

Isolation of aerobic and anaerobic bacteria from suspected enterotoxaemia cases in lambs

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Abstract

Ninety cases of clinically diagnosed enterotoxaemia infection in lambs at AL-Hamdaniya region where studied for isolation of aerobic and anaerobic bacterial causes, faecal samples were collected from all suspected cases during January- June 2008, the results show that 41.6% of the isolates were *Cl. perfringens* as pure single isolates, while mixed infection of *Cl. perfringens* with each of Enterococci and staphylococcus in percentage of 26.04%, 20.83% respectively, also mixed infection of *Cl. septicum* with each of Staphylococcus and *E.coli* were isolated at the percentage of 5.2%, 6.25% respectively. Highest bacterial isolation was from the faecal samples collected during April. McIntosh jar method show isolation of pure culture of anaerobic bacteria (*Cl. perfringens*), while Candle jar method show detection of 56 isolates in mixed cultures of aerobic and anaerobic bacteria.

Keywords: Anaerobic Bacteria, Enterotoxaemia, Lambs.

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عزل الجراثيم الهوائية و اللاهوائية من حالات التسمم المعوي المحتملة في الحملان

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الخلاصة

تم دراسة تسعون حالة اصابة بالتسمم المعوي مشخصة سريريا في الحملان في منطقة الحمدانية لعزل الجراثيم الهوائية واللاهوائية المسببة لتلك الاصابات وقد تم جمع نماذج براز من جميع الحالات المشكوك بها خلال الفترة من كانون الثاني لغاية حزيران ٢٠٠٨، اشارت النتائج الى عزل جراثيم لاهوائية (مطثيات بيرفرنيس) بنسبة ٤١,٦% بشكل مفرد، بينما عزلت جراثيم اخرى من اصابات مختلطة من جراثيم مطثيات بيرفرنيس مع كل من جراثيم انتيروكوكس و جراثيم المكورات العنقودية بنسبة ٢٦,٠٤%، ٢٠,٨٣% على التوالي، وجراثيم المطثيات الانتانية مع كل من جراثيم المكورات العنقودية وجراثيم الاشيريكية القولونية بنسبة ٥,٢% و ٦,٢% على التوالي. وكانت اعلى نسبة عزل للجراثيم من نماذج البراز التي جمعت خلال شهر نيسان. وقد اظهرت طريقة حضن ماكنتوش عزل نقي للجراثيم اللاهوائية لمطثيات بيرفرنيس، بينما امكن الكشف بطريقة ناقوس الشععة عن ٥٦ عزلة من اصابات مشتركة لجراثيم هوائية واخرى لاهوائية.

Introduction

Animal diseases cause enormous economic loss through mortality, inefficient production and increase in the stock replacement rates, which all require additional resources. Control and treatment of the disease also contribute to the

losses. Sheep and lambs are threatened by a number of infectious diseases, among which enterotoxaemia is believed to be the most important disease resulting in heavy economic losses (1).

Enterotoxaemia is one of the important diseases and its also called "over eating disease" its not caused by

overeating but its caused by the toxins produced by the bacterium called *clostridium perfringens* and other aerobic bacteria (2).

Clostridium perfringens is an anaerobic producing toxins that will show signs of enterotoxemia and will frequently cause animal death. It is anaerobic and cannot tolerate oxygen and must be cultivated under growth conditions in which oxygen is removed (3).

Clostridium perfringens is found universally in soil and manure, its also present in certain amounts in the intestinal tract, however when animal overeats more than ¾ pounds /head /day this will lead to excessive bacterial growth and allow the bacteria to produce lethal amounts of toxin which was absorbed into the animal systems (4,5).

Enterotoxaemia can be caused by anaerobic or sometimes aerobic +ve and gram –ve bacilli and cocci (6).

Materials and methods

In the current study 90 lambs presented with signs of clinically diagnosed enterotoxemia at AL-Hamdaniya region.

Samples

Fecal samples were collected from all suspected cases by sterile cotton swabs and placed in an sterile test tubes containing transported media (stewarts media), then processed at the diagnostic bacteriology laboratory, Department of Microbiology, College of Veterinary Medicine, University of Mosul.

Isolation procedures

Each sample was inoculated onto three blood agar plates, one of them was incubated aerobically. While the others were incubated anaerobically by using two methods of incubations; anaerobic jar (McIntosh jar) with Gas Generating Kit, and candle jar incubation.

Chocolate agar was also inoculated by the sample and incubated in 5-10 % CO₂. MaConkeys agar was also used and incubated aerobically. All plates were incubated at 37°C for 24 hours, with further 24 hours incubation if there was no growth. Cultures for anaerobes were incubated for 48-72 hours (7,8).

Steps in identification

Direct smears were prepared and stained with gram stain and spore stain. All morphological arrangement and its reaction with the stain were studied by using light microscope (8,9).

Biochemical reactions, sugar fermentation tests, bile solubility, starch hydrolysis, gelatin digestion, catalase, lecithinase, indole production and nitrate reduction test, which are the most characteristics tests to differentiate between them (8,10).

