RISK FACTORS ASSOCIATED WITH BOTH BLOOD AND INTERNAL PARASITES IN DAIRY FARMS IN KUKU AREA, KHARTOUM STATE

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المستخلص

اظهرت نتائج تقصي الإصابة بطفيليات الدم والطفيليات الداخلية في مواسم مختلفة (البارد الجاف، الحار الجاف ، الحار الرطب) في مزارع الألبان بحلة كوكو في ولاية الخرطوم ، بارتفاع معدل انتشار للإصابة بطفيليات الدم في الموسم البارد الجاف (٤٦%) مقارنة بالموسم الحار الجاف والحار الرطب (١٤,٧٤%، ٥,٦٨%) على التوالي. بينما كانت نسبة الإصابة بالطفيليات الداخلية اقل من نسبة الإصابة بطفيليات الدم (١٥% - ٨,٤٢% -١١,٣٦ %) للموسم البارد الجاف والحار الجاف والحار الرطب على التوالي . وجدت علاقة موجبة (٠,٠١ >P- ٤٨,٤٨٣=χ^2) بين الموسم والإصابة بطفيليات الدم حيث يعتبر الموسم عامل وقائي (٠,٢٤٤ = OR) وعلى العكس لم تتم ملاحظة علاقة موجبة بين الموسم والإصابة بالطفيليات الداخلية (٠,٠٥< P - ٢,٠٥٨=χ^2). وجود القراد ذو أهمية من الناحية الإحصائية(٠,٠١>P - ٢٠,٥٨٣=χ^2) للإصابة بطفيليات الدم ويعتبر عامل خطر(٣,٥٨٦ = OR). حجم كريات الدم المتراص (PCV) ذو علاقة وثيقة (٠,٠١>P - ٦,٥٧٣ - ٩, ٦٧٩ =χ^2) بالإصابة بطفيليات الدم والطفيليات الداخلية على التوالي.

Key words: Blood parasites, internal parasites, Risk factors, Sudan

Abstract

Investigation of blood and internal parasites among different seasons (dry cool, dry hot and wet hot) in dairy herds in Kuku area of Khartoum State, revealed that there was a high prevalence of blood parasites in the dry cool season (46%) compared to dry hot and wet hot season (14.74% and 5.68%, respectively). While the presence of internal parasite was found to be less than infection with blood parasites (15%, 8.42% and 11.36 for dry cool, dry hot and wet hot season, respectively). A positive association (χ^2=48.483, P<0.01) was found between season and presence of blood parasites and season is considered to be a protective factor (OR= 0.244). In contrast, presence of internal parasites was not associated with season (χ^2 = 2.058, P>0.05). Tick infestation was found statistically significant (χ^2 = 20.583, P<0.01) with respect to presence of blood parasites and could be a risk factor (OR=3.586). Packed cell volume (PCV) was found strongly associated with both blood and internal parasites (χ^2= 9.679 &6.573 P<0.01, respectively

Introduction

Animal resources in the Sudan are considered as one of the largest in the Arab and African countries, and estimated according to the records of the Ministry of Animal Resources and Fisheries (2003) as 132,340,000 head including different species. Cattle played a significant role in the economic cycle in rural and urban areas. Intensive and semi-intensive production system of Sudan distributed either within aggregation sites in different locations or in small herds located in different sites around towns. The high needs for animal proteins especially milk and milk products in recent years in Khartoum State oriented the producers to import highly milk producing foreign breeds to meet the human consumption, which had been increased recently as the result of human population increase and urbanization as well as the migration from other states due to natural disasters and conflicts.

The high increase of cross-bred cattle in kuku area played an important role in dissemination of health problems due to the their high susceptibility to different causative agents (particularly parasitic

infections). In addition, the bad husbandry and poor management in the farms complicated the health status of dairy cattle. Other diseases are fairly under control by using vaccines or chemotherapeutic preparations. Parasitic diseases affect the milk industry by the direct effect on milk production, high cost of the treatment and financial implications for farms management to prevent the parasitic infestations for difficulty to control vectors. Therefore, this study was planned to investigate the presence of blood and internal parasites in dairy cattle during different seasons in Kuku area and to analyze the risk factors that associated with parasitic infections.

