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ANTIBIOTIC SENSITIVITY OF SOME BACTERIA ISOLATES ISOLATED FROM SCOURING CALVES FROM SOME FARMS IN OYO AND OGUN STATES, NIGERIA

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Abstract.

Diarrhoea in young pre-weaned calves is one of the most important causes of calf morbidity and mortality. Information on this multi-aetiological condition is scanty in Nigeria. Therefore, we studied the prevalent bacteria and antibacteria susceptibility to various bacterial isolates from faecal samples of suspected cases of calf scours in Oyo and Ogun States of South-western Nigeria. One hundred and twenty calf faecal samples (120) from various breeds of cattle showing clinical manifestation of scours were screened for bacterial agents in two farms in Oyo and five farms in Ogun States. Isolation and identification were done using standard procedures, while antibiotic sensitivity was done using Kirby Bauer's disc diffusion method. *Escherichia coli, Salmonella spp.* and *Campylobacter spp.* were identified. Out of all the calves, 19 calves (15.8%) were positive for bacteria agents, *E. coli* was isolated from 12 calves (10%), *Salmonella spp.* was isolated in 10 calves (8.3%) and *Campylobacter spp.* was isolated in 1 calf (0.8%). Antibiotic sensitivity test revealed that the most sensitive antibiotics were Meropenem (91.3%) and Amikacin (82.6%) while the least sensitive was Ampicillin (21.7%). Therefore Meropenem is the most sensitive antibiotic and recorded the highest percentage sensitivity in all the three bacteria pathogens isolated, while Ampicillin appeared to be the least sensitive antibiotic to virtually all the pathogens isolated. Meropenem is therefore recommended as the drug of choice for the management of bacteria calf scours. The isolation of highly pathogenic *E.coli* 0157 from calf scour cases reaffirmed cattle as a reservoirs host which may enter the food chain and poses a faeco-oral potential public health hazards.

Introduction

Cattle rearing is an essential part of the agricultural production system in Nigeria and contributes an important role in national economy as well as in socio-economic development of millions of rural and semi-urban dwellers. Young cattle are highly susceptible to a range of diseases ranging from viral to parasitic and bacteria diseases. Bacteria infections are an important cause of death in large animal neonates (Fecteau *et al.*, 1997). Calf scour is one of the most common and multifactorial diseases of neonates principally caused by *E. coli* and remains a common devastating disease all over the world (Bhalerao *et al.*, 2000;

Kumar *et al*., 2013) . It constitutes a major health problem by causing heavy morbidity and mortality particularly in the developing countries (Malik *et al*., 2012). The public health implications of this condition are equally important. Numerous infectious agents causing diarrhoea in animals are zoonotic and have been associated with food-borne diseases (Trevejo *et al*., 2005). Calf scour is a complex disease, with many interrelated causes. Agent, host, and environmental factors collectively explain scours, and these factors interact dynamically over the course of time (David and Smith , 2007). Discontinuation or incomplete course of treatment and continuous indiscriminate use of antibacterial drugs against diarrhoeal infection of man and animal might have contributed to a new generation of virulent and resistant type of bacteria. Although routine laboratory isolation and drug sensitivity testing are expensive and impractical, the periodical check of the pattern of the drug sensitivity of organisms is more significant. It is, therefore, important that sensitivity of different bacteria isolated from diarrhoeic calves needs to be studied from time to time in order to formulate appropriate therapeutic regimens (Kaura *et al.,* 1988). A similar work was carried out in Bangladesh where bacteria associated with calf diarrhea was isolated and identified,the bacteria werecharacterised by different cultural, biochemical and serological tests and the antibacterial sensitivity of the isolates was carried out (Ansari *et al*, 2014). Antibiotics have being used for treating calf diarrhea cases in Nigeria for many years, but the bacteria population are altering their genetic composition to develop resistance to most commonly used antibiotics, as a result, generation of bacteria strains are being developed which are multidrug resistant and this unabated increase in incidence of bacteria resistance calls for use of new types of antibiotics by recurrent sensitivity test for prevention and control of bacteria diseases in animals (Nazir, 2004).

Reports on enteropathogens associated with calf diarrhoea are very limited in Nigeria. Therefore, an attempt was made to isolate bacterial pathogens, identify them and carry out sensitivity patterns of isolated organisms to commonly used antibacterial drugs in Nigeria in order to establish and recommend the most appropriate antibacterial agent against calf scour.

