

ENTEROPATHOGENS PRESENT IN DIARRHOEIC CALVES IN DAIRY HERDS IN TANZANIA

J.A. Matovelo, Sokoine University of Agriculture, Department of Veterinary Pathology, P.O. Box 3018, Morogoro, Tanzania.

SUMMARY

In an investigation for enteropathogens associated with diarrhoea in calves in dairy herds, 71 diarrhoeic and 46 non-diarrhoeic calves from 0 to 90 days old were sampled and analyzed for prevalence of various enteropathogens that may be responsible for diarrhoea in calves. The calves came from 4 dairy farms in three different locations. ELISA based techniques were used for detection of K99 *E.coli*, rotavirus, coronavirus and cryptosporidia. Coprological methods were used for detection and identification of coccidia. Further, bacteriological methods were used in screening for *Salmonella* spp. Results showed that diarrhoea in calves below the age of two weeks was relatively uncommon. Various enteropathogens were detected. Rotavirus was detected in 38% of diarrhoeic calves and in 26.1% of the non-diarrhoeic calves examined. Coronavirus was detected in 31% and in 4.3% of diarrhoeic and non-diarrhoeic calves respectively. K99 *E. coli* was detected in 42.1% of diarrhoeic calves and in 19.6% of non diarrhoeic calves. Cryptosporidia was detected in 25.8% of diarrhoeic calves and in 28.6% of non-diarrhoeic ones. Coccidiosis was detected in 51.6% of diarrhoeic calves in contrast to 16.7% in non-diarrhoeic calves. *Salmonella* spp. were detected in 7.3% and 0% of diarrhoeic and non-diarrhoeic calves respectively. Although six different enteropathogens were detected in the calves, causal effects were demonstrated for only three of them. It was statistically demonstrated that, significant differences in prevalence rates of enteropathogens between diarrhoea and non-diarrhoeic calves existed with respect to K99 *E. coli*, coronavirus and coccidia only. There was no significant difference ($P > 0.05$) in the prevalence rates with respect to rotavirus, cryptosporidia and *Salmonella* spp. between the diarrhoeic and non-diarrhoeic calves investigated. It is suggested that some of the detected enteropathogens had no causal effect. Such detections serve to indicate enteropathogens that are probably incidentally present in cases of diarrhoea caused by other agents.

INTRODUCTION

Calf mortality continues to pose a serious constraint in the expansion of the national dairy herd in Tanzania (Das *et al.*, 1988).

Morbidity and mortality studies involving several dairy herds in Eastern Tanzania have shown that as much as 23% of the calves born die before weaning (Shoo *et al.*, 1992). Diarrhoea accounts for 27%

of all deaths (Shaka, 1977; Shoo *et al.*, 1990, 1992). However, there have only been limited aetiological studies on calf diarrhoea in Tanzania (Mtambo *et al.*, 1997). Surveys in other countries show that the most frequent aetiologic agents are enterotoxigenic *E. coli* (ETEC), various serovars of *Salmonella enterica*, rotavirus, coronavirus and *Cryptosporidium* (Morin *et al.*, 1976; Snodgrass *et al.*, 1986; Fuente *et al.*, 1999). Lema and Banda (1991) investigated on the agents associated with diarrhoea in calves and found out that the agents were essentially the same as those identified in temperate countries. However, the relative importance of the enteropathogens identified and the role of coccidiosis in the aetiology of diarrhoea were not addressed in the cited study. This study therefore, sets out to identify the various enteropathogens present in calves with diarrhoea in Tanzanian dairy herds and to determine the relative importance of such pathogens.

MATERIALS AND METHODS

Faecal samples were collected from four different dairy farms. Two such farms were in one location. The other two were each located in different locations. The locations selected represented three different agroecological zones. One in semiarid area at Mpwapwa in Dodoma, one at the Sokoine University of Agriculture (SUA) which is considered to represent an equivalent of a tropical savannah

and the remaining two in the southern highlands at Uyole and Kitulo which is characteristically temperate.

Basically two sample collection visits were made to each farm. At each visit fecal samples were collected from diarrhoeic and non-diarrhoeic calves. The non-diarrhoeic calves were picked up randomly. The faecal samples were collected per rectum, put into plastic tubes, transported in ice packs and stored at 4°C until examined. A total of 71 diarrhoeic and 46 non-diarrhoeic calves were sampled for the investigation.

