BACTERIAL CAUSES OF CALF DIARRHOEA IN DAIRY FARMS IN BAHRI LOCALITY, SUDAN

ABSTRACT

This study was aiming at investigating the bacteriological causes of calf diarrhea in dairy farms in Bahri Locality of Khartoum State during 6 months of the year 2019. A questionnaire survey was done before collection of samples. A total of 60 faecal samples were collected directly from rectums of 60 diarrhoeic dairy calves at the age of one month and less. Samples were collected from calves not subjected for any treatment before collection of samples. Samples were examined bacteriologically using conventional and rapid API methods. The prevalence of calf diarrhoea in dairy farms was 9.4%. Results of the questionnaires of 10 dairy farms revealed that calves at age of one month or less are more susceptible for diarrhea, calves were separated from dams, hygiene is poor in 80% of the farms, records were not available in 70% of the farms, colostrum was fed to caves at first hours of birth in 30% of the farms, all farms experienced calf diarrhea before, 70% of the owners do not vaccinate calves, 90% experienced death of calves due to diarrhea and in 60% of the farms treatment of calf diarrhea was successful. Ninety six bacterial isolates were identified as 48 *Escherichia coli* (50.0%), 16 *Pseudomonas aerogenosa* (16.6%), 10 *Prote*us *mirabilis* (10.4%), 8 *Staphylococcus aureus* (8.3%), 6 *Klebsiella pneumoniae* (6.3%), 4 Salmonella spp., (4.2%), 2 *Staphylococcus epidermidis* (2.1%) and 2 *Bacillus subtilis* (2.1%). Gram negative bacteria represented the higher percentage (87.5%) compared to Gram positive bacteria. *E. coli* was the dominant bacteria. We recommend feeding colostrums during the first day of birth. Other management factors should not be underestimated.

Key words

Areobic bacteria, Bahri Locality, Calf diarrhea, Sudan.

INTRODUCTION

Cattle rearing in Sudan plays an important role in the country's economy and social welfare. In Khartoum State the greatest numbers of dairy farms, contain crossbred animals with varying percentages of foreign blood (Friesian breed). Presence of infectious agents, poor management and poor nutrition are some of the factors that can be pointed out as causes of calf diseases and mortality. Mortality rate among calves in Sudan was estimated to be 10% and in some months it may reach 100% due

to colibacillosis and diarrhoea which are considered the major cause of economic loss in intensive, modern and conventional farming systems [1]. Diarrhea in newborn farm animals, particularly calves under 30 days of age, is one of the most common disease complexes that the large-animal clinician encounters in practice. It is a significant cause of economic loss in cattle herds and continues to assume major importance as livestock production becomes more intensified [2]. The most important enteropathogenic aliments of cattle, sheep and goats, in which diarrhoea is a significant clinical finding include: the viruses (Rotavirus, Coronavirus, Bovine Viral Diarrhoea Virus and Bovine Malignant Catarrhal Fever Virus), fungi (*Candida spp.*), and helminthes (*Ostertagia spp.*, *Nematodirus spp.* and *Tricostrongylus spp.* In goat), protozoa (*Eimeria spp.* and *Cryptosporidium spp.*) and bacteria Enterotoxigenic *E. coli*, *Clostridium perfringens* types B, C, and D, Salmonella *spp*., *Mycobacerium paratuberculosis*, *Proteus spp.*, and Pseudomonas spp. [3]. [4] [5] [6] reported the involvement of *E. coli* as a cause of diarrhoeal disease in calves. *E. coli* also causes haemorrhagic colitis and dysentery. When scour outbreak occurs, producers often focus a great deal of labor and money on treatment of calves with fluids, and antibiotics, but the environment often becomes extremely contaminated very quickly. Calves with *E. coli* scours may be shedding billions of bacteria in single stool [7]. Environmental survival of *E. coli* may play an important role in the persistence and dissemination of this organism [8]. Many of the losses caused by scour can be prevented through good management practices [3].

The objective of this study is to investigate the problem of diarrhoea among calves in dairy herd in Bahri locality in Khartoum State.

MATERIALS AND METHODS

Source of samples

A total of 60 faecal samples were collected from rectums of dairy calves in Bahri locality showing typical signs of calf diarrhoea and didn't receive any treatment.

Sampling procedure

Questionnaires were filled then faecal samples were collected directly from rectums of calves showing typical calf diarrhea and put in sterile containers. The collected samples were put in ice box containing ice, transported to the laboratory of faculty of Veterinary Medicine University of Bahri and examined.

