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Aerobic bacteria associated with calf pneumonia in dairy farms in Bahri Locality, Sudan

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KEYWORDS

Bahri Locality
Calf pneumonia
Dairy calves

ABSTRACT

This study was aiming at: 1. Isolation and identification of aerobic bacteria implicated in dairy calf pneumonia in Bahri locality of Khartoum State (Sudan). 2. General evaluation of the housing condition, type and hygiene level and diseases of dairy calves. Eighty naso-pharyngeal swab samples were collected from untreated dairy calves showing typical signs of pneumonia. The swab samples were bacteriologically examined and 83 bacterial isolates were found. The identified bacteria were: 15 *Staphylococcus aureus* (18.2%), 7 *Staphylococcus epidermidis* (8.4%), 4 *Staphylococcus chromogenes* (4.8%), 9 *Streptococcus pneumoniae* (10.8%), 5 *Streptococcus uberis* (6.0%), 7 *Klebsiella pneumoniae* (8.4%), 13 *Escherichia coli* (15.7%), 11 *Pseudomonas aerogenosa* (13.3%), 4 *Bacillus subtilis* (4.8%), 5 *Micrococcus variens* (6.0%) and 3 *Micrococcus luteus* (3.6%). Gram positive Bacteria represented the higher percentage (59.0%) compared to Gram negative bacteria which represented 41.0% of the total bacteria isolated from nasal swabs. Staphylococci represented the predominant bacteria (31.4%) isolated from naso-pharyngeal swabs compared to other bacteria Streptococci (16.8%), *E. coli* (15.7%), *Ps. aerogenosa* (13.3%), Micrococci (9.6%) *K. pneumoniae* (8.4%) and *B. subtilis* (4.8%). Results of the questionnaire survey in Bahri locality showed that calf pneumonia is one of the main health problems in calves with a prevalence of 45.5%. We conclude the high prevalence of calf pneumonia among dairy calves in Bahri Locality. We recommend feeding colostrum during the first day of birth. Other management factors should not be underestimated.

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INTRODUCTION

Calf diseases that cause morbidity and mortality are the results of complex interaction of the management practices and environment, infectious agents and the calf itself (Wudu *et al*., 2008). Common causes of calf diseases and deaths are diarrhoea, pneumonia, joint problems, umbilical diseases, trauma, congenital abnormalities, nutritional deficiencies, dystocia and other infections (Svensson *et al*., 2003; Singla *et al*., 2013). Calf losses were significantly reduced by introducing new techniques of management including on-time colostrum feeding, housing, feeding and nutrition (Razzaque *et al*., 2009). Pneumonia is a cause of major economic loss for the cattle industry, associated with decreased production, higher levels of mortality and increased veterinary and labour costs. The long-term impact can be equally, if not more, damaging. The fibrosis and loss of functional lung capacity in animals that recover from pneumonia has a negative impact on daily live weight gains. For the beef producer this means a longer finishing time, whilst for those rearing dairy replacements, it means an increase in the age at first calving and the subsequent negative effects this has on production and reproductive performance (Tim, 2007). Pneumonia is inflammation of the pulmonary parenchyma, usually accompanied by inflammation of the bronchioles and often by pleuritis. It is manifested clinically by an increase in the respiratory rate, changes in the depth and character of respirations, coughing, abnormal breath sounds on auscultation, and, in most bacterial pneumonias, evidence of toxemia (Radostits *et al.,* 2007). Pneumonia may be associated with viruses, mycoplasmas, bacteria, or a combination of all three; fungi; metazoan parasites; and physical and chemical agents. The main bacterial causes of pneumonia include: *Pasteurella haemolytica*, *Pasteurella multocida* with or without parainfluenza-3 virus, *Histophilus somnus,* bovine respiratory syncytial virus, bovine herpesvirus 1, parainfluenza-3, adenovirus-1, -2, and -3, rhinovirus, reovirus and *Chlamydia spp*., *Mycoplasma spp*., *Actinomyce arcanobacterium* or *Corynebacterium pyogenes*, *Streptococcus spp*., *Bedsonia sp*., and *Actinobacillus actinoides, Klebsiella pneumoniae, Fusobacterium necrophorus, Trueperella pyogenes, Parachlamydia acanthamoebae* and *Mortierella wolfii* (Peter *et al*, 2017). Zulfekar and Shirin (2012) reported that the most frequent bacteria isolated from cases of pneumonia were *Staphylococcus spp.* and *Pasteurella haemolytica*. Francis and Ameh, (2015) isolated *Staphylococcus aureus, Klebsiella pneumonia, Streptococcus pneumonia, Proteus vulgaris, Pasteurella multocida, Escherichia coli, Corynebacterium spp, Salmonella spp*, and *Enterobacter spp* from cases of pneumonia in Nigeria.