Materials and methods

The study was conducted from June 2014 to February 2015 on the calves from two farms in Oyo and five farms in Ogun state, south western Nigeria. Feacal samples were collected directly from the rectum of each diarrheic calf in the selected farms and were subsequently brought in ice packs to the laboratory of Department of Microbiology, University College Hospital, Ibadan, Nigeria. The faecal samples were stored at -20°C before analysis.

Collection of samples

A hundred and twenty diarrheic faecal samples were collected from the selected farms in the two states. Each sample was aseptically collected in a sterile sample bottle with ice pack and transported immediately to the laboratory of the Department of

Microbiology, University College Hospital, Ibadan for bacteriological examinations. Samples were analysed within 24–48 hours of collection.

Isolation of the bacteria

Bacterial isolation was done from the collected faecal samples. The isolation and identification of bacteria was carried out by standard methods as described by Cowan (1985). Nutrient broth (NB) was used for primary culturing of *E. coli* organisms that were present in the collected faecal samples. For the differentiation of the bacteria MacConkey (MC) agar medium was used followed by Eosin Methylene Blue (EMB) agar medium which was used as a selective medium and these were used according to the methods described by Cheesbrough (1984).

Identification and characterization of other bacteria

The isolated bacteria were characterised and confirmed with distinctive cultural characteristics, morphology and with Gram's staining and biochemical tests according to the methods of Cowan (1985) and Cheesbrough (1984).

Antimicrobial susceptibility tests

Antimicrobial susceptibility tests of all isolates was done on Mueller Hinton agar plates by Kirby Bauer's disc diffusion method according to performance standards of (Clinical and Laboratory Standards Institute (CLSI), 2006). The plates were then examined for the diameters of the zone of inhibition. Isolates were classified as susceptible and resistant according to the standard methods described by (CLSI, 2009). The antimicrobial agents used were gentamicin, ampicillin, amoxicillin, amikacin, ciprofloxacin, ceftriaxone and meropenem.

Results

Out of the total of 120 calves with clinical suspected cases that were screened, 50 from Ogun state and 70 from Oyo state, 19 calves (15.8%) were positive for culture, in all the 19 calves, *E. coli* was isolated from 12 calves (10%), *Salmonella spp.* was isolated in 10 calves (8.3%) and *Campylobacter spp.* was isolated in 1 calf (0.8%). Study of antibiotic sensitivity pattern of all isolates showed variation in their resistance. In general, all isolates of all organisms showed highest sensitivity to meropenem 91.3%, followed by amikacin with 82.6% sensitivity, ciprofloxacin had 60.9% sensitivity, ceftriaxone had 60.9% sensitivity, augmentin had 47.8% sensitivity, gentamicin had 47.8% sensitivity, while antibiotic with least sensitivity to all the isolates was ampicillin with 21.7% sensitivity as revealed in Fig. 1 .

Study of sensitivity pattern of various antibiotics to single isolate of *E. coli* indicated highest susceptibility to meropenem 91.7% and least susceptibility to ampicillin 16.7% and augmentin 16.7%. The general variation in sensitivity and resistance pattern of various antibiotics showed as follows; 8.3% resistance and 91.7% sensitivity to meropenem, 33.3% resistance and 66.7% sensitivity to amikacin, 66.7% resistance and 33.3% sensitivity to ceftriaxone, 66.7% resistance and 33.3% sensitivity to ciprofloxacin, 66.7% resistance and 33.3% sensitivity to gentamicin, 83.3% resistance and 16.7%

sensitivity to augmentin and 83.3% resistance and 16.7% sensitivity to ampicillin as shown (Fig. 2) .

Study of sensitivity pattern of various antibiotics to single isolate of *Salmonella spp.* revealed 0% resistance and 100% sensitivity to amikacin, 10% resistance and 90% sensitivity to ciprofloxacin ,10% resistance and 90% sensitivity to ceftriaxone, 10% resistance and 90% sensitivity to meropenem, 20% resistance and 80% sensitivity to augmentin, 40% resistance and 60% sensitivity to gentamicin, while the antibiotic with the least sensitivity to single isolate of *Salmonella spp.* was ampicillin with 70% resistance and 30% sensitivity, as shown in Fig. 3.

