Bacterial and Parasitological causes and Risk factors associated with Bovine Calf Diarrhoea in Tamboul Locality, Gezira State-Sudan

Abstract: This study was aiming at investigating bacterial and parasitological causes and risk factors associated with bovine calf diarrhea in Tamboul Locality, Gezira state-Sudan. The study was conducted during the year 2021 to cover farms in Tamboul city. Questionnaires were filled and 36 fecal samples were taken from calves at the age less than 5 months, showing clinical signs of diarrhea and not subjected for treatment. Samples were subjected for bacteriological and parasitological examinations. The results showed that 35 (97.2%) of the samples were positive for bacteriological tests and all of the samples were negative for parasitological examinations. The statistical analysis showed a significant variation (P=0.000) in the effectiveness of etiology on occurrence of calf diarrhea in Tamboul city. Gram negative bacteria were isolated from 20 (57.2%) diarrhoeic samples, gram positive bacteria from 4 (11.4%) samples and mixed (gram negative and gram positive) bacteria were isolated from 11 (31.4%) samples. The statistical analysis showed no significance variation ($p>0.05$) between colour of diarrhea and other symptoms (emaciation, anorexia, nasal discharge, fever and skin) and incidence of calf diarrhoea. The results showed that 21 (58.3%) diarrhoeic calves were under 1 month of the age, 9 (25%) were in between 1-3 months and 6 (16.7%) were more than 3 months. By using Chi-square test there was no significance variation ($p>0.05$) between groups of age of diarrhoeic calves and incidence of calf diarrhoea. The results showed that 21 (58.3%) diarrhoeic calves were males and 15 (41.7%) were females. There was no significance variation ($p>0.05$) between groups of age of diarrhoeic calves incidence of calf diarrhea. Also cross breed were more infected than local breed (61.61%) and (38.89%)

respectively. All bacterial isolate were sensitive to Gentamicin and Enrofloxacin and Resistant to Oxytetracycline and Sulphadimidine. Animals' wners should be aware about risk of antimicrobial resistance and we recommend the use of Gentamicin and Enrofloxacin for treatment of calf diarrhea.

Keywords: calf diarrhea, bovine, antibiotic, sensitivity, Tamboul Locality, Gezira State Sudan.

1. INTRODUCTION:

Cattle population in El-gezira State represented 8.41% of the total cattle population in Sudan [1]. Most important causes of calf diseases and deaths are diarrhoea, pneumonia, joint problems, umbilical diseases, trauma, congenital abnormalities, nutritional deficiencies, dystocia and other infections [2] [3]. Neonatal calf diarrhea (NCD) is one of the most serious diseases worldwide among newborn calves (<1 month old). NCD causes notable levels of morbidity and mortality through several complications, such as dehydration, acidosis, and solution depletion [4]. Seventy five percent (75%) of early calf mortality in dairy herds is caused by acute diarrhea in the pre-weaning period and also, a commonly reported disease in young animal and still a major cause of productivity and economic loss to cattle producers and also a cause of high morbidity and mortality in the cattle industry worldwide [5] [6]. Some factors may have significant effect on calf diarrhea occurrence these include: age, sex, location and breed, the age of the calf is the most important factor affecting morbidity and mortality. Approximately 75% of the mortality in dairy animals less than one year of age occurs in the first month of their life [7]. The incidence rate of calf diarrhea in Khartoum State is 60% and 33% of mortality rate among Suckling calves in Khartoum State was caused by calf diarrhea [8].

Calf diarrhea is widespread in Tamboul city and there are very few published previous researches about it. The direct economic loss from calf diarrhea is due to

loss of calves, high cost of treatment and continues complainant of herds man about non efficiency of antibiotics against calf diarrhea is badly needed addressing.

2. MATERIALS AND METHODS:

Study area:

This study was conducted at seven different villages around Tamboul Locality (13˚09' N, 33˚55' E) in Gezira State which lies far 150 km South of Khartoum during the year 2021.

