

CLOSTRIDIUM SORDELLII AS A CAUSE OF ENTERITIS IN LAMBS

RASMA SHALABY

Department of Bacteriology

Animal Health Research Institute, Dokki, Giza

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SUMMARY

During 2004-2005, out of 25 lambs up to 10 weeks old which died suddenly, 13 *Clostridium sordellii* were isolated. The post- mortem findings varied. Losses have been sporadic in some farms in Kafer el-Sheikh governorate and recorded in all age of lambs. Also, 61 fecal samples of diarrhetic lambs at three different age groups, were examined bacteriologically for the presence of clostridia with special emphasis on *C. sordellii*, the isolation rate of *C. sordellii* was higher in all age groups (24.6, 8.3, and 5 %) than other *Clostridium species*. *C.perfringens*, *C. sporogenes*, and *C. bifementians* were isolated and identified in a different percentage. In vitro, the sensitivity of *C. sordellii* isolates to different chemotherapeutic agents was determined .Results of this study indicates that *Clostridium sordellii* should be considered when investigating the cause of sudden death in lambs.

INTRODUCTION

Clostridium sordellii is a gram positive spore forming anaerobe, and is one of the known members of the *Clostridial species* .It is ubiquitous in distribution, and is found in soil and as a part of animal intestinal flora (Manteca et al. 2005).

Clostridium sordellii is a recognized animal pathogen causing enteritis and enterotoxaemia in sheep and cattle, infection in animals is thought to be caused by ingestion (Lewis and Naylar1998).

Clostridium sordellii seems to be cropping up more and more and is associated with sudden deaths in all ages of sheep but particularly in newly born lambs. Its infection is often associated with sudden change of diet. Consequently, it is likely that cases are going undiagnosed. In ewes *Clostridium sordellii* cases are often mistaken for calcium or magnesium deficiency and animals

will die by toxemia. In lambs *Clostridium sordellii* kills by causing inflammation of abomasums and is sometimes dismissed as a metabolic disorder (Macaldowie,2003).The pathogenicity of *C. sordellii* was historically related to its ability to produce three putative virulence factors a lecithinase (phospholipase C),which is similar to alpha-toxin, a major virulence factor from *Clostridium perfringens* (Karasawa et al., 2003) and a lethal factor known as β -toxin . This toxin was found to be dermonecrotic and haemorrhagic when injected intradermally into guinea pigs β -toxin was subsequently found to consist of two types of toxin, a lethal toxin (LT) and haemorrhagic toxin (HT) (Boquet et al., 1999 and Amimoto et al., 2001).The toxins produced by *C. sordellii* affect blood vessels so that they become more permeable. this causes fluid to move out of the blood vessels into surrounding tissue, and in essence, the blood becomes thicker and more concentrated, this leads to signs of disease, in addition, toxins cause necrosis, edema and haemorrhagic fluid accumulation in intestine (Abdulla and Yee, 2000).

This work was directed to the sheep disease problems caused by clostridial organisms with particular attention to the significance of *C. sordellii* as a disease pathogen.

MATERIALS and METHOD

Twenty five lambs from Kafer el-Sheikh governorate which had died either suddenly or very rapidly with diffuse or localized lesion of acute enteritis .Post-mortem examination of these lambs frequently revealed a distention of the abdominal cavity and on occasions the visible mucous membranes were pale .The general appearance of the open carcass showed a degree of toxemia including enlargement of both superficial and deep regional lymph nodes .A slight peritoneal effusion was present with congestion and enlargement of the liver and the kidneys which were pale and autolytic. Musculature was soft and varied from pale pink to dark red. Also, Sixty one faecal samples from diarrheic lambs were collected and classified into three groups according to their ages; the 1st group including 35 faecal samples of 1-2 month old , the 2nd group including 16 faecal samples of 3-4 month old , the 3rd group including 8 faecal samples from lambs up to 6 month old . Intestinal samples from suddenly died lambs, faecal samples from diarrheic lambs were subjected to bacteriological examination for anaerobes. The samples were inoculated onto 5% sheep blood agar and incubated at 37° C for 48 hours anaerobically, suspected colonies were confirmed as anaerobes by their lack of growth in air and identified by using biochemical methods according to Holdeman et al.,(1977), and Koneman et al., (1992) involving gelatin liquefaction, indole production, glucose fermentation, enzymatic tests involving urease

and lecithinase production, the suspected colony was inoculated in Robertson's cooked meat broth with carbohydrate for two days.

