

Isolation of Clostridium species from abomasal lesions of sheep

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Abstract

This is the first study in Iraq aimed to isolate and identify of Clostridium bacteria from the abomasal lesions of sheep.

One hundred abomasal samples collected from slaughtered sheep in slaughterhouse of Al-Qasim city, Babylon governorate in Iraq. abomasal lesions was detected, swabs taken from lesions for bacterial isolation.

Nodules, ulcers and hemorrhage lesions were found in abomasa exam as well as parasites. Direct smear from ulcers showed presence of large gram positive bacilli in large numbers and nodules have been given the higher percentage of bacterial isolation more than from ulcers.

Fourteen isolates (14%) was *C. perferengens* as six isolates from ulcer (42.8%) and eight isolates from nodules (57.14%), while *C.sordelli* only four isolates were found in ulcers at 4%. In this study concluded that *Clostridium spp.* which considered the primary causes of abomasal lesions.

Key words: *Clostridium perfringens*, *Clostridium sordellii* , abomasum ,sheep.

من افات المنفحة في الاغنام Clostridium species عزل انواع من جرثومة المطثيات

الخلاصة:

تعد هذه الدراسة الاولى في العراق تهدف إلى عزل وتحديد انواع من جرثومة المطثيات من الافات المنفحة للاغنام.

جمعت مئة عينة منفحة من الاغنام المذبوحة فيمجزرة من مدينة القاسم محافظة بابل في العراق. تم تحديد الافات، واخذت مسحات لغرض العزل الجرثومي.

الافات التي تم تشخيصها هي العقيدات، والقروح والافات والنزيف بالإضافة الى الطفيليات. المسحات المباشرة من القروح اظهرت وجود عصيات كبيرة ايجابية لصبغة كرام. اعطت المسحات المباشرة المأخوذة من العقد تواجد جرثومي مقارنة بالقروح.

تم عزل 14 عزلة (14%) من جرثومة المطثيات الحاطمة *Clostridium perfringens* ستة عزلات من القروح (42.8%) وثمانية عزلات من العقيدات (57.14%)، في حين وجد أربعة عزلات فقط لجرثومة *Clostridium sordelli* في القروح (4%). يستنتج في هذه الدراسة إلى أن جرثومة المطثيات تعد من الأسباب الرئيسية للافات المنفحة.

Introduction

Drake *et al.*(1) who reported that abomasums are divided into three glandular regions which included: cardiac region, the body or fundic region and the pyloric region that has

pyloric or antral glands. The body region, which is characterized grossly by spiral mucosal folds which protrude into the lumen and run caudally toward the pyloric region,

presents about 90% of the surface area of the abomasums.

The reticulorumen microbes include numerous species of bacteria, protozoa, fungi and archaea, and are dominated by bacteria. The predominant bacterial species in the rumen of sheep belong to genus *Bacteroides* and the phylum firmicutes, particularly *Clostridium* and *Prevotella* (2).

Many studies were done for elucidating the increase number of bacterial population in abomasums, Lawton *et al.* (3) mentioned that the role of parasitic infestation of abomasums which increased the present of bacteria in the hyperacidic abomasum may affect gastrin secretion. Elevated of abomasal pH is a good condition for growth many bacteria. Also Simpson (4) reported that anaerobic bacteria survive in greater numbers as the pH rises. Failure to lyse bacteria may affect adversely the nutrition of the host. The parasites may initiate the pathophysiology through the release of excretory or secretory products which either act directly on parietal cells or indirectly through enterochromaffin-like cells by provoking inflammation or by disrupting the protective mucosal defense system.

Many types of *Clostridium* species were isolated from Abomasal lesions, from ulcer, *Clostridium perfringens* types A and E (5) and *Clostridium sordellii* (6).

Clostridium is a genus of gram-positive, rod shaped and arranged in pairs or short chains., catalase and oxidase negative, spore-forming bacteria belonging to the phylum of Firmicutes, family Clostridiaceae. There are close to 200 species of *Clostridium* (7).

Clostridium bacteria have a specific characteristics which capacity

to form heat resistant spores and their intolerance to oxygen being the principals. Different species still require specific growth conditions, some are psychrophilic while other are mesophilic or even thermophilic (8).

Radostitset *al.* (9) mentioned than cause of abomasitis in sheep is *C. sordellii* which appears to be an emerging pathogen for sheep in Great Britain and possibly Europe in association with a syndrome of abomasitis and sudden death in young lambs and adult sheep. The isolation of *C. sordellii* from abomasum lesions of lambs and the confirmation of the isolates by polymerase chain reaction (PCR) was done by Akan *et al.* (6). A total of 298 abomasal specimens (swabs) obtained from lambs of 4 to 8 months of age were investigated. Due to lack information in the Closteridial caused of abomasitis, so that the study was aimed to isolation of *Clostridium* species bacteriums from abomasal lesions.

