

Detection of Bacterial Contaminant of Newborn Calves and Their DOMS Pens

Sarah Jasim Abdulameer¹, Liqaa Hameed Khamees², Zainab Fadhil Rahman³, Ghassan H. Jameel⁴, Anas A. Humadi*⁵

^{1,3,4} Department of Microbiology, College of veterinary Medicine , University of Diyala, Iraq

² Department of veterinary and Animal Health , Ministry of Agriculture, Diyala Governorate, Iraq

⁵ Department of Pathology and poultry diseases, College of Veterinary Medicine, University of Diyala, Iraq

E.mail: anashumady@yahoo.com

Abstract

The aim of this scientific job is to describe reasons for pathogen loading in the environment and ways to reduce this loading and determination of these pathogens . Most pathogens are opportunistic exploit the contamination of pens bed and hutches by different types of calves secretions and inflammatory exudates from the uterus and mammary gland . The large isolated pathogens were *Escherichia coli* and *Staphylococcus aureus* and one isolate represent a genus *Klebsiella* . The main drugs were more active against these bacteria after the sensitivity test has been done ; Enrofloxacen , Flurphenicol, Colistin Sulfate and combination of Sulfadiazine Vs Trimethoprim. Amoxicillin , Ampicillin and Tetracycline were less active.

In recommendation, good ventilation, exposure to sun light and proper disinfection of the pens will share in decrease the rate of infections when occurred through indirect contact. Sensitivity test has been applied from time to time for passing the drug resistance.

Keywords: pens contamination, calves diseases, *Escherichia coli* , *Staphylococcus aureus* , *Klebsiella*.

INTRODUCTION

The feed ,water, equipment used for handling and distributing feed and water, materials and equipment used for cleaning, and the physical housing environment which provides refuge are considered as calf's environment . Calf health is directly influence by some attributes of the environment like ventilation and impact behavior of individual and group housing figure(1). The good or bad management of the environment has a significant influence on the calf health or disease (Bartels *et al.*, 2010).

Animal health is the major reason to manage the load of an pathogen within the calf environment, but it is important to refer to the some implications correlated with some calf pathogens with public health of employees and the community. Specific pathogens are familiar and associated with diarrheal disease should be infect calves are includes *Escherichia coli*, *Salmonella*, Rotavirus, Coronavirus, *Campylobacter*, Cryptosporidia and Coccidia. Occasional cases of diarrhea may be due to *Giardia* spp or *Clostridium* spp (Hammit *et al.*, 2008). Nielsen *et al.*, (2002) were refer to many pathogens can persist in the environment for weeks to years leading to some colonization and causes some diseases if not properly managed.

Through transporting of the calves to the housing area, some potential pathogens are being introduced from housing area containing adults to the calf environment. Often calves arrive from multiple sources to ranches and could be carry a variety of pathogen types with

different strains come from exposing to the environmental changes from time to time (Wray *et al.*, 1990a).

Escherichia coli is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium that is commonly found in the lower intestine of warm-blooded organisms [Singleton,(1999) ; Tenaillon *et al.*,(2010)]. Some serotypes of *E. coli* can cause serious food poisoning in their hosts but, most strains are harmless , and occasionally are responsible for food contamination (Vogt and Dippold ,2005). Fecal substances are considered as the main source of *E. coli* into the environment. Three days enough to grow massively in fresh fecal matter under aerobic conditions , but it is decline in numbers slowly with time (Russell and Jarvis , 2001).

Enterotoxigenic *E. coli* caused diarrhea in newborn calves and the disease considered as an infectious bacterial disease and harmful . Special attributes of virulence that *E. coli* had been carried and allow them to colonize the small intestine and produce an enterotoxin which stimulate the internal epithelia of the intestine to secret high quantity of fluid into the intestinal lumen. These bacterium are shed onto the house floor by infected animals in the herd and are ingested by newborn calves with the contaminated feed and water (Bartels *et al.*,2010). Dehydration, strong ion acidosis, and electrolyte abnormalities are frequently developed after occurrence of diarrhea result in , a state of negative energy balance followed by signs of weakness ,imbalance, recumbency, shock and death (Smith, 2009).

Staphylococcus aureus is a Gram-positive round-shaped bacterium, and is a usual member of the microbiota of the body, sometimes isolated from the upper respiratory tract and the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen (Masalha *et al.*,2001). It produces a multiple virulence factors as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies and inhibit immunity result in promote infections (Tong *et al.*,2015).

Klebsiella is a genus of a group of rod-shaped bacteria of the family Enterobacteriaceae .They are gram-negative, facultative anaerobic, nonmotile bacteria . They found in the soil and water surfaces and on plants, and some strains are considered as a part of the normal flora of the human and animal gastrointestinal tract (Podschun and Ullmann, 1998) ; Rogers,2022). Struve and Krogfelt (2004) were refer to the species *Klebsiella pneumoniae* is an important opportunistic pathogen and is transmitted from infected animal to health through direct contact and mostly infect the urinary and respiratory systems . Podder *et al.*,(2014) and Davidson *et al .*, (2015) were proved that *K. variicola* is caused bovine mastitis like other pathogenic bacteria.