Suspected colonies were small 2 to 3 mm in diameter, grayish white with a low convex surface and an irregular margin. The colonies were surrounded by a weak narrow zone of haemolysis. Colonies suspected of being *C. sordellii* were subcultured for purity. The isolates were shown to be lecithinase, gelatinase, glucose, urease and indole-positive, but lipase and lactose negative, these findings are consistent with the biochemical pattern exhibited by *C. sordellii* (Holdeman et al., 1977 and Koneman et al., 1992). Lethal toxicity of the *C. sordellii* was assayed on mice, where mice were injected intraperitoneally, the lethal toxicity

showed that all mice inoculated with the culture died after 24-72 hours. *C. perfringens* isolates were typed by dermonecrotic test in guinea pigs according to Sterne and Batty (1975). The antibiotic of the recovered *C. sordellii* was done using the disc diffusion method described by Koneman et al., (1992) and Quinn et al., (2002)

RESULTS

The prevalence rate of *C. sordellii* isolated from suddenly dead lambs is shown in Table (1). Samples collected showed 13 positive samples for *C. sordellii* in an incidence of 52% which was higher than the incidence of other Clostridium isolates

Table (1): Incidence of Clostridium species among suddenly died lambs

	Clostridium species	No (%)
single isolates	<i>C. sordellii</i>	13(52)
	<i>C. perfringens</i>	6(24)
	<i>C. perfringens</i> + <i>C. sordellii</i>	3(12)
	<i>C. perfringens</i> + <i>C. sporogenes</i>	1(4)
mixed isolates	<i>C. sordellii</i> + <i>C. sporogenes</i>	1(4)
	<i>C. perfringens</i> + <i>C. bifementans</i>	1(4)
Total		25

Table (2) shows distribution of different *Clostrid.* by while it was detected as a mixed infection, *col* isolates among different ages of diarrhetic an incidence of 3.2 % and 1.6 % in each of 0, lambs. *C. sordellii* was isolated and identified second and third groups of ages. *C. perfringens* in pure form 15 (24.6%), 5(8.3%) and 3(5%) as *C. sporogenes*, and *C. bifermentans* were isolate a single isolate in the three age groups respective- and identified in different percentages.

Table (2): Frequency distribution of Clostridia among diarrhetic lambs

Age	No. of examined samples	No. (%) of positive cases	Isolated Clostridium	No (%)
1-2 m	35	23 (37.7)	<i>C. sordellii</i>	15 (24.6)
			<i>C. perfringens</i> type D	8 (13.1)
3-4m	16	9 (14.7)	<i>C. sordellii</i>	5 (8.3)
			<i>C. perfringens</i> type D	2 (3.2)
			<i>C. sordellii</i> + <i>C. perfringens</i> type A	2 (3.2)
			<i>C. sordellii</i>	3 (5)
Up to 6 m	10	6 (9.8)	<i>C. perfringens</i> type D + <i>C. bifermentans</i>	2 (3.2)
			<i>C. sordellii</i> + <i>C. sporogenes</i>	1 (1.6)
Total	61	38 (62.2)		

Table (3) shows the sensitivity of different *C. sordellii* isolates to chemotherapeutic agents, *C. sordellii* isolates were highly sensitive to penicillin, clindamycin, tetracycline, and gentamicin.

Table (3) :Antibiogram of *C. sordellii* isolates from lambs (10 isolates)

Antimicrobial Agent	Concentration (ug)	No	(%)	AA
Ampicillin	10	4	40	R
Streptomycin	10	2	20	R
Penicillin	10	9	90	S
Chloramphenicol	25	5	50	S
Lincomycin	15	9	90	S
Tetracycline	30	7	70	S
Oxacillin	1	0	0	R
Erythromycin	15	1	10	R
Bacitracin	10	4	40	R
Cephalothin	30	3	30	R
Gentamicin	10	6	60	S
Lincomycin	15	8	80	S

No of sensitive isolates

(%) Percentage of sensitive isolates in relation to the used isolates

AA Antibiogram Activity S Sensitive R Resistant

DISCUSSION

Clostridium sordellii, is an important veterinary pathogen, causing abomasal bloa, haemorrhage, ulcers, liver disease, myositis, and sudden death in sheep. *C.sordellii* has also been reported in human maternal deaths with a disease presenting as a toxic shock-like syndrome Unfortunately, the knowledge about *C.sordellii* disease remains poorly understood. Myonecrosis is apparently rather infrequent, and death may be due to septicemia. In the present study, a result given in Table (1) reveals that 13 out of 25 lambs were positive for *C. sordellii* which proved to harbor a higher incidence (52%) from suddenly died lambs. This was due to the action of toxin produced by the organism which affects the capillary permeability of the intestinal tract as mentioned by Qa'Dan, et al., (2001). The first reported isolations have been recorded by Richards and Hunt (1982). They described cases of sudden death in 1 month-old lambs and supported their diagnosis by isolating *C. sordellii* from the liver.

Various reports have identified *C. sordellii* as being one of several agents responsible for sudden death in sheep and recovered *C. sordellii* from 14 of 128 cases of sudden death in sheep (El. Edrissi et al., 1992), PoPoff (1987) investigated a series of sudden death in sheep and isolated *C. sordellii* from 12 of 19 examined animals. However *C. perfringens* was also isolated. A single death was

reported by Amando and Nazareth (1986), while Amando (1990) reported four isolations of *C. sordellii* from cases of haemorrhagic enteritis in sheep.