Material and methods

One hundred abomasal samples collected from slaughtered sheep in slaughterhouse of Al-Qasim city, Babylon governorate in Iraq. They included males and females with age range between 3.5 months and 3 years. At slaughter house. abomasal samples collected immediately after slaughter, after take out the contents of the stomach and intestines of the carcass, by a clean knife, abomasum separated from its anterior part associated with omasum and its posterior part with small intestine, and which linked in both ends, and each sample is placed in nylon bags and transported immediately by ice box into the Laboratory Graduate Studies at the College of Veterinary Medicine, University of Al-Qadissiya

Two techniques were used for isolation of bacteria from abomasal lesions. Direct Swab from ulcerated lesion, while from nodulated lesion swabs taken after clean and washed nodules with distilled water. Then nodules opened by sterile needle open, after that swab collected.

Direct smear was taken from abomasal lesions after opening the sample, smear impression were done from lesions and which stained by Grams stain and exam under light microscope.

Bacterial culturing:

Swabs from abomasal lesions inoculated in cultural medium which included, sheep blood agar, Columbia blood agar, cooked meat broth, brain heart infusion broth and nutrients broth. All media were incubated either flushed with anaerobic gases or put in anaerobic jar provided with gas pack in 37°C for 48hrs and mentioned the growth, if there is no growth the tubes leaved for seven days in incubates, then examined for growth (10).

Anaerobic bacteria culture methods :

Many types of anaerobic methods were used for culturing of bacteria in current study which includes: Anaerobic Jar provided with Gas Pak Anaerobic System. The gases kit which provided nitrogen, carbon dioxide and hydrogen gases that essential for growing many strict anaerobic bacteria. Anaerobic screw tube methods, this best anaerobic method was showed in current study for culturing anaerobic bacteria with flushed with mixture gases, as well as anaerobic culture by use olive oil. This method was mentioned by Eyre (11) for increasing anaerobic condition and prevent further the entry of oxygen for cultural medium specially broth

medium, added 1-2 cm of sterile olive oil and incubated anaerobically.

All morphological features of colony growing were documented (types of growth, color, size and shape of colonies and hemolysis). Sub culture of bacteria, for increase of purity of the bacteria sub culturing were done on sheep blood agar, Also used m-CP Agar Base for *Clostridium perefrens*.

The growth bacteria were identified as follow, Gram' s stain: Spore stain by malachite green, Biochemical tests: Catalase test, KOH test, Oxidase test, Indole test, Nitrite test, Urease test, Litmus skim milk test, Esculin hydrolysis, Gelatin hydrolysis, Motility test, Lecithinase production test, Chopped Meat Medium.

Statistical analysis:

Data was analyzed using Chi-square test was applied for the statistical analysis of the data (12).

Results

The total abomasal lesions were found at percentage 56%, which divided in to three types of lesions were detected during current study, as well as, presences of parasites which are clearly appeared. the highest rates of lesions were found in abomasums which are nodules at proportion (48.23%) followed by ulcers are (23.52%), also parasites showed in proportion (17.64%) and lowest lesion was recorded is hemorrhage (10.58%) Direct smear was directly taken from abomasal lesions, from nodules, many types were detected in different shapes. Grams positive and negative bacilli was found in nodules, as well as, spirals and wing gull bacterium were showed. also some nodules have cocci and coccobacilli bacteria.

Direct smear From ulcers also showed presence large gram positive bacilli in large numbers.(figure, 4-1).