Results

It was obvious from table 1, *Clostridium perfringens* was the major offending bacteria which contributed 40 (41.6%) of the total isolates. *Clostridium perfringens* and *Enterococci* represents 25 (26.04%) of the total isolates, in addition to other mixed infection with *Clostridium perfringens* plus *Staphylococcus aureus* contributed 20 (20.83%) of the total isolate while other type of *Clostridium spp.* Includes *Cl. septicum* + *Staphylococcus aureus*, *Cl. septicum* + *E.coli* which contributes 5 (5.20%), 6 (6.25%) respectively. Regarding to the negative result which includes 12 cases only.

Anaerobic bacteria (*Clostridium spp.*) was diagnosed depending on cultural characteristics and biochemical reactions with sugar fermentation table 2.

Table 1: showed percentage of anaerobic isolates from enterotoxemic cases.

Bacterial isolates	Number of isolates	% of isolates
<i>Cl. perfringens</i>	40	41.6%
<i>Cl. perfringens</i> + <i>Enterococci</i>	25	26.04%
<i>Cl. perfringens</i> + <i>Staph.aureus</i>	20	20.83%
<i>Cl. septicum</i> + <i>Staph.aureus</i>	5	5.20%
<i>Cl. septicum</i> + <i>E.coli</i>	6	6.25%
Negative result	12	10.8%
Total no.	96	100%

Table 2: Biochemical test results used for identification of *Clostridium spp.* from enterotoxemic cases.

Biochemical test	Type of bacteria	
	<i>Cl. perfringens</i>	<i>Cl. septicum</i>
Gelatin liquefaction	+	+
indole	-	-
lecithinase	+	-
glucose	+	+
maltose	+	+
lactose	+	+

(+) positive test, (-) negative test.

This study also included percentage of infection in enterotoxemia in lambs in Al-Hamdaniya region in 6 months in 2008. As in table 3, its obvious that increase in infection percentage in January, February and April which contributed 90.9%, 91.6% and 92.6% respectively, while this study showed decreased in infection in March, May and June which constitutes 80%, 62.5% and 80% respectively.

Table 3: The percentage of bacterial isolates during 6 months in 2008.

Month	Total number of samples	Positive results	%of infection
*January	11	10	90.9%
*February	24	22	91.6%
March	10	8	80%
*April	27	25	92.6%
May	8	5	62.5%
June	10	8	80%
Total number	90	78	86.6%

*Increased enterotoxemic cases in these months.

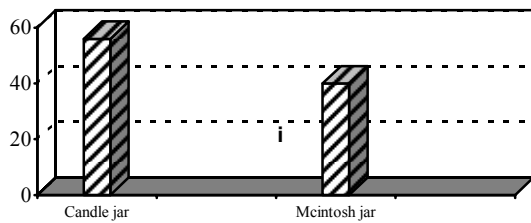


Figure 1: The efficient methods for detecting anaerobic bacteria using the Candle Jar and McIntosh Jar, incubation method.

This figure illustrates the efficiency of McIntosh Jar which was used for isolation of pure culture of anaerobic bacteria which constitutes 40 isolates including *Clostridium perfringens*, while other method (candle jar) detects 56 isolates in mixed cultures (aerobic and anaerobic bacteria).

Discussion

Enterotoxemia, or overeating disease, is a major killer disease of lambs from shortly after birth through the entire feeding period. Its characterized by acute indigestion, convulsion and other nervous system signs, colic and sudden death. It most commonly affects single lambs, nursing ewes that are heavy milk producers and feeder lambs on high energy diets (10).

These can be used as clinical clues for presence of anaerobes like *Cl. perfringens* which is an anaerobic bacteria that resides in soil and manure and forms spores that are highly resistant to disinfectants. This bacteria enters the body through mouth from contamination teats, the bacteria grow rapidly and produce toxin, which cause rapid death (11-13).

Out of 90 cases investigated enterotoxemic lambs, *Cl. perfringens* was showed a higher percentage which

included 41.6% that may represents the most common cause of enterotoxemia in lambs, this result was in agreement with other ones (14-16) which represents 66.5%, 84.6% and 80% respectively, while other workers (17,18) who isolated *Clostridium perfringens* in lesser percentages 38%, 39% consequently.

As in table 1, the isolation of *Clostridium perfringens* grow also mixed with other bacteria like Enterococci, *Staphylococcus aureus* that representing 26.04% and 20.83% respectively. This mixed infection may be due to presence of this type of bacteria normally in the intestine or due to contamination from the surrounding environment (19,20).

Depending on the obtained cases (samples), the average of infection during 6 months in 2008 between January and June is 86.6% as show in table 3, this result is higher than that recorded by (21) which was 73.37%. The percentage of infection was increased in January, February and April 90.9%, 91.6% and 92.6% respectively and that result was consistent with (21) which showed increased in infection between January, February and especially in April 26.5%, 77.27% and 80.95% respectively, this difference may be due to changing in weather and diet from dry to wet one (22).

It was obvious from Fig. 1, Mixed isolates (aerobic and anaerobic) were dominant results that encountered 56 isolates, while pure isolates were contributed 40 isolates. This variation in results may be due to clinical circumstances and culture method were employed, also further studies are needed to improve the real pathogenic causes of enterotoxemia as toxin production isolates, as well as experimental infection trials.

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