Study of sensitivity pattern of various antibiotics to single isolate of *Campylobacter spp.* showed 0% resistance and 100% sensitivity to meropenem, 0% resistance and 100% sensitivity to amikacin, 0% resistance and 100% sensitivity to ceftriaxone, 0% resistance and 100% sensitivity to ciprofloxacin, 0% resistance and 100% sensitivity to gentamicin, 0% resistance and 100% sensitivity to augmentin, while a single isolate of *Campylobacter spp.* showed 100% resistance and 0% sensitivity to ampicillin as shown (Fig . 4).

The sensitivity pattern of various antibiotics tested on all isolates from location A (Ogun state, Nigeria) indicated as follows; highest sensitivity of isolates were seen with augmentin, meropenem and amikacin, while the least sensitive among all the antibiotics used in this study location was ampicillin. Augmentin had 16.7% resistance and 83.3% sensitivity to isolates, meropenem had 16.7% resistance and 83.3% sensitivity to isolates, amikacin had 16.7% resistance and 83.3% sensitivity to isolates, ceftriaxone had 33.3% resistance and 66.7% sensitivity, ciprofloxacin had 33.3% resistance and 66.7% sensitivity, gentamicin had 50% resistance and 50% sensitivity, while ampicillin being the least sensitive antibiotic had 66.7% resistance and 33.3% sensitivity to all isolates in this location as shown (Fig. 5).

The sensitivity pattern of various antibiotics tested on all isolates from location B (Oyo state, Nigeria) indicated the most sensitive of all the antibiotics tested to be meropenem, while the least sensitive was ampicillin. The details is as follows; meropenem had 5.9% resistance and 94.1% sensitivity, amikacin had 18% resistance and 82% sensitivity, ceftriaxone had 41.2% resistance and 58.8% sensitivity, ciprofloxacin had 41.2% resistance and 58.8% sensitivity, gentamicin had 53% resistance and 47% sensitivity, augmentin had 64.7% resistance and 35.3% sensitivity, while ampicillin being the least sensitive antibiotic had 82.4% resistance and 17.6% sensitivity to all isolates in this location as shown in Fig 4.

Discussion

To have an effective managementof calf scour cases, study of bacteriological profile with their antibiotic sensitivity pattern would offer an important role. Calf scour still remains an essential cause of morbidity and

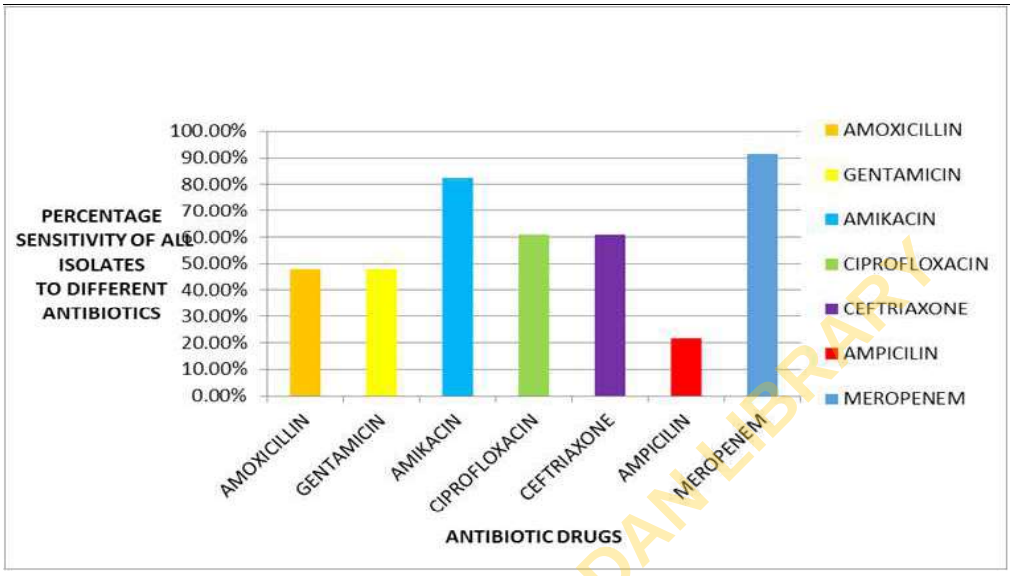

Figure 1: Summary of antibiotics sensitivity result for the three organisms isolated