Materials and Methods

Study area

The study was conducted in Khartoum State which is situated in Northern Sudan between latitude 16°N and 14°N. It is regarded as one of the areas of intensive and semi-intensive production system in Sudan. The climate is an arid type which is characterized by a wide range of daily and seasonal temperature. A temperature of 45°C may occur during the summer with hot dry weather and low humidity. During winter the weather is cool and dry with a mean daily temperature of 24°C. The maximum rainfall is from mid July to mid September, in this season there is an increase in relative humidity with a maximum of 68% in August.

Study population

Cattle in dairy farms of Kuku area in Khartoum state were sampled regardless to the sex and age. The description of the study population in the study site is shown in Table 1.

Sampling

Selection of the dairy farms was done according to the willingness of the owners to participate (convenience sampling, Thrusfield (1996). A total of 100, 95 and 88 of animals were sampled among different seasons from 10 dairy farms in Kuku area.

Blood samples collection

A total of 283 blood samples in different seasons were collected in the morning form the jugular vein of the cattle using vacutainer with EDTA as an anticoagulant. Samples were placed in ice pox at 4 C° and transferred as soon as possible to the laboratory before processing parasitological examinations.

Faecal samples collection

A total of 283 fresh faecal samples in different seasons were collected. Each sample was collected directly from the rectum in plastic bags. The bags were labeled and immediately transferred to the laboratory for faecal examinations.

Packed Cell Volume (PCV)

The capillary tubes were put in the haematocrit centrifuge which was run for 5 minutes. Then the tubes were taken and put into the haematocrit reader to get results.

Parasitological examinations

Wet mount

One drop of fresh blood was placed on a slide, covered with a cover-slip and examined microscopically for detection of the motility fot the trypanosomes at 10×40 magnification.

Buffy coat examination

Buffy coat examination was done using capillary tubes, and then haematocrite centrifuge was used for centrifugation for 5 minutes. After centrifugation the capillary tubes were placed on clean slide and covered with one drop of distilled water, then examined microscopically at 10×40 magnification to detect trypanosomes and microfilariae (Woo, 1970).

Thin blood film

Thin blood films were prepared on slides, dried and fixed with absolute alcohol, stained with 5% diluted Giemsa stain solution for 45 minutes. Stained blood films were washed using distilled water, dried and examined microscopically at 10×100 magnification for detection of blood parasites.

Table 1: Description of the dary cattle examined in Kuku area

Unit	Season		
	Dry cool (%)	Dry hot (%)	Wet hot (%)
Total No. of animals examined	100	95	88
Breed			
local breed	2 (2.00 %)	2 (2.11%)	2 (2.27%)
cross breed	(98.00%)	93 (97.89%)	86 (97.73%)
Sex			
male	2 (2.00 %)	2 (6.32%)	2 (2.27%)
female	98 (98.00%)	93 (97.89%)	86 (97.73%)
Age (years)			
0-3	7 (7.00 %)	6 (2.11%)	4 (4.55%)
4-6	64 (64.00%)	47 (49.47%)	44 (50.00%)
> 6	29 (29.00%)	42 (44.21%)	40 (45.45%)

Dry cool: February-March Dry hot: May-June Wet hot: August-September

Faecal examinations

Flotation method

Two to three grams of the faeces were taken in a mortar, the samples were covered with saturated sodium chloride solution. The solution was powered through a tea sieve in to a beaker to remove the large particles. Some of the solution was poured into small bottle until it was completely filled to make a convex meniscus at the top. Then it was covered with a clean grease-free slide. The slide was removed after 10 minutes and examined under low power × 20 magnifications. The examination was done systemically to cover all the cover slip for detection of the eggs (Angus, 1978).

Sedimentation method

Tow-three grams of the faeces were mixed with water and put it in tubes. Then, the tubes were centrifuged using centrifugation for 5 minutes for three times. The deposits were taken and placed on slides with covers slip and examined microscopically at high power × 40 magnifications for detection of the eggs (Angus, 1978).

Data analysis

Stata 6.0 for Windows 98/95/NT was used for data analysis. Chi-square (χ^2) was used for assessing the statistical associations of various factors for presence of blood and internal parasites. Logistic regression model was employed to obtain the odds ratio (OR) only for those factors which gave statistical significance by using the Chi-square (χ^2). When (OR) was greater than one, the factor is considered to be a risk factor. While, OR was less than one, the factor is considered to be a protective factor.