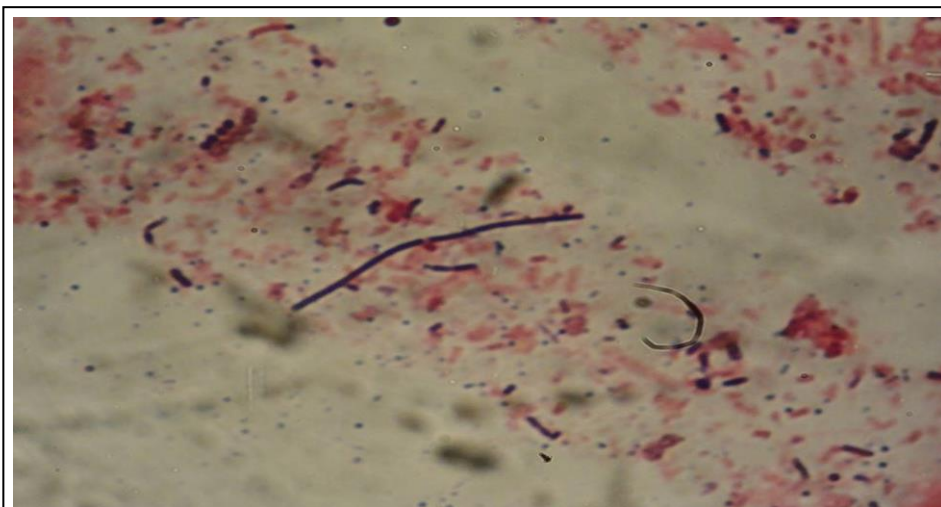


Fig.(4-1). Direct smear from ulcer shows large Grams positive bacilli bacteria)×100.

During the current study, two species of *Clostridium* were isolated from ulcers and nodules of abomasum. The higher percentage of bacteria isolation were found in nodules more than ulcers. Fourteen isolates (14%) was *C. perferengens* as six isolates from ulcer (42.8%) and eight isolates from nodules (57.14%),while *C.sordelli* only four isolates were found in ulcers at 4%.

Growth condition of a bacteria are strict anaerobe, All isolates of *Clostridium periferngensis* gram positive large bacilli form short chains in blood agar and single bacilli in broth medium, no showed spore forming during studies in ordinary medium(Fig,4-1). Growth good on sheep blood agar with double zone of hemolysis, and it is gave cylindrical growth on nutrient agar. Growth on broth medium concentrated at bottom. Growth on m-CP Agar BaseFormula, (Tryptose medium) showed pin point colony of clostridium change the medium from blue to yellow color.

The almost of isolates on sheep blood agar have been given continuous growth on the line of culturing, mucoid and it change became rough colonies.

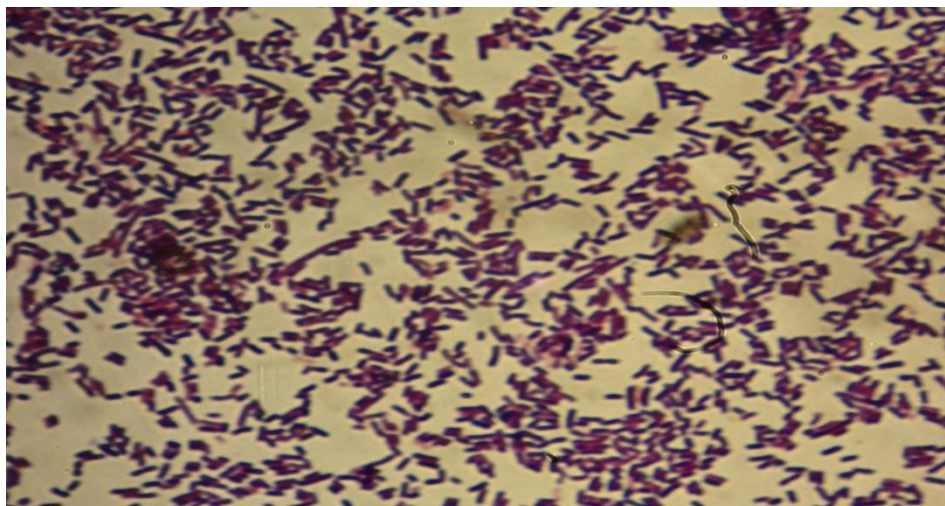


Fig.(4-2):Grams positive bacilli (*Clostridium perferngens*) Gram-stain $\times 100$.

No reaction were exhibited for catalase and oxidase in exam isolates, negative results for 3% KoH test. All isolates of bacteria gave negative results for indole test, while giving positive results for nitrate reduction and gelatin hydrolysis, some strain of bacterium are hydrolyzed of esculin, in skim milk test showed coagulate and formation of gas. Although bacteria give cylindrical growth on nutrient agar but no motility were showed. No growth was showed on bile esculin agar, all isolates gave positive results for lecithenase test.

Results of carbohydrate fermentation were showed fermentation for glucose, lactose, maltose, sucrose and glycerol. The bacterium is no fermented mannitol, xylose and arabinose but for salicin some isolates fermented.

Clostridium sordelli only isolated from ulcer is 4 isolates(4%). No isolate were detected in other lesions. After 48 hours of incubation, gray-white colored, convex, rough colonies were showed on cultural media, Slight haemolysis were observed on sheep blood agar. Also growth on broth media and cooked meat agar were good observed. The bacterium is Gram positive, cylinder-shaped bacilli in short chains. Central spores were observed(Fig.4-6). No growth were observed in aerobic condition.