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 streaked onto blood agar, McConkey's agar, and nutrient agar using sterile wire loop. The cultures were incubated aerobically at 37^{o}C 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 4^{o}C.

Biological and biochemical identification of the bacteria

The purified isolates were identified as previously described by [9][10]. The identification include: 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).

API 20E (BIOMERIEUX, France)

According to [11], API 20E is a standardized identification system for Enterobacteriaceae and other Gram-negative rods which uses 23 miniaturized biochemical tests and a data base. The API 20E strip consists of 20 microtubes containing dehydrated substrates. These microtubes are inoculated with a bacterial suspension, prepared in API 20E medium that reconstitutes the tests. During incubation metabolism produces colour changes that are either spontaneous or revealed by the addition of reagents. The reactions were read according to the reading table and the identification is obtained by referring to the analytical profile index or using the identification soft-ware. Tests included in API 20E strips were Ortho-nitro-phenyle-galactoside (ONPG), Arginine (ADH), Lysine (LDC), Ornithine (ODC), Sodium citrate (CIT), Sodium thiosulphate (H2S), Urea (URE), Tryptophane (TDA), Indole (IND), Sodium pyruvate (VP), Kohn's gelatin (GEL), Glucose (GLU), Mannitol (MAN), Inositol (INO), Sorbitol (SOR), Rhamnose (RHA), Sucrose (SAC), D-melibiose (MEL), Amygdalin (AMY), Arabinose (ARA), Oxidase

(OX), Nitrate (NO3-NO2), Motility (MOB), MacConkey growth (MAC) and Oxidation-fermentation test (OF). The incubation boxes (trays and lids) were prepared and 5ml of distilled water was distributed into the honey–combed wells of the trays to create a humid atmosphere. The strains references were recorded on the elongated flab of the trays. The strips were removed from their packaging and placed in the incubation boxes. The organisms were sub cultured onto blood agar and incubated at 36C ± 2 for 18–24 hours. They were checked to be belonging to Enterobacteriaceae Family (morphology, Gram stain, catalase, oxidase, etc.) and they were pure cultures. Ampoles of API 20E medium were opened and homogeneous bacterial suspension prepared. These suspensions were used immediately after preparation. Both tubes and cubules were filled with the inoculated API 20E media by using a pipette. Anaerobiosis was ensured in the ADH, LDC, ODC, URE and H2S tests by filling the cupules with mineral oil to form a convex meniscus. The incubation boxes were closed and incubated at 36^{o}C ± 2 for 18–24 hours. After the incubation period, the reaction was developed by adding one drop of each of the following reagents and then results were read by referring to the reading tables.

Identification of Staphylococcal Isolates Using API staph (BIOMERIEUX, France) Identification System

According to [12] API staph (Analytical Profile Index for identification of the Genus Staphylococcus) is a standardized system for the identification of the Genera: Staphylococcus, Micrococcus and Kocuria, which uses miniaturized biochemical tests and specially adapted database. Pure staphylococci isolates were sub cultured on blood agar and incubated at 36oC ± 2 for 18–24 hours. The identification test of staphylococci isolates was conducted according to the manufacturer BIOMERIEUX protocol. Homogeneous bacterial suspension was obtained by using API staph medium. Both tubes and cubules of API staph were filled with the inoculated API staph media. Anaerobios was ensured in the ADH, LDC, ODC, URE and H2S tests by filling the cubules with sterile mineral oil to form a convex meniscus. The incubation boxes were closed and incubated at 36^{o}C ± 2 for 18–24 hours. Identification was obtained according to the numerical profile of API staph.

Statistical Analysis

Statistical analysis was done through Microsoft office Excel 2007.

RESULTS

Questionnaire survey of dairy farms in Bahri locality

Analysis of the questionnaire of 10 dairy farms in Khartoum State illustrated that: 100% of the housing systems were loose corral, 70% of the stall surfaces were clay and 20% uses concrete surfaces. The general evaluation of the housing condition was good for 70% of the farms and poor for the rest. Eighty percent of the farms were suffering from mastitis and other infections and 50% were suffering from Tick-borne diseases. Veterinary services were available in 80% of the farms and hygienic level was poor in 80% of the farms and was good in the rest. Calves' health records were available in 70% of the farms. Calves diseases in the farms were diarrhoea in 100%, Pneumonia 80% and Tick-borne disease in 50%. Colostrum was not presented during first hours of birth in 70% of the farms. Vaccination system was not adopted in 70% of the farms. Concerning calf diarrhoea 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 calf diarrhoea and the risk decreases in older ages. All owners confessed losses of calves due to calf diarrhoea and that they adopted different treatment trials of the disease. The majority (80%) of the owners used Septrin for treatment of calf pneumonia and 50% used Sulpha and Enrofloxacin. Only 20% of the owners used fluid therapy. Summary of the questionnaire was illustrated in table (1).