Results

The presence of blood parasites was 46% (n= 100), 14.74% (n= 95) and 5.68% (n= 88) in the dry cool, dry hot and wet hot season, respectively. While, 15%, 8.42% and 11.36% were recorded for infection with internal

parasites in the dry cool, dry hot and wet hot season, respectively (Table 2). Mixed infection was only found in the dry cool and wet hot season (7% and 1.13%, respectively).

High prevalence of *Theileria* species infection was reported in the dry cool season (39%) compared to dry hot and wet hot season (14.74% and 5.68%, respectively). In contrast, prevalence of *Babesia* species infection and filarial worm infection were only recorded in the dry cool season (6% and 1%, respectively) (Table 3). On the other hand, *Coccidia* species infection was observed in different seasons at a prevalence of 6%, 3.16% and 5.68% for the dry cool, dry hot and wet hot season, respectively. High prevalence of *Fasciola* species infection (7%) was reported in the dry cool season; while high prevalence of *Schistosoma* species infection (5.26%) was reported in the dry hot season (Table 4). A positive association (χ^2= 48.483, $P< 0.01$) was found between season and blood parasites and Odd Ratio (OR= 0.244) indicated that the season is a protective factor (infection was less in hot season compared to dry cool season) (Figure 1). In contrast, there was no association (χ^2 = 2.058, $P> 0.05$) between the season and internal parasites infection(Figure2)

The age was found to be associated with blood parasites infection (χ^2= 6.211, $P< 0.05$) and could be a protective factor (Odd Ratio (OR) = 0.643) (old animals are at lower risk to be infected compared to young animals). Negative association (χ^2= 0.138, $P> 0.05$) was recorded for breed and presence of blood parasites infection. Statistical analysis of breed and age and internal parasites infection indicated that there was no association (χ^2= 0.149, $P >0.05$ and χ^2 = 2.475, $P > 0.05$, respectively). A positive association (χ^2= 20.583, $P< 0.01$) was obtained for tick infestation and blood parasites infection, this was confirmed by the result of Odd Ratio (OR= 3.586) which revealed that the tick infestation could be a risk factor. A strong association (χ^2=9.679, $P < 0.01$) was recorded between infection of blood parasites and Packed Cell Volume (PCV) and the same result was obtained for infection with internal parasites (χ^2= 6.573, $P< 0.05$), all results are given in Table 5.

Table 2: Summary of the results of blood and internal parasites infection among different seasons in dairy farms in Kuku area

Unit	Season		
	Dry cool (%)	Dry hot (%)	Wet hot (%)
Total no. of animal examined	100	95	88
Packed Cell Volume (PCV)			
1. normal	20(20.00)	29(30.53)	18(20.45)
2. abnormal	80(80.00)	66(69.47)	70(79.55)
Tick infestation			
1. mild	51(51.00)	93(97.89)	87(98.86)
2. moderate	49(49.00)	2(2.11)	0(0.00)
3. high	0(0.00)	0(0.00)	1(1.14)
Blood parasites			
1. positive	46(46.00)	14(14.47)	5(5.68)
2. negative	54(54.00)	81(85.26)	83(94.32)
Internal parasites			
1. positive	15(15.00)	8(8.42)	1(11.36)
2. negative	85(85.00)	87(91.58)	78(88.64)