In Sudan Eltigany and Elayis (2021) mentioned that the prevalence of calf pneumonia in Bahri Locality was 8.9%. Abdullatief *et al.,* (2014) reported that pneumonia is the most important cause of calf mortality beside diarrhoea in dairy farms at Khartoum State. Different managemental and environmental factors were reported to affect significantly, calf morbidity and mortality, these include: colostral feeding, housing, calving assistance, production system, herd size, season and hygiene of micro- environment (Shiferaw *et al*., 2002).

This study was aiming at the isolation and identification of aerobic bacteria implicated in pneumonia of dairy calves in Bahri locality, Sudan.

MATERIALS AND METHODS

Source of samples

A total of 80 naso-pharyngeal swabs were collected from untreated dairy calves in Bahri locality showing typical signs of pneumonia during the year 2020.

Sampling procedure

Questionnaires were filled then swabs samples were collected from nostrils of calves showing typical signs of pneumonia and didn't receive any treatment. The collected samples were put in an ice box containing ice and transported to the laboratory of college of Veterinary Medicine University of Bahri. The swabs were placed in tubes containing nutrient broth, incubated at 37C^{o} and examined on the next day. The next day the swabs were removed from the incubator and then cultured, characterized and identified.

Isolation, identification and characterization of bacterial isolates

All media (Oxoid media) were prepared and sterilized according to the manufacturer instructions. For the primary isolation of bacteria, a loop full of the enriched broth was streaked onto blood agar, Mac-Conkey's agar, and nutrient agar using sterile wire loop. The cultures were incubated aerobically at 37ºC for 18-24 hours. Cultures on semi-solid media were examined grossly for colonial morphology and haemolysis on blood agar. Whereas, broth media were checked for turbidity, change in colour, accumulation of gases in CHO media and for sediment formation. One half colony from each plate was used for performing gram staining. Purification was based on the characteristics of colonial morphology and smear. This was obtained by sub culturing of a typical discrete colony on blood agar plate. Pure cultures were preserved on slants of blood agar and egg media at 4ºC.

Biological and biochemical identification of the bacteria

The purified isolates were identified as described by Smith *et al.* (1986) and Barrow and Feltham (2004). The identification included: Gram's reaction, presence or absence of spores, shape of organism, motility, colonial characteristics on different media, aerobic and anaerobic growth, sugars fermentation ability and biochemical tests (staining of smear, catalase test, oxidase test, coaggulase test, oxidation fermentation test, motility test, glucose breakdown test and fermentation of carbohydrates).

Questionnaire survey of dairy farms in Bahri locality

A questionnaire on 11 dairy farms in Bahri locality was conducted before collection of samples. The questionnaire included information about housing type, housing condition, common diseases, availability of veterinary services, calves' health, previous cases of pneumonia and morbidity, mortality and treatment of pneumonia.

RESULTS

Questionnaire survey of dairy farms in Bahri locality

Table 1 shows the analysis of the questionnaire of 11 dairy farms in Bahri Locality illustrated that: 100% of the housing systems were loose corral, 81.8% of the stall surfaces were clay and 18.2% uses concrete surfaces. The general evaluation of the housing condition was good for 54.5% of the farms and poor for the rest. Seventy-two point two of the farms were suffering from Tick-borne diseases and other infections and 45.5% were suffering from mastitis. Veterinary services were available in 90.2% of the farms and hygienic level was poor in 72.2% of the farms and was good in the rest. Calves' health records were available in 36.4% of the farms. Calves diseases in the farms were diarrhoea in 72.7% of the farms, Pneumonia in 54.5% and Tick-borne disease in 45.5%. Colostrum was not presented during first hours of birth in 72.7% of the farms. Vaccination system was not adopted in 72.7% of the farms. Concerning calves' pneumonia 100% of the farms experienced previous cases of the disease. All owners considered that the three first weeks of calves' age are the most hazardous in cases of calves' pneumonia and the risk decreases in older ages. All owners confessed losses of calves due to calf pneumonia and that they adopted different treatment trials of the disease. The majority (54.5%) of the owners used Penicillin for treatment of calf pneumonia and 36.4% used Tetracycline and Enrofloxacin.