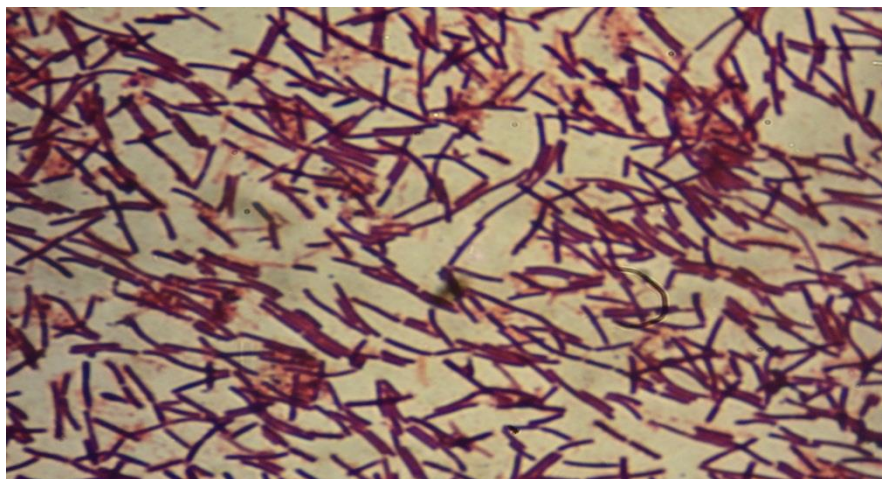


Fig.(4-3):Grams positive bacilli (*Clostridium sordelli*) Gram-stain $\times 100$.

Like *Clostridium perfringens*, it is gave negative results for catalase and oxidase in exam isolates, negative results for 3%KOH test. All isolates of bacteria gave positive result for indole test and gelatin hydrolysis, while give negative results for nitrate reduction and esculin hydrolysis, coagulation and digestion with no formation of gas observed in skim milk test. No growth was showed on bile esculin agar, all isolates gave negative results for lecithinase test.

Carbohydrate fermentation are showed positive for glucose, maltose, and glycerol. The bacterium is no fermented lactose, mannitol, sucrose, xylose, arabinose and salicin.

Discussion

In Iraq, little information and was no expanded study was available for detecting the bacterial causes of abomasal lesions in ruminant. While the studies was excepted on parasitic causes and their effect on abomasal mucosa.

The lesions were recorded in abomasum of sheep are nodules

(48.23%), ulcers (23.52%), parasites (17.64%) and hemorrhage (10.58%).this results of current study differed with the study was conducted by Khodakaram-Taftiet *al.*(13) on prevalence of abomasal abnormalities in sheep, in Iran, whose found that ulcers formed 75% and nodular lesions are 79%, while Jassim and Alkhaled (14)found five abomasal infections in sheep, the lesions were predominantly ulcers and parasites with few being nodules, hemorrhage and a few thickness. Ulcers (21.4%), parasites, (21.4%), mild to severe hyperemia (8.9%), thickness of abomasal wall was(10.1%)nodules was (12.5%) these results compatible with result of our study excepted percentage of abomasal nodules have lowest than in current results.

Other study conducted by (15) in abomasal lesions of cattle found many lesions in abomasums are ulcer and erosions, (10.97%), hyperemia (9.7%), parasites (5.28%),lower than found in the current study.

In present study the success of the method was used for isolation of anaerobic bacteria from abomasal lesions was by screw glass tubes which allows very good anaerobic conditions to be continuously maintained and to the use of a pre-reduced medium with a composition similar to that of the natural environment of the organisms. This results are compatible with researchers Griswold *et al.*, (16) ; Guo *et al.*, (17) they mentioned that many ruminal bacteria are strict anaerobes that require special technique for culturing. Also (18) said that main source of abomasal bacteria is rumen and it localized in abomasal mucosa when there are appropriate conditions, change of abomasal pH and parasitic infestation. (4); (19) mentioned that anaerobic bacteria survive in greater numbers as the pH rises, in parasitized sheep with bacterial densities becoming similar to ruminal populations at an abomasal pH of 4 and above. the viable anaerobic bacterial populations are similar to those in rumen fluid. Failure destruction of bacteria by acidic abomasum may affect adversely the nutrition of the host.