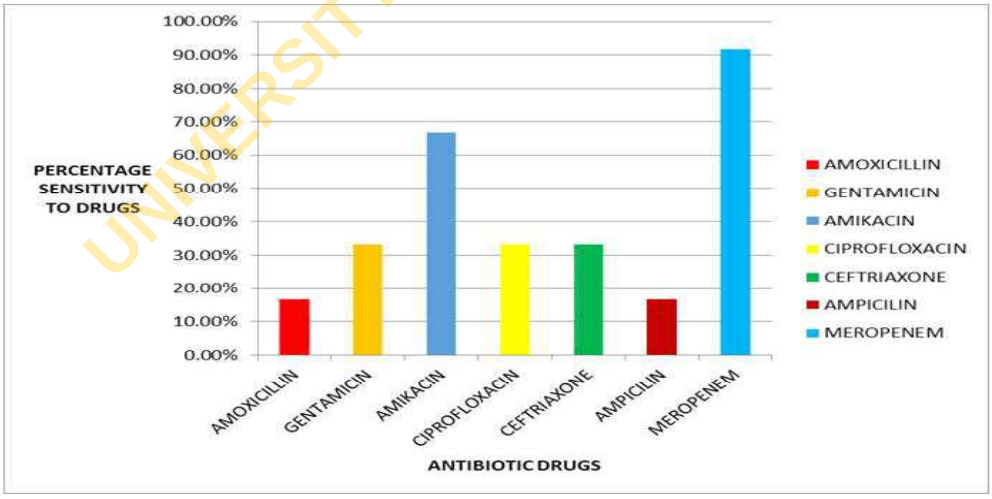

Figure 2: Antibiotics sensitivity result for *Escherichia coli* isolates

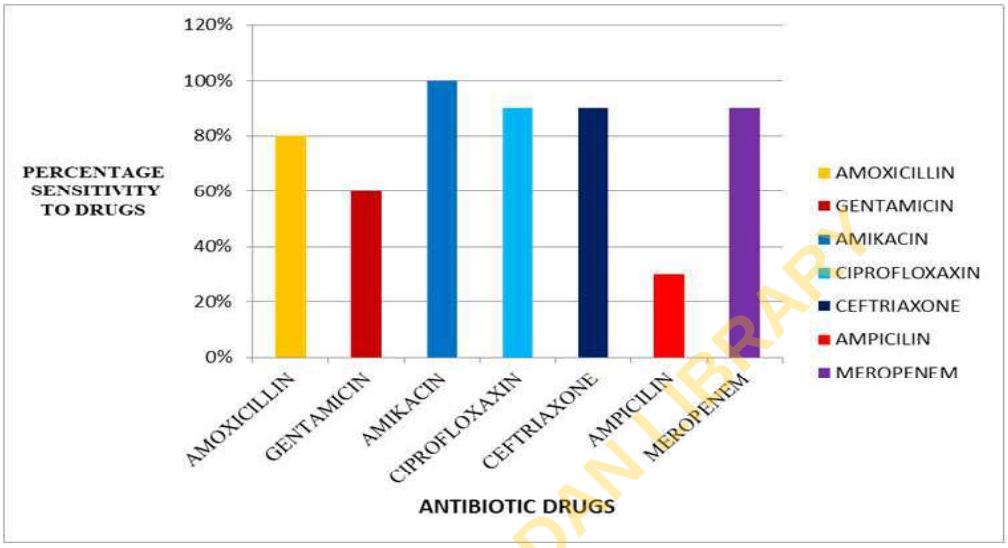

Figure 3: Antibiotics sensitivity result for *Salmonella* isolates