Table 1: Summary of the questionnaire survey of 10 dairy farms in different areas in Bahri locality

Unit	Frequency (%)	Unit	Frequency (%)
Housing type		**Colostrum during first hours of birth**	
-Free stall	0 (0%)	Yes	3 (30%)
-Loose corral	10 (100%)	No	7 (70%)
-Stanchion	0 (20%)	**Vaccination system**	
Stallsurface		Yes	3 (30%)
Concrete	2 (20%)	No	7 (70%)
Clay	7 (70%)	**Previous cases of Calf diarrhoea**	
Sand	1 (10%)	Yes	10 (100%)

Housing condition		No	0 (0%)
Excellent	0 (0%)	**Population at risk**	
Good	7 (70%)	One week old	5 (50%)
Poor	3 (30%)	Two week old	8 (80%)
Common diseases		Three week old	10 (100%)
Tick-borne diseases	5 50%)	One month old	9 (90%)
Mastitis	8 (80%)	More than One month old	8 (80%)
Other diseases	8 (80%)	**Losses due to Calf diarrhea per year**	
Veterinary services		Yes	10 (100%)
Yes	8 (80%)	No	0 (0%)
No	2 (20%)	**Treatment of calf Calf diarrhoea**	
Hygiene Level		Septrin	8 (80%)
Excellent	0 (0%)	Sulpha	5 (50%)
Good	3 (30%)	Enrofloxacin	5 (50%)
Poor	8 (80%)	Fluid therapy	2 (20%)
Diseases		**Calves' health Records**	
Diarrhoea	10 100%)	Yes	3 (30%)
Pneumonia	8 (80%)	No	7 (70%)
Tick-borne diseases	5 (50%)		

Prevalence of calf diarrhoea

Out of 635 dairy calves investigated in Bahri locality, only 60 calves showed the signs of calf diarrhoea. The prevalence of calf diarrhoea was 9.4% (Table 2).

Table 2: Farms, healthy and diarrhoeic calves investigated for calf diarrhea in Bahri locality:

Farms	Calves above 1month old healthy calves	One month old healthy calves	Less than 1 month old healthy calves	Less than 1 month old diarrhoeic calves	Total
1	100	60	30	10	
2	200	50	1	16	
3	40	10	12	8	
4	4	3	1	4	
5	5	3	2	2	
6	4	0	3	0	
7	3	2	1	2	
8	4	0	3	0	
9	7	6	1	8	
10	15	5	0	10	
Total	382	139	54	60	635

Bacteria isolated from diarrhoeic samples collected from Bahri locality:

In this investigation a total of 96 bacterial isolates were obtained from 60 diarrhoeic samples collected from dairy calves in Bahri locality. According to the cultural characteristics, bacterial morphology and biochemical reactions results (Table 3) and API rapid test results (Tables 4 and 5) the identified bacteria were: 48 *Escherichia coli* (50.0%), 16 *Pseudomonas aerogenosa* (16.6%), 10 *Prote*us *mirabilis* (10.4%), 8 *Staphylococcus aureus* (8.3%), 6 *Klebsiella pneumoniae* (6.3%), 4 Salmonella spp., (4.2%), 2 *Staphylococcus epidermidis* (2.1%) and 2 *Bacillus subtilis* (2.1%) (Table 6). Gram negative bacteria represented the higher percentage (87.5%) compared to Gram positive bacteria. *E. coli* was the dominant bacteria.

Table (3): 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	Pink	Bright pink
Haemolysis on blood agar	+	+	+	-
Gram reaction	-	+	-	-
Shape	Rod	Cocci	Rod	Rod
Spore	-	-	-	-
Motility	+	-	+	+
Catalase	+	+	+	+
Oxidase	-	-	+	-
Indole	+	-	-	+
Methyl red	-	+	-	-

VP	-	-	-	-
Citrate	-	-	+	-
H_2S	-	-	-	-
O/F	+	+	+	+
Glucose	+	+	-	+
Lactose	+	+	-	+
Coaggulase	-	+	-	+

Table 3 (Continued): Cultural characteristics, bacterial morphology and biochemical tests of the isolated bacteria.