Bacteria isolated from nasal swabs collected from Bahri locality

In this investigation a total of 83 bacterial isolates were obtained from 80 naso-pharyngeal swab samples collected from pneumonic calves in Bahri locality. According to the cultural characteristics, bacterial morphology and biochemical reactions results shown in Table (2). The identified bacteria were: 15 *S. aureus* (18.2%), 7 *S. epidermidis* (8.4%), 4 *S. chromogenes* (4.8%), 9 *Str. Pneumoniae* (10.8%), 5 *Str. uberis* (6.0%), 7 *K. pneumoniae* (8.4%), 13 *E. coli* (15.7%), 11 *Ps. aerogenosa* (13.3%), 4 *B. subtilis* (4.8%), 5 *M. variens* (6.0%)

Table 1: Summary of the questionnaire survey of 11dairy farms in different areas in Bahri locality

Unit	Frequency (%)	Unit	Frequency (%)
Housing		**Calves' health**	
Housing type		*Records*	
-Free stall	0 (0%)	-Yes	4 (36.4%)
-Loose corral	11 (100%)	-No	7 (63.6%)
-Stanchion	0 (0%)	*Diseases*	
Stallsurface		-Diarrhoea	8 (72.7%)
-Concrete	2 (18.2%)	-Pneumonia	6 (54.5%)
-Clay	9 (81.8%)	- Tick-borne diseases	5 (45.5%)
-Sand	0 (0%)	*Colostrum during first hours of birth*	
Housing condition		-Yes	3 (27.3%)
-Excellent	0 (0%)	-No	8 (72.7%)
-Good	6 (54.5%)	*Vaccination system*	
-Poor	5 (45.5%)	-Yes	3 (27.3%)
Common diseases		-No	8 (72.7%)
-Mastitis	5 (45.5%)	*Previous cases of pneumonia*	
-Tick-borne diseases	8 (72.7%)	-Yes	11 (100%)
-Other diseases	8 (72.7%)	-No	0 (0%)
Veterinary services		*Population at risk*	
-Yes	10 (90.9%)	-One week old	5 (45.5%)
-No	1 (9.1%)	-Two weeks old	8 (72.7%)
Hygiene Level		-Three weeks old	11 (100%)
-Excellent	0 (0%)	-One month old	10 (90.9%)
-Good	3 (27.3%)	-More than one month old	9 (81.8%)
-Poor	8 (72.7%)	*Losses due to pneumonia per year*	
		-Yes	11 (100%)
		-No	0 (0%)
		Treatment of calf pneumonia	
		-Penicillin	6 (54.5%)
		-Tetracycline	4 (36.4%)
		-Enrofloxacin	4 (36.4%)

and 3 *M. luteus* (3.6%) (Table 3). Gram positive Bacteria represented the higher percentage (59.0%) compared to gram negative bacteria which represented 41.0% of the total bacteria isolated from nasal swabs. Staphylococci represented the predominant bacteria (31.4%) isolated from nasal swabs compared to other bacteria Streptococci (16.8%), *E. coli* (15.7%), *Ps. aerogenosa* (13.3%), Micrococci (9.6%) *K. pneumoniae* (8.4%) and *B. subtilis* (4.8%) (Figure 1).