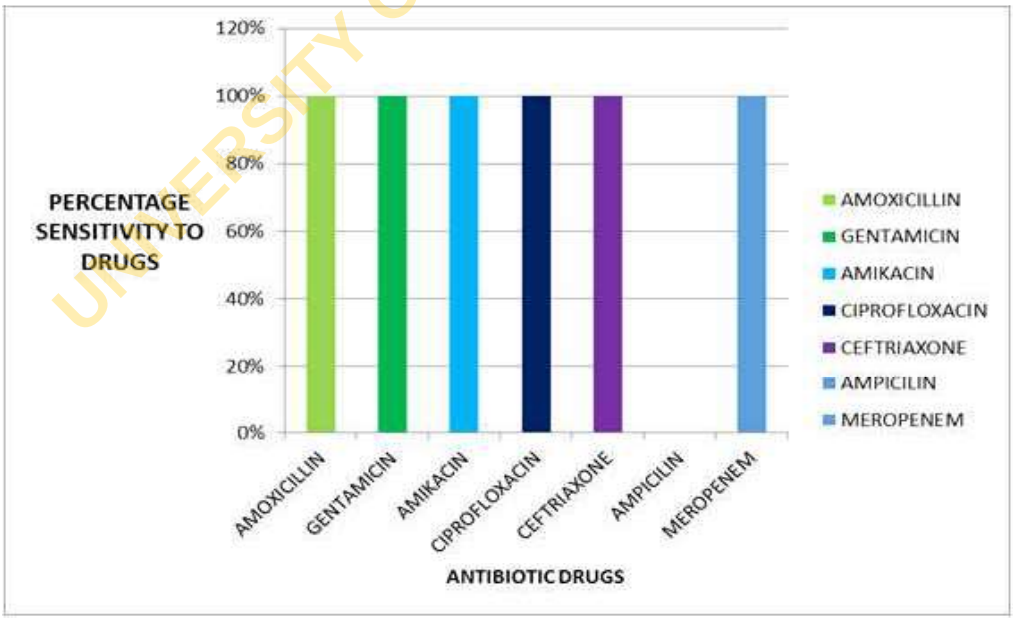

Figure 4: Antibiotics sensitivity result for *Campylobacter* isolates

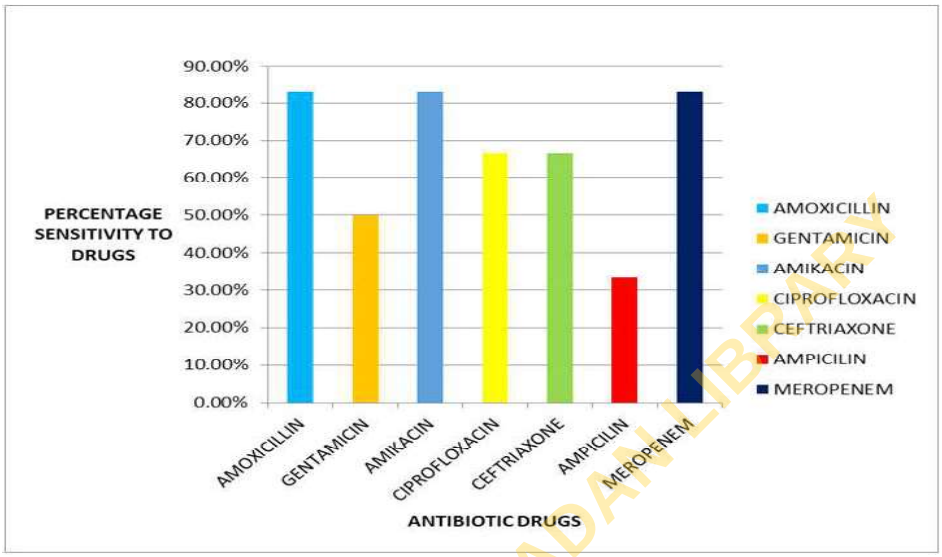

Figure 5: Antibiotics sensitivity result for all isolates from location A (Ogun State)