Test	***B. subtilis***	**Salmonella**	***Proteus mirabilis***	***S.* epidermidis**
Aerobic growth	+	+	+	+
Colonies on MacConkey	Bright pink	Pink	Pink	Pink
Haemolysis on blood agar	+	-	+	-
Gram reaction	+	-	-	+
Shape	Rod	Rod	Rod	Cocci
Spore	+	-	-	-
Motility	+	+	-	-
Catalase	+	+	+	+
Oxidase	-	-	-	-
Indole	+	+	-	-
Methyl red	-	+	+	+
VP	-	-	-	-
Citrate	-	-	-	-
H_2S	-	-	-	-
O/F	+	+	+	+
Glucose	+	-	-	-
Lactose	+	-	-	-
Coaggulase	-	-	-	-

Table (4): Api Staph Identification system's results

Test	***S. aureus***	***S. epidermidis***
(0)	-	-
D-glucose (GLU)	+	+
D-fructose (FRU)	+	+
D-mannose (MNE)	+	-
D-maltose (MAL)	+	+
D-lactose (LAC)	+	+
D-trehalose (TRE)	-	-
D-mannitol (MAN)	+	-
Xylitol (XLT)	-	-
D-melibiose (MEL)	-	+
Potassium Nitrate (NIT)	+	+
B-Naphthyl phosphate (PAL)	+	+
Sodium pyruvate (VP)	+	+

D-raffinose (RAF)	-	-
D-xylose (XYL)	-	-
D-saccharose (SAC)	+	+
Methyl-αD-Glucopyranoside (MDG)	+	-
N-acetyl-glucoseamine (NAG)	+	+
L-arginine (ADH)	-	-
Urease (URE)	+	+

Table (4) (Continued): Api Staph Identification system's results

Test	*E. coli*	*K. pneumoniae*
Ortho-Nitro-Phenyl-Galactoside (ONPG)	-	+
Arginine (ADH)	-	-
Lysine (LDC)	+	+
Ornithine (ODC)	+	-
Na citrate (CIT)	-	+
Na thiosulphate (H_2S)	-	-
Urease (URE)	-	+
Tryptophane (TDA)	-	-
Indole (IND)	+	-
Voges-Proskauer (VP)	-	+
Gelatinase (GE)	-	-
Glucose (GLU)	+	+
Mannitol (MAN)	+	+
Inositol (INO)	-	+
Sorbitol (SOR)	+	+
Rhamanose (RHA)	+	+
Sucrose (SAC)	+	-
Melibiose (MEL)	+	+
Amygdalin (AMY)	-	-
Arabinose (ARA)	+	+
Nitrate reduction (NIT)	-	-

Table (5): Total number and percentage of bacteria isolated from diarrhoeic samples collected from dairy farms in Bahri Locality.

Isolated bacteria	No. / %	Isolated bacteria	No. / %
E. coli	48 (50%)	*S. aureus*	8 (8.3%)
Ps. aerogenosa	16 (16.6%)	Salmonella spp.	4 (4.2%)
Proteus mirabilis	10 (10.4%)	*S. epidermidis*	2 (2.1%)
K. pneumoniae	6 (6.3%)	*Bacillus subtilis*	2 (2.1%)
Total	**80**		**16**

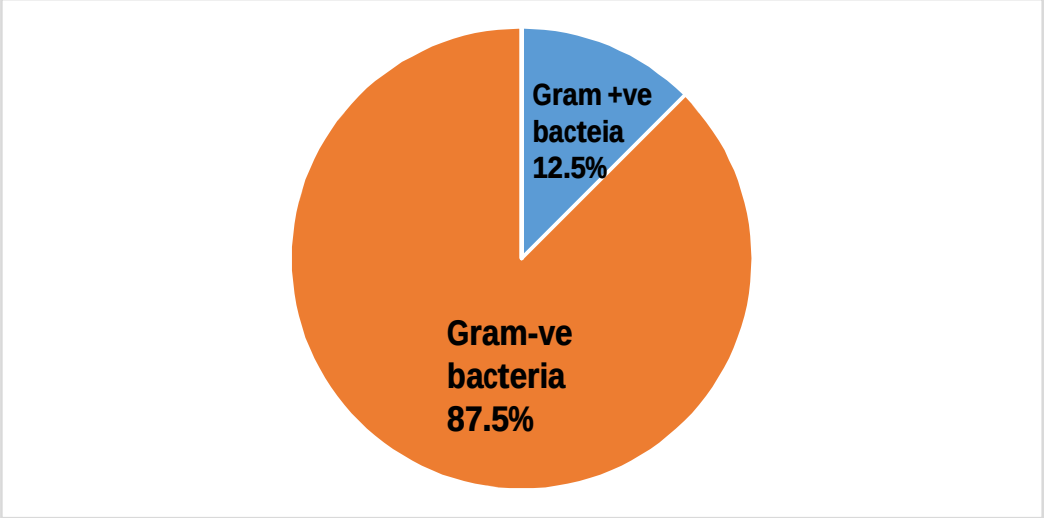

Fig. (1): Gram-negative and Gram-positive bacteria isolated from diahhoeic calves in Bahri Locality.