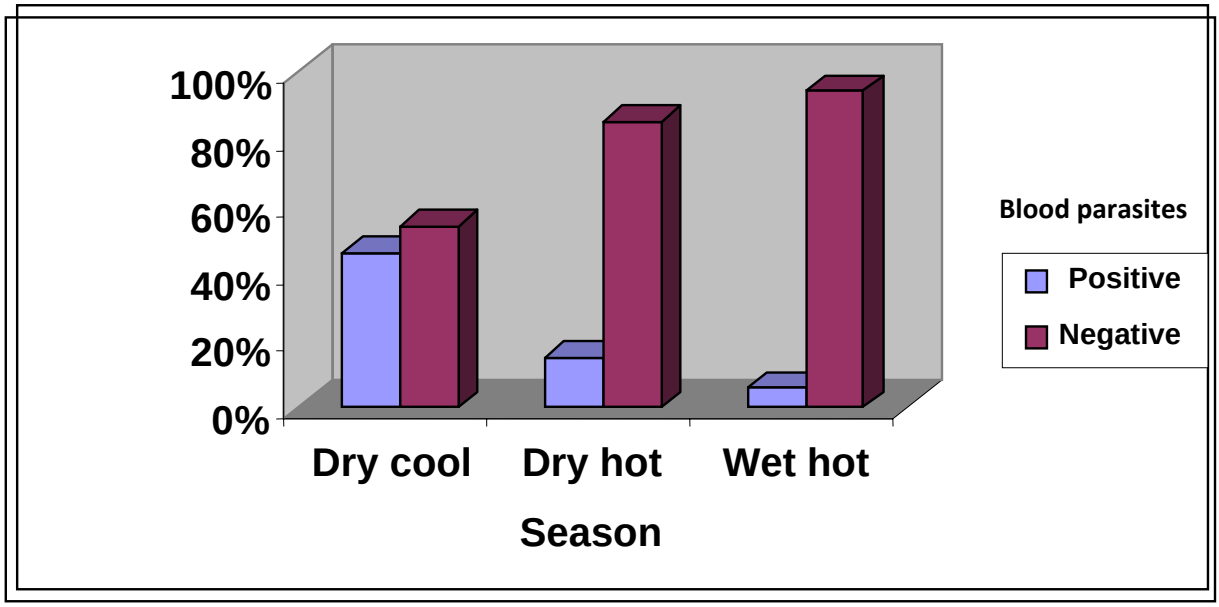
Dry cool: February-March Dry hot: May-June Wet hot: August-September

PCV: Adult cow: normal 28.4-38.8 abnormal < 28.3 - Calf: normal 32.0-39.7 abnormal < 32.1.

Diagnosis of blood parasites infection based on blood film, wet mount and puffy coat.

Diagnosis of internal parasites infection based on the faecal examinations using floatation and sedimentation.

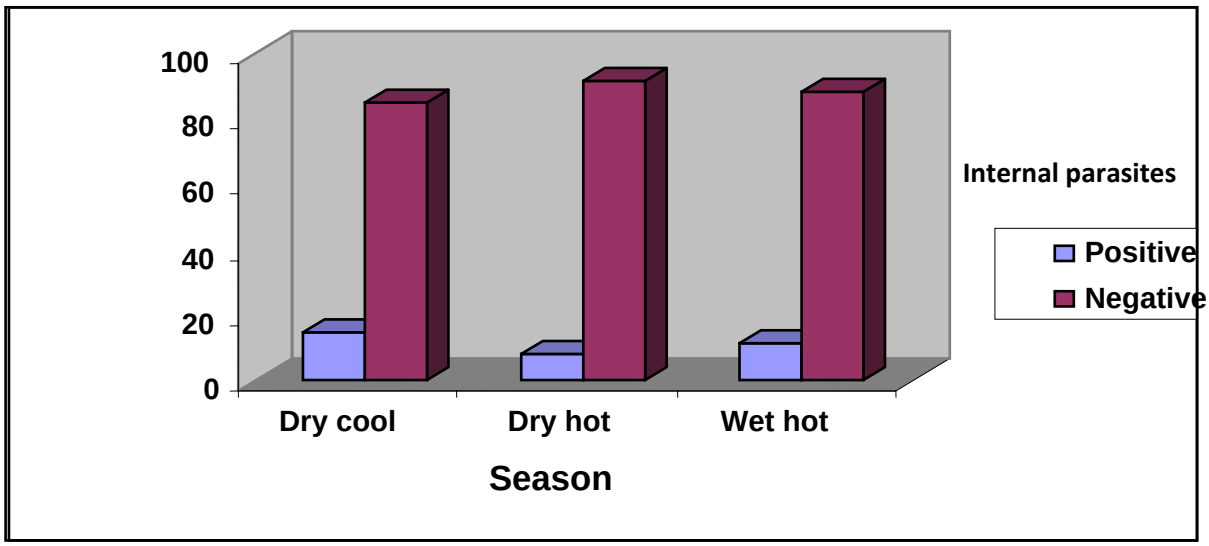
Figure 1: The effect of season on blood parasites infection in dairy farms in Kuku area



Chi-square (χ^2) = 48.483 (highly significant, $P < 0.01$)

Odd Ratio (OR) = 0.244 (OR < 1, a protective factor).