Stiles *et al.*(20); Hamouda *et al.*(21) mentioned that the *Clostridium* genus represent ubiquitous bacilli commonly found in soil, water, and gastrointestinal tracts of insects and animals, as well as humans. This genus grow in low-oxygen environments; however, the clostridia are better adapted for anaerobic life with varying aerotolerance among different species. *Clostridium perfringens* produces

enteric diseases in cattle, sheep and goats.

Many studies was done for isolation of *Clostridium perfringens* from abomasum in ruminants from lesions and healthy specimens. (22) isolated *Clostridium perfringens* type-D from calves affect with ulcerative abomasitis, which these calves examined in Veterinary School, Minas Gerais Federal University, Brazil. Also Roeder *et al.* (5) isolated two types of *Clostridium perfringens* which are A and E from ulcers and erosions of abomasum of calves. Also *Clostridium perfringens* was isolated from Belgian blue calves suffering from braxy-like disease with sudden death and acute hemorrhagic abomasitis, in good condition with no clinical signs noted a few hours prior to death (23), and (24) mentioned that *Clostridium perfringens* is a common inhabitant of the avian and mammalian gastrointestinal tracts and can behave commensally or pathogenically. Some enteric diseases caused by type A. *Clostridium perfringens*, including bovine Clostridialabomasitis, remain poorly understood.

Valgaeren *et al.*(25) isolated *Clostridium perfringens* from healthy abomasa and abomasal ulcers of calves this same results of current study, the bacterium was isolated from many lesions of abomasum.

Mortimer and Ellis(26) found that infected rate by *Clostridium perfringens* in examined calves as following, abomasitis is 3.2% and abomasal ulcer 4.3% this results agreement with results of present study

which ensured that *Clostridium perfringens* causes abomasal ulcer and abomasitis.

Mills *et al.*(27) put out the main bacterial etiology of abomasal ulcer in cattle, who found the two main bacteria in ulcers are *Clostridium perfringens* and *Campylobacter jejuni*.

Ghorbanpoor, *et al.*(28) studied the correlation between abomasal ulcer and presence of *Clostridium perfringens*, whose showed on statistical difference of their bacterium and induced ulcers by isolating it from abomasal contents(3.3%) and about tissues(10%).

Direct smear was taken for diagnosis of *Clostridium perfringens* from lesions was conducted by (29) found that impression smears from affected parts of abomasum and small intestine were taken; fixed and stained routinely with gram. All smears showed clusters of gram positive capsulated bacilli with round ends. Also same results found by (22) mentioned that direct smears important to diagnose *Clostridium perfringens*, whose showed by using Gram stain to direct impression smears of abomasal mucosa has been revealed short, thick, non-sporulated Gram positive rods.

The results of biochemical were compatible with(30), *Clostridium perfringens* is Gram positive bacilli, catalase, oxidase and indole test are negative, negative to 3% KOH test, positive results for nitrate reduction and gelatin hydrolysis, and fermentation of carbohydrates same were found.

Couchman *et al.* (31) elucidated that *Clostridium sordelli* is commonly found in soil and in the intestines of animals, gram-positive, anaerobic bacillus reported as an unusual cause of Clostridial myositis in humans and animals. enteritis, and sudden death in cattle and sheep. It has been associated with malignant edema, myositis, hemorrhagic. Many studies was done for isolation *Clostridium sordelli* from abomasum. (32); (33) were isolated *Clostridium sordelli* from abomasitis and abomasal ulceration in cattle, while(6) diagnosed the *C. sordelli* the first time in Turkey. A rate of 5.7% of *C. sordelli* isolated from abomasal lesions in abattoirs indicates that this agent could cause potential health problems and finally losses in sheep.

Richards and Hunt (34) and Clark(35) identified this organism in cases of bovine abomasitis, who showed changes in abomasum infected by *Clostridium sordelli*, wall of abomasum is thickened due to a combination of emphysema and oedema, erosions and congestion are present in the abomasal mucosa.

Morriet *et al.* (36) reported *C. sordelli* seen in the stomach of the affected animals, bacteria isolated from the drinking water. is ubiquitous and distributed widely in the environment their natural habitats are soil, water, and the gastrointestinal tract of animals and humans.

Lewis and Naylor (37) pointed out that *Clostridium sordelli* can be diagnosis by direct smear was taken from lesions by impression smears from affected parts of abomasum. All

smears showed Gram-positive, rod shaped bacteria capsulated bacilli.

The results of biochemical were done for diagnosis of *Clostridium sordelli* is agreement with (32); 38) who found the bacterium gave negative results for catalase and oxidase, positive result for indole test and gelatin hydrolysis, while give negative results for nitrate reduction, esculin hydrolysis and lecithinase test. And results of carbohydrate fermentation also give same result.

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