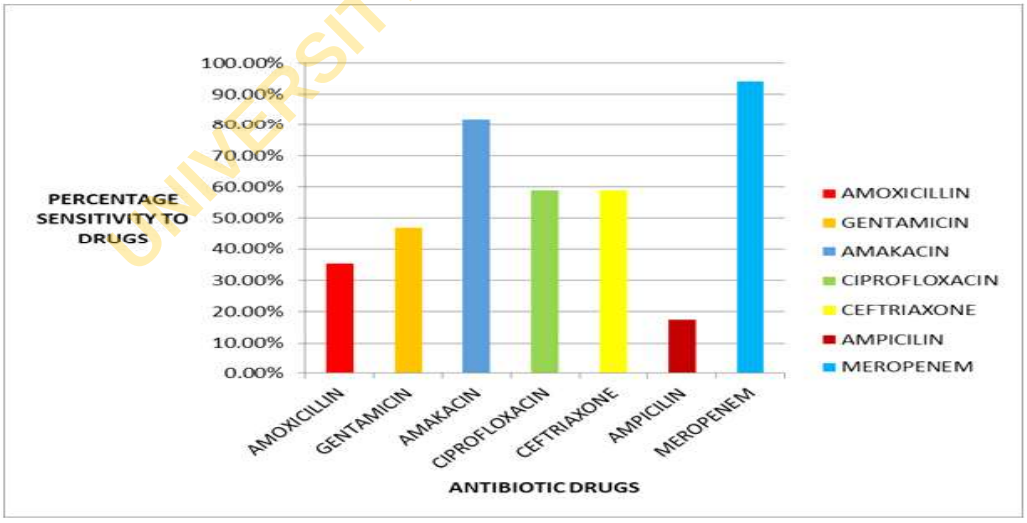

Figure 6: Antibiotics sensitivity result for all isolates from location B (Oyo State)

mortality in the first few months of a calves life. The search for an improved alternative treatment is still on-going and essential; due to the associated high mortality and its adverse socio-economic consequences as a result of wide spread multi drugs antibiotic resistance. Out of 120 calves that were sampled, *E. coli* appeared to be an important causative agent either alone or in combination with *Salmonella spp* and or *Campylobacter spp* in the etiology of calf diarrhea.

Many researchers reported higher prevalence of *E.coli* in calf scour cases, and this has been ascribed to the organism being the most prevalent and rapidly colonizes the alimentary tracts of Infants, as such,its frequency of isolation is greater than other bacteria (Smith and Halls, 1967). Hussain and Saikia (2000) also found *E. coli* to be the causative agent in majority of calf scour cases in India, about (73.12%). Navade *et al* (2000) noticed 53.37% prevalence of *E.coli* in neonatal diarrhoeic calves. Gupta *et al.* (2006) also observed only 23.72% prevalence rate of *E.coli*in diarrhoeic calves. Ahmed *et al.* (2010) reported prevalence of *E.coli*isolates (36.84%) from feacal samples of diarrhoeic lambs in Sokoto state, Nigeria. Similar reports have been made by Arshad *et al.* (2006) and Smith *et al.* (2009). The findings of these workers are more or less similar to the findings of our study. There is variation in the susceptibility of isolates against all the antibiotics used. The antibiogram study revealed that isolates of bacterial organisms from calf scour generally were most sensitive to meropenem, amikacin, moderately sensitive to ceftriazone and ciprofloxacin and less sensitive to ampicillin and augmentin. These findings corroborate the results of Nazir (2007), Ahmad *et al.* (1986) who stated that calf isolates were highly sensitive to ciprofloxacin, levofloxacin and resistant to ampicillin, erythromycin, gentamicin and amoxycillin. These results were slightly not in agreement with the findings of Tripathi and Soni (1982), Joshi *et al.* (1986), and Joon and Kaura (1993) who reported that most of the bacteria isolated from calf diarrhoea were highly sensitive to tetracycline, chloramphenical, and streptomycin; moderately sensitive to ampicillin, amoxicillin, less sensitive to penicillin, gentamycin and kanamycin. The difference in the sensitivity test could be due to difference in time when antibiotic resistance was less common. The variation in the susceptibility of various isolates to different antibiotics may be due to recent and reoccurring abuse and indiscriminate use of antibiotic in managing different diseases of cattle. These findings corroborate the result of Nazir (2007) and Ahmed *et al.* (1986) who stated that bacterial calf isolates were highly sensitive to ciprofloxacin, levofloxacin and resistant to ampicillin, erythromycin, gentamicin and amoxycillin. The findings of the present study indicate that the use of meropenem, amikacin and ciprofloxacin may have the preference to be the choice in clinical control of *E.coli, Salmonella,* and *Campylobacter* causing calf diarrhoea in Oyo and Ogun States, South western Nigeria.

Conclusions

This study has been able to identify most common bacterial pathogens in calf scour

cases with recommendation of meropenem and amikacin as antibiotics that should be used alone or in combination for managing calf scour cases.

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