Discussion

Diarrhoea in newborn farm animals, particularly calves under 30 days of age, is one of the most common disease complexes that the large-animal clinician encounters in practice. It is a significant cause of economic loss in cattle herds and continues to assume major importance as livestock production becomes more intensified. The effective treatment and control of herd epidemics of diarrhoea in calves can be frustrating and unreliable. Considerable progress has been made in the treatment of the effects of diarrhoea such as dehydration and acidosis but less so in the control of these disease complexes [3]. According to Questionnaire survey of dairy farms, the general evaluation of the housing condition was poor for 30% of the farms, 70% of the stall surfaces were clay and the hygiene level was poor in 80% of the farms. According to [13] findings, these factors increase the incidence of any disease, especially calf scour. Matching with [14] who found that diarrhoea 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 (100%). In the

present survey's results concerning calf diarrhoea, 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 in accord with the findings of [15]. According to owners' records, there were high mortality rates due to calf diarrhoea and in support of [14] who estimated that 75% of early calf mortality in dairy farms is caused by acute diarrhoea in the pre-weaning period. The analysis of the data on treatments adapted to the affected calves in the areas of the study showed different drugs with different percentages of adoption: Septrin (80%), Sulphonamides and Enrofloxacin (50%) and fluid therapy. These treatment strategies were also recommended by [13] with different routes of administration of drugs. The same author also mentioned that, diarrhoeic calves are commonly treated with oral fluids and electrolytes and left with the cow. However, it is a common practice to reduce the milk intake of diarrhoeic hand-fed dairy calves for up to 24 hours or until there is clinical evidence of improvement. In this study the prevalence of calf diarrhea in Bahri Locality was 9.4%. Higher prevalence rate (19.1%) was recorded by [6] in Khartoum State. [16] reported the prevalence rate of 17% among calves in El Bagair area In Khartoum Locality due to diarrhoea. [3] mentioned that the prevalence of calf diarrhea may reach 75%.

Out of 60 diarrhoeic samples obtained from dairy farms in Bahri Locality a total of 96 bacterial isolates were obtained. The identified bacteria were: 48 *Escherichia coli* (50.0%), 16 *Pseudomonas aerogenosa* (16.6%), 10 *Prote*us *mirabilis* (10.4%), 8 *Staphylococcus aureus* (8.3%), 6 *Klebsiella pneumoniae* (6.3%), 4 Salmonella spp., (4.2%), 2 *Staphylococcus epidermidis* (2.1%) and 2 *Bacillus subtilis* (2.1%). [6] isolated *Escherichia coli* (76.0%), *Klebsiella pneumoniae* (6.4%), and *Proteus mirabilis* (8.8%) from diarrhoeic calves in Khartoum State. In some cases more than one isolate was recovered from the same sample. In this study Gram negative bacteria represented the higher percentage (87.5%) compared to Gram positive bacteria. *E. coli* was the dominant bacteria. [6] found that the Gram-negative bacteria were the most prevalent bacteria among bacteria isolated from diarrhoeic calves in Khartoum State. This result is also similar to that reported by [1]. In this study we found that *E. coli* represented the predominant bacterial spp. among Gram-

negative aerobes. The result is similar to that found by [6][17][13][5][4][18][19][20].

CONCLUSION AND RECOMMENDATIONS

From this study we conclude that the main health problem in dairy calves is calf diarrhoea (100%). The prevalence rate of calf diarrhoea in Bahri locality is 9.4%. *E. coli* represents the predominant Bacterial spp. (50%), isolated from diarrhoeic calves.

We recommend further studies in different farms and an extensive study of the significance of *E. coli* in calf diarrhoea. Further studies should be carried out to investigate the predisposing factors related to the incidence of neonatal calf diarrhoea and to identify different causes of calf diarrhoea. Pregnant cows should be isolated within the last two weeks before calving. Moreover, feeding colostrums during the first day is strongly advised.

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