Figure 2: The effect of season on internal parasites infection in dairy farms in Kuku area



Chi-square (χ^2) = 2.058 *(*not significant, $P > 0.05$)

Table 3: The prevalence of blood parasites infection among different seasons in dairy farms in Kuku area

Season	No. of animal Examined	Prevalence (%)			Over all prevalence
		Theileria spp.	*Babesia spp.*	*Filaria spp.*	
Dry cool	100	39.00	6.00	1.00	46.00
Dry hot	95	14.74	0.00	0.00	14.74
Wet hot	88	5.68	0.00	0.00	5.68

Prevalence of blood parasites infection based on thin blood film, wet mount and puffy coat

Dry cool: February-March Dry hot: May-June Wet hot: August-September

Table 4: The prevalence of internal parasites infection among different seasons in dairy farms in Kuku area

Season	No. of animal Examined	Prevalence (%)				Over all prevalence
		Coccidia spp.	*Fasciola spp.*	*Schistosoma spp.*	*Paramphistomum spp.*	
Dry cool	100	6.00	7.00	1.00	1.00	15.00
Dry hot	95	3.16	0.00	5.26	0.00	8.42
Wet hot	88	5.68	4.55	1.14	0.00	11.36

Prevalence of internal parasites infection based on faecal examination using floatation and sedimentation methods

Dry cool: February-March Dry hot: May-June Wet hot: August-September

Table 5: The association between blood and internal

Factor	Chi-square (χ^2)		*P*-value	
	Blood parasites	Internal parasites	Blood parasites	Internal parasites
Breed	0.138	0.149	0.711	0.699
Age	6.211	2.475	0.045*	0.290
Tick infestation	20.583	-	0.000**	-
Packed Cell Volume (PCV)	9.669	6.573	0.008**	0.037*

Parasites infection and various factors in dairy farms in Kuku area

* Significant difference (P <0.05) while ** highly significant difference (P<0.01)

Age: (OR= 0.643), protective factor while tick infestation (OR= 3.586), risk factor for blood parasites infection

Discussion

The results of this study showed that infection with blood and internal parasites were common in selected dairy herds in the study site (46% and 15% at the dry cool season, respectively. Similarly, investigations on blood and internal parasites in different production system in Sudan had been made by different researchers. Saad (2004) stated that *Theileria*, *Babesia*, and Microfalria were prevalent in dairy farms in the White Nile and Gizera States. El Hussein *et al.* (1991) recorded *Theileria annulata* infection in 37% of apparently healthy cattle in River Nile state, Northern Sudan. He also found that the prevalence was higher in adult cross-breed rather than indigenous cattle. Presence of blood parasites in the study site was most likely attributed to the management. From an epidemiological point of view, husbandry and general conditions in dairy farms were strongly associated with the presence of both infectious and non-infectious diseases. It was observed that tick infestation was one of the great problems in dairy farms in Kuku area due to lack of application of proper management practices and misuse of acaricides. Moreover, the pens were poorly designed, made of mud and metals which act as a hiding place and source of ticks infestation. It was also observed that there was no routine screening for blood parasites or prophylactic use of anti-protozoal drugs in the study site. On the other hand, infection with internal parasites was well documented from different production system of Sudan. An investigation of diseases in dairy cattle in the White Nile and Gezira States by Saad (2004) revealed that the prevalence of fascioliasis was 34.4% and 14.4%, respectively, while paramphistomiasis prevalence was 40% and 17.6%, respectively. In addition, the same author recorded a low prevalence of schistosomiasis (4%) in the White Nile area. The presence of infection with internal parasites in Kuku area is mostly due to poor hygiene in the farms resulting from infrequent removal of animal dung's as well as the animals were crowded in the center of pens where there was a partial shade. Further more, the animals were fed on fodder purchased from the market which increases the risk of infection

with internal parasites as contamination with infective stages can happen at any point. It was observed that in Kuku dairy farms there was a continuous introduction of new animals particularly from areas which are known to be endemic for schistosomiasis and facioliasis such as Gezira and White Nile areas.

Our study revealed that there was no association between the season and internal parasites infection (χ^2= 2.058, P>0.05). This finding is in contrast with Fischer and Say (1989), who stated that the out breaks of internal parasites infection in pasture were associated with the dry season and the beginning of the rainy season. Based on that they recommended that the application of anti-helmentics should be given at the end of the rainy season and another dose at the end of the dry season. The disagreement is attributed to the type of production system as most of the studies on the effect of the season on the prevalence of internal parasites had been done in pastoral production system. This study was conducted in semi-intensive production system where environmental conditions and feeding are maintained at adequate levels throughout the year. An association was reported between season and infection with blood parasites (χ^2= 48.483, p< 0.01,(OR) = 0.24 and infection was low in the the hot season). It was suggested that the environmental factors in grazing season might influence the maturation of parasites in salivary glands of ticks (Kamio *et al*., 1990).