MATERIALS AND METHODS

This study was conducted for four months a period from 1 January 2022 to 30 April 2022. Ten pens were included in this job contains from 4-5 cows and their calves have been bred in open system. Swabs had been taken from the contaminated bed from diarrheic calves and discarded milk from cows infected with mastitis figure (1 and 2). Different steps were taken for isolation and identification of the pathogens as:

1. Thirty fresh samples were taken from the anus of infected calves and from the area around and kept in a sterile container until used.
2. Bacteriological analysis and preparing of cultivation procedures to all the samples were done to isolate the bacteria by using different types of nutrient agars.
3. All procedures of sensitivity test were done on Mueller Hinton Agar by using different types of common drugs.

RESULTS

Contaminated pens with different types of pathogens lead to multisource diseases in newborn calves in addition to economic losses, so the application of public health routes result in good breeding and gain moderate benefits.



Figure (1): represent the contamination of the floor with different types of animal secretions.

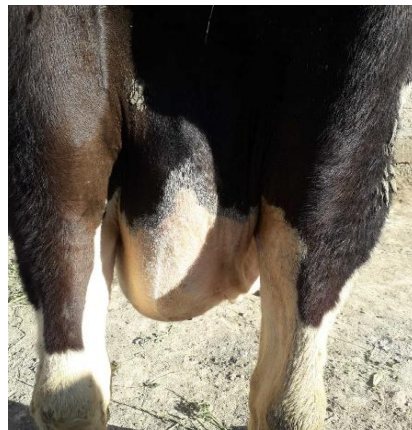


Figure (2): Represent cow infected with unilateral (at left side) mastitis.

The figures (3,4 and 5) were revealed the shapes of different isolated bacteria from the infected animals and contaminated floor of the pens with animal secretions.



Figure (3) : revealed *Escherichia coli* colony on MacConkey agar



Figure (4) : revealed *Staphylococcus aureus* colony on Mannitol salt agar.



Figure (5) : revealed *Klebsiella* colony on MacConkey agar

The figures (6, 7 and 8) were revealed the effect of different types of drugs to each isolate.



Figure (6): Revealed the effect of different types of drugs on *Escherichia coli* Bacteria determined with zone of inhibition.



Figure (7): Revealed the effect of different types of drugs on *Staphylococcus aureus* Bacteria determined with zone of inhibition.



Figure (8): Revealed the effect of different types of drugs on *Klebsiella* Spp. determined with zone of inhibition.

The table (1) was revealed the types of isolated Bacteria (positive) and negative isolates.

The number of samples	<i>E. coli</i> (+ve)	<i>Staphylococcus aureus</i> (+ve)	<i>Klebsiella</i> spp. (+ve)	Negative Isolates
30	17	4	1	8

The table (2) was revealed the types of drugs used in the experiment and their effects determined by the diameter of zoon of inhibition.

Table 2: Revealed the isolated *Escherichia coli* and its sensitivity to common drugs.

Type of isolated Bacteria	Antibiotics	Abbreviate	Disc potency	Diameter of zoon of inhibition	Rate of resistance %
<i>E. coli</i>	Tetracycline	TE-10	10 mcg	14 mm	-
	Ampicillin	AMP	10 mcg	14 mm	-
	Amoxicillin / Clvaulanic acid	AmC-30	20/10 mcg	17 mm	-
	Enrofloxacin	No1	10 %	30 mm	-
	Flurphenicol	No2	10%	36 mm	-
	Colistin Sulfate	No3	0.05 IU	35 mm	-
	Sulfadiazine / Trimethoprim	No4	200 / 40 mg	27 mm	-

Table -3: Revealed the isolated *Staphylococcus aureus* and its sensitivity to common drugs.

Type of isolated Bacteria	Antibiotics	Abbreviate	Disc potency	Diameter of zoon of inhibition	Rate of resistance %
<i>Staphylococcus aureus</i>	Tetracycline	TE-10	10 mcg	zero	100%
	Ampicillin	AMP	10 mcg	10 mm	-
	Amoxicillin / Clvaulanic acid	AmC-30	20/10 mcg	14 mm	-
	Enrofloxacin	No1	10 %	20 mm	-
	Flurphenicol	No2	10%	33 mm	-
	Colistin Sulfate	No3	0.05 IU	27 mm	-
	Sulfadiazine / Trimethoprim	No4	200 / 40 mg	23 mm	-

Table -4: Revealed the isolated *Klebsiella* Spp. and its sensitivity to common drugs.