Study population and sample size:

A total of 36 fecal samples were collected from calve with age ranged between 1 day to 150 days and with different sex and breed.

Clinical investigation:

The body temperature, pulse rate and respiratory rate of each diarrheic calf of each diarrheic calf were measured by using methods described by [9].

Sample collection:

A total of 36 fecal samples were collected using clean containers and 36 fecal swabs using sterilized loops according to [10].

Sedimentation test for parasitic infestation:

5-10 gram of fecal sample was mixed in 10 ml of distilled water into test tube of 10 ml capacity. Then the tube was centrifuged at 3000 rpm of several minutes. The extra water was poured out, then a drop of the precipitate was taken and placed on a slide. Cover slip was placed on the on the sample and then examined under the microscope with a 4x lens [10].

Media preparation and sterilization:

28 gram of nutrient agar powder was dissolved in 1L of distilled water. After mixing and dissolving them completely, the suspension was sterilized by autoclaving at 121℃ for 15 minutes. The liquid was poured into the petri dish and waited for the medium to solidify. Once the agar solidifies, the agar was ready to

use [11] and Plates after cultured were incubated at 37° C for 24-48 hours to bacterial growth.

Gram stain:

Gram stainwas used for diagnosing contagious skin necrosis. After slide prepration, Ammonium oxalate –crystal violet stain was applied to smear for 90 second. Then Lugol's iodine solution was added for one minute , this was washed with distilled water and decolorized with alcohol for 2-3 seconds and again washed thoroughly with water , counter staining was done with diluted carbol fuchsin for 15 seconds then washed and drained or blotted to dry. Gram positive organisms were reed as either blue or purple and gram negative organisms were red in color [12].

Comment [i1]: Stain was

Comment [i2]: preparation

Antimicrobial susceptibility testing:

Antimicrobial susceptibilities test for isolates were performed by disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guideline [13] [14]. The antimicrobial discs tests were Gentamicin, Oxytetracycline, Enrofloxacin and Sulphadimidine. Colonies were picked from culture of each bacterial grown on nutrient agar, sub cultured into nutrient agar and incubated at 37°C for 2 h. The inoculum was well distributed on the surface of the medium and a pipette was used to Withdraw off excess culture. The inoculated plate was left to dry at room temperature for 15 minutes. Then antibiotic disks was placed on the agar and pressed gently by sterile forceps. The inoculated plate was then incubated at 37°C for 24-48. The diameter of the zone of growth inhibitions caused by each antibiotic to each bacterial growth was measured in (mm) and the results were registered and the score of inhibition zone diameters of each drug was interpreted according to [15] recorded in table (1).

Table (1): The score of inhibition zone.

Zone diameter	Score
<8mm	Resistant (R)
9-15mm	Slightly sensitive (+)
16-22mm	Sensitive (++)
>23mm	Very sensitive (+++)

Data Analysis:

Data collected from this study was compiled using an appropriate statistical package SPSS of social sciences version 26 and levels of significance was taken at ($P \leq 0$ .05).

3. RESULTS:

Among the 36 diarrhoeic samples diagnosed for calf diarrhea oetiology, 35 (97.2%) were positive for bacteria and all samples were negative for parasitological tests. statistically the result showed high significant variation in effect of etiology on occurrence of calf diarrhea in Tamboul Locality (P=0.000). According to types of bacterial infection, Chi-square revealed significant difference and recorded 20 (57.2%) samples were gram negative bacteria followed by 11 (31.4%) samples were mixed (Gram negative + Gram positive) bacteria and 4 (11.4%) samples were Gram positive bacteria (Figure, 1).