The samples were screened for rotavirus, coronavirus, and the K99 *E. coli* by monoclonal antibodies in qualitative enzyme linked immunoassay (ELISA) kits supplied as "Pathasure Bovine Enteritis" ELISA kits (Cambridge Veterinary Sciences Limited, Henry Crabb Road, Littleport, Ely, Cambs CB 6 1SE UK). The same samples were also screened for *Cryptosporidia* using anti-*Cryptosporidium* monoclonal antibodies specific for *Cryptosporidium parvum*, *Cryptosporidium muris* and *Cryptosporidium baileyi* in ELISA kits from the same supplier. Further, some of the diarrhoeic and non diarrhoeic fecal samples were cultured on Blood agar, Selenite broth and McConkey agar for isolation of *Salmonella* spp.

Fecal samples were also screened for coccidia. The screening for

coccidia was done by direct examination of faecal samples suspended in saturated salt solution (Anon, 1986) followed by counting of *Eimeria* oocysts on the McMaster slide (Anon, 1986). Differentiation of oocysts into species, was by examination of oocysts induced to sporulate following incubation of faecal samples in 2% potassium dichromate in shallow layers in petridishes kept at room temperature for 4 days and by micrometric determination of the average dimensions of oocysts (Anon, 1986).

The number of samples analyzed for different enteropathogens depended on the availability of analysis kits and other reagents. 71 samples from diarrhoeic and 46 samples from non-diarrhoeic calves were investigated for K99 *E.coli*, rotavirus and coronavirus. 62 samples from diarrhoeic and 42 samples from non-diarrhoeic calves were investigated for coccidia and cryptosporidia. Whereas, 41 samples from diarrhoeic and 5 samples from non-diarrhoeic calves were investigated for *Salmonella* spp.

The normal approximation method was used for the analysis of the differences in proportional prevalences of enteropathogens between the diarrhoeic and non-diarrhoeic calves.

RESULTS

The faecal sample analysis revealed that on the farms selected, calf diarrhoea was most common in animals aged between one and three months (Table 1). The classic neonatal diarrhoea usually observed during the first week of life was far less common.

The relative importance of the different pathogens detected in the cases of diarrhoea as portrayed by their frequency of detection are summarized in Tables 1, 2 and 3.

Coccidia were detected in 53.2% of diarrhoeic calves in contrast to detection in 16.7% of non-diarrhoeic calves investigated. K99 *E. coli* was detected in 42.3% of the diarrhoeic calves and in 19.6% of the non-diarrhoeic calves. Coronavirus was detected in 31% and 4.3% of diarrhoeic and non-diarrhoeic calves respectively. Rotavirus was detected in 38% and 26.1% of diarrhoeic and non-diarrhoeic calves respectively. Whereas, cryptosporidia were detected in 25.8% and 28.6 of diarrhoeic and non-diarrhoeic calves respectively. *Salmonella* spp. were detected in 7.3% of the diarrhoeic calves and none were detected in the samples from non-diarrhoeic calves (Tables 1 & 2).

Table 1: Distribution of enteropathogens in diarrhoeic calves according to age

Enteropathogen	Age of calves			Number of calves examined	Overall percentage of positive cases for all ages
	0-14 days	15-30 days	1-3 months		
Coccidia	1	16	16	62	53.2
K99 <i>E. coli</i>	4	10	16	71	42.3
Coronavirus	3	5	13	71	29.6
Rotavirus	1	8	18	71	38
Cryptosporidia	3	5	8	62	25.8
Salmonella	2	1	0	41	7.3
Total	12	25	37	N.A.	N.A.

N.A. = Not Applicable

Table 2: Actual counts on the distribution of enteropathogens in non-diarrhoeic calves according to age

Enteropathogen	Age of calves			Number of calves examined	Overall percentage of positive cases for all ages
	0-14 days	15-30 days	1-3 months		
Coccidia	0	4	3	42	16.7
K99 <i>E. coli</i>	0	4	5	46	19.6
Coronavirus	0	1	1	46	4.3
Rotavirus	0	6	6	46	26.1
Cryptosporidia	0	6	6	42	28.6
<i>Salmonella</i> spp.	0	0	0	5	0

A statistical analysis of the sample proportions for diarrhoeic and non-diarrhoeic calves revealed that the prevalence of enteropathogens was significantly higher in diarrhoeic calves than in non-diarrhoeic ones for only three of the enteropathogens detected (Table 3). The prevalences of K99 *E. coli*, coccidia and coronavirus were significantly higher in diarrhoeic

calves when compared to prevalences in non-diarrhoeic calves. There was no significant difference between diarrhoeic and non diarrhoeic calves with respect to the prevalence of rotavirus, cryptosporidia and *Salmonella* spp. (Table 3). Further, there was no significant difference in the prevalence of enteropathogens between age groups for the non-

diarrhoeic calves (Table 2). Table 4 shows the relative importance of the different species of *Eimeria* where it is reflected by the frequency of detection amongst cases with coccidia as encountered during this study.