Type of isolated Bacteria	Antibiotics	Abbreviate	Disc potency	Diameter of zoon of inhibition	Rate of resistance %
<i>Klebsiella</i> Spp.	Tetracycline	TE-10	10 mcg	zero	100%
	Ampicillin	AMP	10 mcg	zero	100%
	Amoxicillin / Clvaulanic acid	AmC-30	20/10 mcg	zero	100%
	Enrofloxacin	No1	10 %	34 mm	-
	Flurphenicol	No2	10%	33 mm	-
	Colistin Sulfate	No3	0.05 IU	26 mm	-
	Sulfadiazine / Trimethoprim	No4	200 / 40 mg	22 mm	-

DISCUSSION

Livestock feces can be considered as an important potential source of pollution by pathogenic bacteria with their environmental resistance genes (ERGs). Continuous use of antibiotics without programming or specific diagnosis to the pathogen will predispose to antibiotic pollution through feces, and promotes the accumulation and spread of pathogenic bacteria and ERGs in the environment (Zhu *et al.*, 2013; Wang *et al.*, 2017).

Three pathogenic *Bacteria* as *E. coli*, *Staphylococcus aureus* and *Klebsiella* spp. were isolated from the infected animals and contaminated areas of pens. Proper coat of calves flooring correlated with the types of good or bad breeding because it is indirectly responsible for transmission of some infections through feed contamination. These results agree with Gulliksen *et al.* (2009) study whom refer to the type of floor which facilitates the cleaning and disinfection as solid concrete and smooth rather than the slatted flooring.

E. coli and *Salmonella* are the common bacteria which are responsible for calf scour in addition to some parasites and viruses. Dehydration is the main cause of death of infected calves after the progression of shock (Smith, 2009). Weak nursing and dehydration of infected calves would lead to weakness followed by staggering gait and may result in sudden death (Gould, 2014).

E. coli is always present in the intestinal tract and is usually the agent that causes a secondary infection following viral agents or other intestinal irritants. *E. coli* scours is characterized by diarrhea and progressive dehydration. Death may occur in a few hours before diarrhea develops (Wani *et al.*, 2004).

The result in table (1) revealed the effect of different types of drugs on *E. coli* and determined by the zone of inhibition, so all drugs were effective but the Flurphenicol, Colistin Sulfate and Enrofloxacin were more effective than others as the combination of Amoxicillin Vs Clvaulanic acid and Ampicillin.

Eibl *et al.* (2021) were authorized that Quinolones, sulfonamides and penicillins were more effective in treatment of calf diarrhea rather than the others. Fluoroquinolones, sulfonamides, and penicillins were the most frequently specified classes for the antibiotic treatment of diarrhea in newborn calves (Zwald *et al.*, 2004; Berge *et al.*, 2005).

This outcome is some time similar or differ to previous studies because, it is depend upon the sensitivity of microorganism and misuse of the drugs.

Regarding to the *Staphylococcus aureus* Bacteria; it is mostly responsible for clinical bovine mastitis. Physical, chemical and microbial changes occurred in bovine mastitis which is characterized by the inflammation of mammary gland and lead to pathological changes in the mammary tissue. The main causative agents in cattle responsible for mastitis are staphylococci and streptococci. *Escherichia coli* and *Streptococcus uberis* are originating from the cow's environment and becoming important causes of clinical mastitis. A significant reduction in productivity, affecting both the quality and quantity of milk after each udder infection and also causing culling of animals at an unacceptable age. Feeding practices includes (the sort of the feed and the quality), animal husbandry, hygiene and general health care are shared in reducing the incidence of udder infections. Proper antimicrobials which are used in treating udder infection can assist to eliminate or at least decrease the incidence the disease (Kumar *et al.*, 2020).

The results in table (2) were refer to the effect of different types of drugs; the Flurphenicol, Colistin Sulfate, Sulfadiazine Vs Trimethoprim and Enrofloxacin were more effective than others as the combination of Amoxicillin Vs Clvaulanic acid and Ampicillin. The Tetracycline is hasn't any effect on the isolated *Staphylococcus aureus*. MacDiarmid (1978) was refer to the drugs were considered as the more common effective against *Staphylococcus aureus* includes penicillins, aminoglycosides and macrolides; oxytetracycline, chloramphenicol, trimethoprim, and several sulphonamides.

Pyörälä (2009) was suggest Penicillin G in treatment of clinical mastitis caused by *Staphylococcus aureus* beside the alternative drugs as Cloxacillin Macrolides and Lincosamides and this result came in agree with our private study.

Regarding to *Klebsiella* Bacteria, one isolate from 22 positive isolates and might be associated with respiratory infection in newborn calves and mastitis in cows. Eight negative isolates might be due to viral infection or overtake of milk nursing.

In recommendations, calf scour is a clinical sign of different types of diseased animal were reflect diarrhea with or without bad smell and with or without pathogenic microorganisms. In order to prevent the spreading of diseases between the animals in closed breeding system we must kept the pens clean, wide, and separation of infected animals from healthy in addition to prevent mixing between small and adult animals.

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