Results in Table (1) show also the isolation of *C.perfringens* in an incidence of 24 %, as a sole isolate. Other species of clostridia were also isolated and identified as a mixed infection, *C.perfringens* combined with *C. sordellii* *C.bifermentans* and *C.sporogenes*. Such findings were also reviewed by Al- Moshat and Taylor (1983) who reported a case of fatal enteritis in a ewe from which *C. sordellii* was isolated with other pathogens as *E. coli* ,Fusobacterium necrophorum , *C.bifermentans* and *C.sporogenes* . These latter isolates were considered to be of doubtful significance. Also such finding are supported by, the greatest losses of lambs caused by *C. sordellii* which could be attributed to sudden changes in feeding management, feeding routine, or abrupt changes in weather .In particular, cases in sheep have been confirmed in Egypt ,(Ahmed ,2004). In this concern, Abu Samra et al., 1984 isolated 10 strains of *C. sordellii* from necrotic area of the liver of 12 sheep that died suddenly. *C. sordellii*, like other clostridia is ubiquitous and distributed widely in the environment. This study recorded *C. sordellii* in 13 of 25 lambs in two years (2004-2005) suggesting that *C. sordellii* may be a significant pathogen of sheep and the economic impact of the disease in this class of

lambs might be considerable. Results given in Table (2), show the isolation of anaerobic microorganisms in the three groups where the results proved that the frequency of *C. sordellii* isolation was higher in the three age groups of lambs (24.6, 8.3, and 5%) than other clostridial species

This result comes in agreement with Yatin et al., (2000) who isolated *C. sordellii* from lambs with abomasal blood, haemorrhage and ulcer cases in an incidence of 19.5%. Such finding is also supported by Moris et al., (2002) who isolated *C. sordellii* from 1 year old sheep. These findings agree with data illustrated by Mahmoud, (1991) who found *C. sordellii* in apparently healthy sheep concluding that the pathogenesis of *C. sordellii* is controversial as the organism is considered to be a part of the normal flora. At the same time Ahmed (2004) isolated *C. sordellii* from diarrhetic sheep in an incidence of 5.9 %.

Unlike most other pathogenic clostridia affecting sheep, *C. sordellii* appears to affect sheep of all age groups.

It is uncertain what factors are responsible for initiating the rapid multiplication of the bacteria and the subsequent toxin production .

A careful examination of the present cases suggests that ingested large quantity of concentrate, excess fermentable carbohydrates, sudden change

es of pH, particle size of food or coarseness of ration may inflict mucosal damage of animal. *C. sordellii* produces two toxins, the haemorrhagic toxin (HT) and the lethal toxin, which show similarity to the A and B toxins of *C. difficile*. A powerful clostridial toxins when inducing disease in sheep produce more than one toxin. None however produces toxins similar to HT and LT produced by *C. sordellii*. This difference would account for the susceptibility of *C. sordellii* infection in sheep correctly (Martinez and Wilkins, 1992).

The type of chemotherapeutic agent should be selected on the basis of its sensitivity which should be detected by laboratory examination. Table (3), showed susceptibility of *C. sordellii* different chemotherapeutic agent, to point out the role of these agents in control of *C. sordellii*. Recorded isolates of *C. sordellii* from lambs showed high susceptibility to penicillin, tetracycline, clindamycin and gentamicin. These results coincide with that recorded by Bitti, et al. (1997) who found that the treatment with penicillin, clindamycin and gentamicin gave the best result.

CONCLUSION

In this trial, *C. sordellii* field isolates were predominating of *C. sordellii* isolates suggesting the importance of this agent in sheep diseases in Egypt.

Additional studies are recommended to know the real prevalence of pathogenic *C. sordellii*. The routine use of anaerobic bacteriological methods must be used to evaluate the role of *C. sordellii*.

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كلوستريديم سورديلي كمسبب لالتهاب الحوي في الحملان

د/بسمه شلبي

قسم البكتريولوجي معهد بحوث صحة الحيوان

في الفترة من ٢٠٠٤-٢٠٠٥ نسبت كلوستريديم سورديلي في نفوق ٢٥ حمل فجأة سن ٨ أسابيع في بعض مزارع كفر الشيخ . وتم الفحص الأكلينيكي للحالات وكانت الحالات المصابة في الحملان من كل الأعمار وتم فحص عينات براز من حالات اسهال في ثلاث مجموعات مختلفة من الحملان التي تم فحصها بكتريولوجيا لوجود الكلوستريديم مع التركيز علي كلوستريديم سورديلي.

وتم عزل الكلوستريديا سورديلي في المجموعات الثلاثة بنسبة ٢٤.١٪ و ٨٣٪ و ٥٪ بالمقارنة بميكروبات الكلوستريديم الأخرى وتم عزل وتصنيف ميكروب كلوستريديم بيرفريجينز وبيوروجنز وبيفيرماننز في نسب مختلفة وتم عمل اختبار الحساسية لعزولات كلوستريديم سورديلي وكان الهدف من الدراسة الفاء الضوء على أهمية ميكروب كلوستريديم سورديلي كمسبب للموت المفاجيء في الحملان.