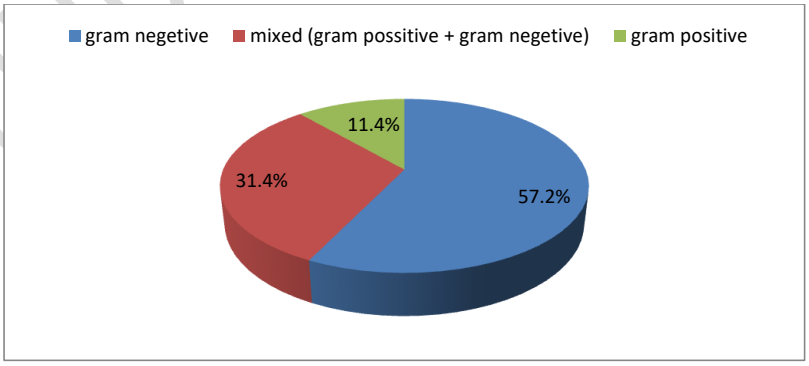

Fig. (1): Bacteria isolated from diarrhoeic calves in Tamboul Locality

Depend on symptoms of infected calves, the result showed no significance variation ($p>0.05$) and high percentage was characterized by Yellow diarrhea, emaciation and anorexia (58.34%) followed by brown diarrhea, nasal discharge and fever (33.33%) and the least signs observed was Black diarrhea with lesions over the skin (8.33%) (Figure, 2).

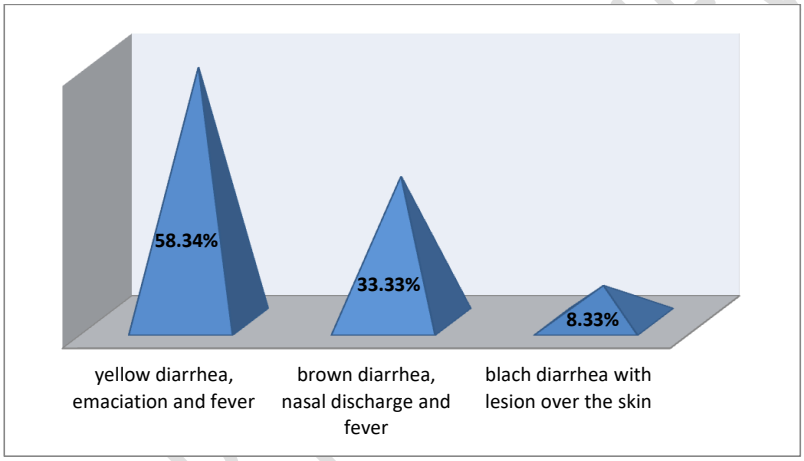

Figure (2): percentage of symptoms of diarrhoeic calves.

The age in this study was divided into three groups; less than 1 month, 1-3 months, and more than 3 month. There was no significant variation between the groups, but higher incidence 21 (58.3%) was noticed in group of age less than 1 month, followed by 9 (25%) in the group of age 1–3 months and just 6 (16.7%) in the group of age more than 3 months. Statistically no significant difference in the effect of sex, breed and clinical parameters on incidence of calf diarrhea but visually males were more infected than females and they were 21 (58.3%) and 15

(41.7%) respectively. Also cross breed were more infected than local breed (61.61%) and (38.89%) respectively.

Five antimicrobial agents were used for sensitivity testing and these were Gentamicin (50 µg), Enrofloxacin (50 µg), Oxytetracycline (50 µg) and Sulphadimidine (50 µg). Chi-square test showed high significant variations in effect of antibiotic and antimicrobial agents on inhibition bacteria caused calf diarrhea (P=0.000); According to inhibition zone diameter (IZD) by millimeters the current study showed all bacterial isolates were sensitive to Gentamicin (32.1 mm) and Enrofloxacin (17.4 mm) and Resistant to Oxytetracycline (4.1 mm) and Sulphadimidine (3.5 mm) (Figure, 3).

