Microbiological Diseases." 1st Iowa State University Press Blackwell Science.
3. Uzal A and Songer J Glenn (2008): Diagnosis of *Clostridium perfringens*

intestinal infections in sheep and goats. journal of Veterinary Diagnostic Investigation Vol. 20 Issue 3, 253-265

4. **Van Baelen D and Devriese L A (1987):** Presence of *Clostridium perfringens* exotoxin in intestinal samples from farm animals with diarrhea of unknown origin. *J. Vet. Med. (B)* 34, 713-716.
5. **Kusiluka L J M and Kambarage D M (1996):** Diseases of Small Ruminants in Sub-Saharan Africa: A Handbook. VETAID.
6. **Filho E J F, Carvalh A U O, Assis R A, Lobato F F, Rachid M A, Carvalho A A, Ferreira P M, Nascimento R A, Fern A A and Vidal J E (2009):** Clinicopathologic Features of Experimental *Clostridium perfringens* Type D Enterotoxaemia in Cattle *Veterinary Pathology*; 46(6): 1213 - 1220.
7. **Koneman E , Allen S , Damsel ver and Sommers H (1988):** Colour Atlas and text book of Diagnostic Microbiology 2NDJ.B Lip. Co.London.
8. **Levett P N (1991):** Identification of clinically important fluids for detection of *Clostridium perfringens* type D epsilon toxin in sheep with experimental enterotoxemia anaerobes. In *Anaerobic Microbiology. A practical approach.* (I R I, Press, Oxford). Pp. 29-49.
9. **Smith L D and Holdeman L V (1968):** The pathogenic Anaerobic Bacteria. Pp. 201-205. 1st Ed Charles C. Thomas Publisher, Springfield, Illionis, USA.
10. **Manual of Clinical Microbiology 7th edition (1999):** Performance standards for antimicrobial susceptibility tests . American Society for Microbiology. P1531-1533.
11. **Winter A and Charnley J (1999):** The Sheep Keeper's Veterinary Handbook. The Cotswold Press.
12. **Maxwell D and Knights G (2005).** Livestock health: Pulpy kidney in sheep and goats. DP&IF Note, File No: SW0055. Department of Primary Industries and Fisheries, Queensland Government, Australia.
13. **Watson P J and Scholes S F (2009):** *Clostridium perfringens* type D epsilon intoxication in one-day-old calves. *Vet Rec.*, 164(26): 816 - 818.
14. **Sohair Y Mohamed , Aly S M and Enany M E (2004):** Pathological studies on Clostridial infection among sheep in Suez canal area. *J. Egypt. Vet. Med. Assoc.* 64, No 1:163-177
15. **Al-Gaabary M H (2000):** Morbidity and fatality rates in an outbreak of enterotoxaemas in sheep. 9th Sci. Cong. Fac.Vet.Med. Assiut. Univ., pp,287-291.
16. **Tooloei M and Masodei M H (2008):** The Distribution and Prevalence Rate of Enterotoxaemia in Sheep in East Azerbaijan Province, Northwestern Iran, in Spring 2008 . *journal of Animal and Veterinary Advances* Volume: (7) Page No.: 1434-1439 .
17. **Hassan A Nibal , Amer H A and Mansour A M (2005):** Pathological studies on the sudden death of buffalo calves infected with *Clostridium perfringens* type A. *Egypt. Comp. Path.* Vol. 18 No. 2 ,40-57.
18. **Mahmoud B S (1991):** Isolation and identification of clostridium in apparently healthy slaughtered sheep and goat . Ph D. bacteriology thesis, fac. Vet. Med. Cairo Univ.
19. **Rawhia Doghaim; Hala El Miniawy and Nabil A Hasan (2000):** Actiopathology of enteritis in sheep caused by anaerobic and parasitic agents: A light and scanning electron microscopic study. *Assiut Vet. Med. J.* 44 (87)
20. **Nillo L (1987):** Toxigenic characteristic of *Clostridium perfringens* type in enterotoxaemia of domestic animals. *Canad. J. Vet. Res.*, 51 (2) , 244-228 .
21. **Merck & Co Inc (2008):** The Merck Veterinary Manual. Whitehouse Station, NJ Merial Ltd USA. All Rights Reserved. Published in educational partnership with

22. *Layana J E, Mariano E F M and Uzal F A (2006):* Evaluation of different fluids for detection of *Clostridium perfringens* type D epsilon toxin in sheep with experimental enterotoxaemia . *Anaerobe J .*, Volume 12, Issue 4, Pp 204-206
23. *Perelman B, mitnts S, Zjut M, Kuttin E and Machny S (1991):* An unusual *Clostridium colinum* infection in broiler chickens . *Avian Pathology* , 20:475-480.
24. *Das B C, Dutta G N, Dervriese L A and Phukan A (1997):* Antimicrobial susceptibility of *Clostridium perfringens* isolated from necrotic enteritis cases of poultry . *Indian Veterinary Journal*, 74(7) 553-555.
25. *Abdel- Rahman, A A , Fatma A Moustafa and Neveen A H (2006):* Detection of the prevalence and Pathogenicity of *Clostridium perfringens* and *Clostridium spiroforme* Associated diarrhea in Rabbits. *Assiut Vet. Med. J.* Vol. 52 No. 108
26. *Songer G (1998):* *Clostridium perfringens* New ways to type strains of a deadly bacteria ag.arizona.edu/pubs/general/resrpt1998/clostridium.html (520) 621-2962

الملخص العربي

ميكروب الكلوستيريديوم بيرفيرجينس المصاحب لحالات النفوق المفاجئ في الأغنام

كوثر حسين أحمد صباح

معهد بحوث صحة الحيوان بالإسماعيلية

نظرا للدور الهام للبكتيريا اللاهوائية في حالات النفوق المفاجئ وحالات التسمم المعوي فقد شملت الدراسة الفحص البكتريولوجي لعدد ٣٥٠ من الأغنام (١١٢ من الحملان الصغيرة و ٢٣٨ من الأغنام البالغة من مزرعة مشروع تطوير النظم الزراعية حيث تم عزل ميكروب الكلوستيريديوم بيرفيرجينس من الحملان الصغيرة بنسب ٦ و ٤٠ % و ٩ و ٣٦ % من حالات النفوق المفاجئ والحالات المصاحبة على التوالي . ومن الأغنام البالغة ٥ و ٣١ % و ٨ و ٢٩ % في حالات النفوق والإصابة . اظهر الفحص البكتريولوجي عزل الكلوستيريديوم بيرفيرجينس من نوع ب و ج من الحملان الصغيرة بنسب ٦ و ٦٦ % و ٣ و ٣٣ % على التوالي ومن الأغنام البالغة بنسب (٦ و ٩٣ %) و (٤ و ٦٠ %) على التوالي ومن خلال إجراء اختبار الحساسية للعترات المعزولة ضد بعض المضادات الحيوية تبين حساسيتها العالية لكل من الاموكسيسيلين والنورفلوكساسين والامبسلين والكلورامفينيكول بينما كانت مقاومة لكل من الستربتومايسين والجنتاميسين. وللوقاية من هذا المرض يفضل تحصين الامهات الحوامل في الثلث الاخير من الحمل ضد ميكروب الكلوستيريديوم بيرفيرجينس.