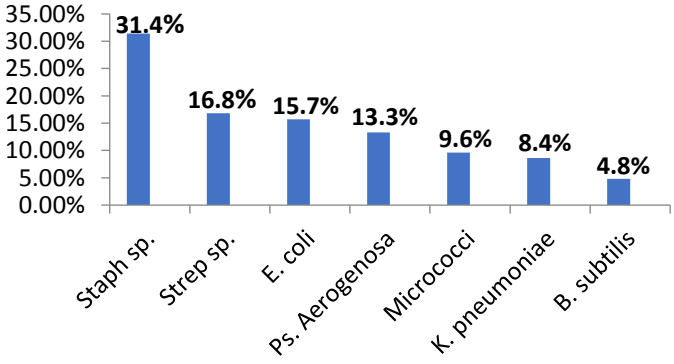

Figure 1: Bacteria isolated from pneumonic dairy calves' naso-pharyngeal swabs collected from Bahri locality.

Table 2: Cultural characteristics, bacterial morphology and biochemical tests of the isolated bacteria.

Test	***E. coli***	***S. aureus***	**Ps. *aerogenosa***	***K. pneumoniae***
Aerobic growth	+	+	+	+
Colonies on MacConkey	Bright Pink	Pink	Bright Pink	Pink
Haemolysis on blood agar	+	+	+	-
Gram reaction	-	+	-	-
Shape	Rods	Cocci	Rods	Rods
Spore	-	-	-	-
Motility	+	-	+	-
Catalase	+	+	+	+
Oxidase	-	-	+	-
Indole	+	-	-	+
Methyl red	+	+	-	-
VP	-	-	-	-
Citrate	-	-	+	+
H_2S	-	-	-	-
O/F	+	+	+	+
Glucose	+	+	-	+
Lactose	+	+	-	+
Coaggulase	-	+	-	-

Table 2 (continued): Cultural characteristics, bacterial morphology and biochemical tests of the isolated bacteria.

Test	***Str. uberis***	***M. luteus***	***M. variens***	***B. subtilis***
Aerobic growth	+	+	+	+
Colonies on MacConkey	Pink	Pink	Pink	Pink
Haemolysis on blood agar	-	+	-	+
Gram reaction	+	+	+	+
Shape	Cocci	Cocci	Cocci	Rods
Spore	-	-	-	-
Motility	-	-	-	+
Catalase	-	+	+	+
Oxidase	-	+	+	-
Indole	-	-	-	-
Methyl red	-	-	-	-
VP	+	-	-	-
Citrate	-	-	-	-
H2S	-	-	-	-
O/F	+	+	+	+
Glucose	+	+	+	+
Lactose	+	+	+	+
Coaggulase	-	-	-	-

Table 2 (continued): Cultural characteristics, bacterial morphology and biochemical tests of the isolated bacteria.

Test	***Str. pneumoniae***	***S. epidermidis***	***S. chromogenes***
Aerobic growth	+	+	+
Colonies on MacConkey	Pink	Pink	Pink
Haemolysis on blood agar	+	-	-
Gram reaction	+	+	+
Shape	Cocci	Cocci	Cocci
Spore	-	-	-
Motility	-	-	-
Catalase	-	+	+
Oxidase	-	-	-
Indole	+	-	-
Methyl red	-	-	-
VP	-	-	-
Citrate	+	-	-
H2S	-	-	-
O/F	+	+	+
Glucose	+	+	+
Lactose	+	-	+
Coaggulase	-	-	-

Table 3: Bacteria isolated from pneumonic dairy calves' naso-pharyngeal swab samples collected from Bahri Locality.

Bacterial species	Number	Percentage
S. aureus	15	18.2%
S. epidermidis	7	8.4%
S. chromogenes	4	4.8%
Str. Pneumoniae	9	10.8%
Str. uberis	5	6.0%
M. luteus	3	3.6%
M. variens	5	6.0%
B. subtilis	4	4.8%
E. coli	13	15.7%
Ps. aerogenosa	11	13.3%
K. pneumoniae	7	8.4%
Total	**83**	**100%**

DISCUSSION

Calf pneumonia is multifactorial disease caused by environmental factors (crowding, humidity, temperature, air quality, stress) and infectious agents (Peter *et al*, 2017).

In this study which lasted for 6 months, 11 dairy farms in Bahri locality of Khartoum State were investigated for the problem of calf pneumonia.