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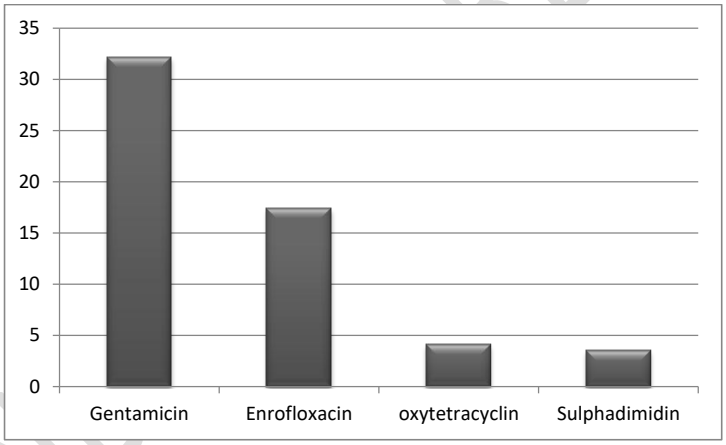

Figure (3): Inhibition zone diameter (IZD) of isolated bacteria.

4. DISCUSSION:

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 [16]. In this study all diarrhoeic samples were negative to parasitological tests while 97.2% were positive to bacterial examination. Among bacterial isolate 57.2% were Gram negative bacteria followed by 31.4% were mixed (Gram negative + Gram positive) bacteria and only 11.4% isolate were Gram positive bacteria. This agrees with previous research in 2013 where a team of researchers in USA reported that parasitical etiology of calf diarrhea is rare unless its co-infection with bacterial agents [17]. In addition that the wide using of anthelmintic agents among herds plays a huge role in the elimination of parasite. The study also agreed with [18] who recorded the infectious agents cause diarrhea in calves alone or as mixed infections. Also similar result was recorded by [19] [20] [21] [22] who reported *E. coli* and Salmonella as the two most common and economically important agents that cause calf diarrhea. In this study, by using Chi-square test there was no significance variation between age groups of diarrhoeic calves, but higher incidence was noticed in age (< 1 month), followed by (1–3 month) and the least infected were at the age (> 3 month). This result agreed with result reported by [23] who found that there was no association between occurrence of *E. coli* and age of diarrheic calves. The study disagreed with [24] [25] [26] who mentioned that most cases of diarrhea occurred in calves less than 30 days of age. The reason of the difference is due to dispute the environment, sample size and time of study. In present study the most signs and symptoms associated with calf diarrhea were yellow diarrhea, emaciation and anorexia followed by brown diarrhea, nasal discharge with fever and the least sings black diarrhea with lesions over skin. This result is almost identical to that result registered by [27] [28]. In this study there was no significance difference ($p>0.05$) between male and female in incidence of diarrhea and this matching the results of [29] who reported that detection the sex factor failed to show significant

difference (P > 0.05) with the prevalence of *E. coli* among calf diarrhea. In this study all bacterial isolates were sensitive to Gentamicin and Enrofloxacin. Similar result reported by [30] [31] who reported that *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated from diarrhoea were sensitive to Gentamicin. Also [32] reported the sensitivity of *E. coli* to Gentamicin and Salmonella to Enrofloxacin. In this study all bacterial isolates showed resistance to Oxytetracycline and Sulphadimidin. The finding agreed with [22] who reported that invitro *E. coli* was highly resistant to Oxytetracycile. Oxytetracycline and Sulphadimidin are widely used at random by animals' owners in the study area and that will decrease their effectiveness against bacterial calf diarrhoea infections, so other alternative drugs as Gentamicin and Enrofloxacin should be used.

5. CONCLUSION AND RECOMMENDATIONS:

Calf diarrhea is widespread in Tamboul area and there are a lot of cases in veterinary clinics especially in calving seasons. All diarrhoeic samples were negative to internal parasite while 97.2% were positive to bacterial growth. Gram negative bacteria represented 57.2%). Yellow diarrhea, emaciation and anorexia were the most symptoms associated with calf diarrhea. There was no significant difference (P>0.05) between risk factors of calf diarrhoea (age, sex, breed). All bacterial iolates were sensitive to Gentamicin and Enrofloxacin while they were resistant to Oxytetracycline and Sulphadimidine.

Awareness of the animals' owners about the prudent use of antimicrobials can reduce the risk of anti-microbial resistance. Gentamicin and Enrofloxacin should be used for prevention and treatment of calf diarrhoea.

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