As seen from the results of this study, there was no significant association observed due to the breed and infection with internal parasites. In contrast, an epidemiological investigation conducted in dairy farm in Uganda (Magona and Mayende, 2002), indicated that infection with Fasiola and gastrointestinal nematode infection is higher in exotic breeds compared to the local breed (Zebu and Sahiwal). Moreover, Duval (1997) explained that an animal which had never been exposed to infection with worms can not develop resistance and immunity. Local breed have a high ability to prevent or limit establishment or subsequent development of worms infection due to the long, previous and continuous exposure to infection with internal parasites. The breed was also not associated with

infection with blood parasites (χ^2= 0.138, p> 0.05). The result disagrees with Bock (1999), who investigated the effect of cattle breed on the transmission rates and innate resistance to *Babesia* species. He observed that cross-bred cattle showed a maximum depression in packed cell volume (PCV) due to infection with *Babesia* species, while pure-bred cattle have a high degree of resistance to babesiosis. Another research work from Costa Rica (Perez et al., 1994) indicated that the breed was determined as a risk factor in the sero-prevalence of *Anaplasma marginale* and *Babesia bovis*. The disagreement which was observed in this study is attributed to the fact that most of cattle population (98%) in dairy farms in Kuku area was cross-bred and this might have affected the outcome of statistical analysis. There is another effect due to the sample size: in cross-sectional study there is no evidence that the factor is strongly associated with the occurrence of disease when the sample size is low.

Age was significantly associated with blood parasites infection (χ^2= 6.211, p< 0.05, OR = 0.643) (old animals are at lower risk to get infection as compared to young animals) . The same result was obtained from Latif *et al*. (1979) who stated that calf at 3, 6, 12 and 18 months demonstrated the presence of *Babesia* infection, while after 22 months no parasites could be demonstrated. Perez et al. (1994) also explained that cattle (over one year) as well as calves less than one year were highly susceptible to Anaplasmosis and Babesiosis. Moreover, Ahmed (1997) reported that the infection with tropical theileriosis was higher in calves rather than adult cattle among the cattle admitted to the National Veterinary Teaching Hospital, Khartoum during the period 1990-1995. No association was obtained for age and internal parasites infection (χ^2= 2.475, P>0.05). This result is in contrast with Duval (1997) who stated that age as well as weight of animals determine susceptibility to parasites. Young animals do not have a great deal of immunity to parasites during the first year in the pasture. His study also revealed that adult animals are much less susceptible to most parasites, unless they are in poor living conditions. The same justification which was made for the effect of breed

on presence of internal parasite concerning the results of data analysis and sample size was also observed. Tick infestation was positvely (χ^2= 20.583, *P*< 0.01, OR= 3.586) associated with infection of blood parasites. Similarly, Aderiano *et al*. (1994) found a significant association between the presence of ticks and infection with *Protozoa* and *Rickettsiae*. A strong association was also obtained for Packed Cell Volume (PCV) with regard to blood parasites infection (χ^2= 9.679, *p*< 0.01). A study by Shiono *et al*. (2004) indicated that anemia is the most important clinical manifestation in cattle infected with *Theileria* species. Another author Gill *et al*. (1997) confirmed that anemia and jaundice were regarded as the major pathological changes occur due to *Theileria annulata* infection. An association was recorded for packed cell volume (PCV) and internal parasites infection (χ^2= 6.573, P<0.05, OR=1.852). This finding has been previously confirmed by Magona and Mayende (2002), who reported high percentage of anemia in exotic breed (Friesian) compared to the local breed (Zebu and Sahiwal) due to the infection with both blood and internal parasites during their investigation in dairy farms in Uganda.

In conclusion, the infection with both blood and internal parasites were prevalent in dairy farms in Kuku area which is considered as one of semi-intensive production system in the country. Based on that, it is highly recommended that good managements and adequate nutrition as an improvement of the general conditions in the dairy farms are required in order to avoid parasitic infections. An attempt should be made to increase the awareness of the dairy farms owners regarding the economic impact of parasitic infections.

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