According to the questionnaire survey of dairy farms, the general evaluation of the housing condition was poor for 45.5% of the farms, 81.8% of the stall surfaces were clay and the hygiene level was poor in 72.7% of the farms. According to Radostits *et al.* (2000) finding, these factors increase the incidence of any disease, especially calf pneumonia. Matching with Svensson *et al*. (2003) who found that diarrhea is one of the most common diseases reported in calves up to three months old. The present survey results proved that the main health problem in calves was calf diarrhoea (72.7%). According to Peter *et al*. (2017), several owners' practices may help to decrease health problems among herds, for example the availability of the veterinary services, adoption of vaccination program and colostrum giving to the calves during first hours of birth. In this study all owners considered that the two first weeks of calf's age are the most hazardous and the risk decreases with old ages, and this is in accordance with the findings of Curtis *et al*. (1988). The analysis of the data on treatments adopted to the affected calves in the areas of the study showed different drugs with different percentages of adoption: Penicillin (54.5%), Tetracycline (36.4%) and Enrofloxacin (35.8%), These treatment strategies were also recommended by Peter *et al*. (2017) with different routes of administration of drugs.

In this investigation a total of 83 bacterial isolates were obtained from 80 nasal swab samples collected from pneumonic calves in Bahri locality. The identified bacteria were: 15 *S. aureus* (18.2%), 7 *S. epidermidis* (8.4%), 4 *S. chromogenes* (4.8%), 9 *Str. Pneumoniae* (10.8%), 5 *Str. uberis* (6.0%), 7 *K. pneumoniae* (8.4%), 13 *E. coli* (15.7%), 11 *Ps. aerogenosa* (13.3%), 4 *B. subtilis* (4.8%), 5 *M. variens* (6.0%) and 3 *M. luteus* (3.6%). Zulfekar Ali and Shirin Sultana (2012) also isolated Staphylococcus spp. and *Pasteurella haemolytica* from cases of calf pneumonia. Francis and Ameh (2015), Benesi *et al*. (2010), Hartel *et al*. (2004), Autio *et al*. (2007), Angen *et al*.(2009) and Oliveira *et al*. (2016) isolated *Staphylococcus aureus, Klebsiella pneumonia, Streptococcus pneumonia, Proteus vulgaris, Pasteurella multocida, Escherichia coli,* Corynebacterium spp., Salmonella spp, and Enterobacter spp. from cases of calf pneumonia in Nigeria. Nicholas *et al.* (2003) reported that facultative anaerobes; *Mannheimia* (*Pasteurella*) *haemolytica*, *Pasteurella moltocida*, *Haemophilus somnus*, *Arcanobacter pyogenes*, *Pseudomona saeruginosa*, *Escherichia coli*, *Actinobacillus pleuropneumoniae*, *streptococcus spp., staphylococcus species, Moraxella spp, salmonella spp.* are the most common bacteria involved in pneumonia.

Dwight *et al*. (2004) reported that the majority of pathogens of the respiratory tract of cattle belong to the *Streptococcus spp.*. Isam Eldeen (2003) isolated *Streptococcus* species from cattle in Khartoum State that suffered from pneumonia.

Klebsiella pneumonia represented 8.4% of the isolated bacteria, Carter (1986) reported that *Klebsiella* spp. can be found

associated with some diseases as secondary invaders but may also act as primary aetiological agents of diseases.

Pseudomonas aeruginosa represented 13.3% of the isolated bacteria, Carter (1986) reported that *Pseudomonas aeruginosa* isolated frequently from wound infections in a number of domestic animals.

CONCLUSION AND RECOMMENDATIONS

According to the results of Questionnaire survey in Bahri locality of Khartoum State, calf pneumonia is one of the main health problems in calves with the prevalence of 45.5%. The mortality rate among pneumonic calves is higher during second and third weeks of age. Staphylococci represented the predominant bacteria isolated from nasal swabs of pneumonic calves compared to other bacteria such as Streptococci, *E. coli*, *Ps. aerogenosa*, Micrococci, *K. pneumoniae* and *B. subtilis*.

Further studies should include a survey of more animals in different farms and the significance of bacteria in calf diarrhoea. Further studies should be carried out to investigate the predisposing factors related to the incidence of neonatal calf pneumonia and to identify different causes of calf diarrhoea. Pregnant cows should be isolated within the last two weeks before calving. Moreover, feeding colostrum during the first day is strongly advised. Other management factors should not be underestimated.

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