All calves except one exhibited infection by more than one species of *Eimeria*. *Eimeria bovis* was found to be the most common species of

coccidia followed by *Eimeria zuernii* and *E. cylindrica*. *E. canadensis*, and *E. auburnensis* in rapidly decreasing proportions (Table 4).

Overall *E. bovis* was present in 73.8% of all cases in which coccidia were detected, second in the list is *E. zuernii* detected in 53.9% of the cases. The least common ones being *E. subspherica* and *E. pellita*.

Table 3: Relative prevalence of different enteropathogens in diarrhoeic and non-diarrhoeic calves

Enteropathogen	Actual counts of positive samples against the number of samples investigated			
	Diarrhoeic calves	Non-diarrhoeic calves	Difference between the two groups	Level of significance
K99 <i>E.coli</i>	30/71	9/46	Significant	P < 0.05
Coronavirus	21/71	2/46	Significant	P < 0.01
Rotavirus	27/71	12/46	NS	P > 0.05
Cryptosporidia	16/62	12/42	NS	P > 0.05
Coccidia	33/62	7/42	Significant	P < 0.01
Salmonella spp	3/41	0/5	NS	P > 0.05

NS = Not Significant (P > 0.05)

Table 4: Relative importance of various *Eimeria* spp. in bovine coccidiosis in the investigated farms

Location	No. of calves with coccidia	Actual counts of number of calves demonstrating the various <i>Eimeria</i> species in the 32 diarrhoeic and 7 non-diarrhoeic calves.							
		<i>E. bovis</i>	<i>E. zuernii</i>	<i>E. auburnensis</i>	<i>E. canadensis</i>	<i>E. cylindrica</i>	<i>E. ellipsoidal</i>	<i>E. subspherica</i>	<i>E. pellita</i>
Diarrhoeic calves	32	29	19	7	8	10	3	2	3
Non-diarrhoeic calves	7	5	4	0	2	3	0	0	4

DISCUSSION.

According to Morin *et al.*, (1976), Moon *et al.*, (1978) and Abraham *et al.*, (1992), calf diarrhoea is a health problem with highest impact in the first 5 weeks of life. All these studies were restricted to viral and bacterial enteropathogens.

However, the classic neonatal diarrhoea that is mostly attributed to viral and bacterial enteropathogens that is usually observed during the first week of life (Snodgrass *et al.*, 1986; Fuente *et al.*, 1999) was found to be less common in this study. On the contrary, diarrhoea was found to feature more prominently after the first two weeks of life and a higher prevalence was observed during the calves' second and third months of life.

Detection of the bacterial and viral enteropathogens in diarrhoeic calves past the age of one month is unusual, but similar observations were made by Abraham *et al.*, (1992) who detected small numbers of bovine enteric coronavirus, rotavirus and K99 *E. coli* in calves as old as 8 weeks. However, this study has shown that proportional detections of bacterial, viral and protozoal enteropathogens in calves past the age of one month are about the same in both the diarrhoeic and non-diarrhoeic calves. This observation suggest that some of the detected enteropathogens had no causal effect. Such detections

serve to indicate enteropathogens that are probably incidentally present in cases of diarrhoea caused by other agents.

Further, it has been shown that coccidia were the single most important cause of diarrhoea in calves at the investigated farms. In the same process, the relative importance of various *Eimeria* species as causes of calf diarrhoea in Tanzania's dairy herds has been demonstrated.

Salmonella spp. were isolated from only three of the diarrhoeic calves involved in this study. However, both the existence and importance of *Salmonella* spp. in Tanzanian dairy herds has been reported in previous studies by Lema and Banda (1991). These workers were able to isolate *Salmonella* spp. from 19.02% of the diarrhoeic faecal samples they investigated.

The relative paucity of diarrhoea in calves below the age of two weeks, is likely to be an attribute of the method of calf rearing in the farms. In all the farms involved, calves were allowed to stay and suckle colostrum directly from their dams, a practice whose favourable effects have been well demonstrated in studies where the health performance of suckled calves has been compared to that of pail fed ones (Selman *et al.*; 1970; 1971).

It is apparent therefore that, amongst other issues, the survival of calves and consequently the

expansion of the national dairy herd can be improved by moving to control the enteropathogens responsible for diarrhoea in calves.

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