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Prevalence of Calf Scour and Associated Risk Factors in Model Dairy Farms of Holeta Town and Adea Berga District, Central Ethiopia

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Abstract: A cross-sectional study was conducted in Holeta town and Adea Berga District on model dairy farms from November 2020 to April 2021 with the aims of determining the prevalence of calf scour and its associated risk factors and identification of helminth parasites (Ostertagia and Cooperia) and *E. coli*. Descriptive statistics were used to determine the prevalence and Pearson's Chi-square (χ^2) test was applied to assess the association between the prevalence of calf scour and different risk factors. The overall prevalence of calf scour was found to be 57 (48.7%). Of the total positive samples 29.8%, 35.1%, 22.8 and 12.3% were found to be positive for *E. coli*, Ostertagia, Cooperia and mixed (Ostertagia and Cooperia), respectively. The results of chi-square (χ^2) analysis revealed that among the different risk factors associated with the occurrence of calf scour only colostrum feeding (P=0.001; χ^2=10.191), breed (P=0.001; χ^2=10.191), body condition (P=0.00; χ^2=21.680) and hygiene (P=0.037; χ^2=6.594) had significant difference in the prevalence of calf scour. Of the total 117 examined calves 17 (14.5%) were positive for *E. coli*. Among the different risk factors associated with the occurrence of colibacillosis age (P=0.000; χ^2=23.576), colostrum feeding (P=0.002; χ^2=9.199), breed (P=0.002; χ^2=9.199) and hygiene (P=0.000; χ^2=16.453) had significant difference in the prevalence of colibacillosis. Of the total 117 examined calves 40 (34.2%) were positive for helminth parasites. Of the helminth positive samples 50%, 32.5% and 17.5% were positive for Ostertagia, Cooperia and mixed (Ostertagia and Cooperia), respectively. Among the different risk factors associated with the occurrence of helminthiais age (P=0.012; χ^2=6.307) and body condition (P=0.00; χ^2=22.756) had significant difference in the prevalence of helminthiais. In general, the current study revealed that there is alarmingly high prevalence of calf scour in the study areas. Hence, improved calf management practices should be implemented so as to minimize the occurrence of calf scour in the study areas.

Key words: Adea Berga · Cooperia · *E. coli* · Holeta · Ostertagia · Prevalence

INTRODUCTION

Ethiopia is endowed with abundant livestock resources of varied and diversified genetic roles with specific adaption to its wide range of agro ecologies [1].This great livestock potential is not properly exploited due to many prevailing socio-economic values and attitudes, traditional management methods, limited genetic potential and rampant disease. Gastrointestinal parasite infections are a problem for both small and large scale farms; however, their impact is greater in sub-Saharan Africa. The prevalence of gastrointestinal parasites and the severity of infection vary considerably depending on the genera of helminth and protozoan parasites involved, animal species, local environmental conditions such as humidity, temperature, rainfall, vegetation and management practices [2- 4].

The most important disease problems in the young calf are pneumonia and diarrhea. Enteric disease is a major health problem in calves and diarrhea is associated with reduced weight gain and increased mortality rates in cattle production [5,6]. Furthermore, diarrhea in young calves has been found to increase the risk of other diseases later in life [7].

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Calves are at greatest risk of developing diarrhea during the first month of life and the risk then decreases with age [8,9]. The etiology of calf diarrhea is multifactorial and may include infective, environmental, nutritional and management factors such as calves being born from a heifer [10], being born during the summer [11,12] suckling [11,13], low serum IgG concentrations [14] and large herd size [15].

The pathogens associated with calf diarrhea are rotavirus, corona virus, *Salmonella, E. coli* species, protozoan parasites, *Eimeria* and *Cryptosporidium* species [16,17]. The pathogenic effect of gastro-intestinal parasites may be sub clinical or clinical. Young animals are most susceptible. The effect of these parasites is strongly dependent on the number of parasites and the nutritional status of the animals they are infecting. The major clinical signs are weight loss, reduced feed intake, diarrhea and mortality reduced carcass quality and reduced wool production/quality [18]. Young animals do not have a great deal of immunity to parasites during their first year at pasture. The second year, they have partial immunity and, although they may appear healthy, they eliminate many eggs. Adult animals are much less susceptible to most parasites, unless they are in poor living conditions [19].

The etiologic diagnosis is not determined for a large percentage of cases of neonatal diarrheas. Although calf scour is an important cause of calf morbidity and mortality in Ethiopia in general and in the study area in particular, little research work has been worked previously and very little attention has been given to the disease and losses.

Therefore, the objectives of this research were to estimate the prevalence of calf scour and various associated risk factors in Holeta town and Adea Berga District model dairy farms and to identify helminth parasites (Ostertagia and Cooperia) and *E. coli*.

MATERIALS AND METHODS

Study Area: The study was conducted in Holeta town and Adea Berga district which are located in Oromia regional state. Holeta is located 45km west of Addis Ababa at altitude 2400 meter above sea level. In these areas the rainfall pattern is bimodal, with short rainy period from February to April and a long rainy season from mid- June to September. The annual temperature ranges between 18°C to 24°C and the rainfall of the area ranges from 1000 to 1100 mm. Adea berga is located in central highland of Ethiopia at 9°16'N latitude and 38° 23' longitude,70 km west of Addis Ababa and 35 km North west of Holeta on the main road of mugger. It lies at altitude of 2500 meter above sea level. It characterized by cool subtropical climate with mean annual temperature and rain fall of 18ºC and1225mm respectively.

Study Population: The study population consisted of calves with less than one year of age belonging to Holeta town and Adea Berga District model dairy farms (large and small scale dairy holders). Examined calves were categorized into two age groups: less than or equal to one month (≤1 month) and greater than one month (1 month-12 months) which was determined by asking the owner of the animal orally and by observing calf age records.

Study Design and Sampling Methodology: Cross-sectional study was conducted in Holeta town and Adea Berga District model dairy farms from November 2020 to April 2021 and a purposive sampling method was used to collect samples from calves. Moreover, the sexes, feeding types of colostrum, body conditions, hygienic condition of the house and breed types of calves were registered [20].

Questionnaire Survey: A pretested questionnaire was administered to dairy farm owners to assess the general calf husbandry practices. Generally the questionnaire includes all practices in the farm that included about calf health care, hygiene, health problem, colostrums feeding type and duration as well as types of diarrhea that affect the growth of calves.

Sample Size Determination: The sample size was determined using the formula recommended by Thrusfield [21]. Since there was no previous investigation conducted on the same title in the study area and expected prevalence of calf scour was 50%. Thus, by giving attention to this consideration the sample size was calculated based on the 95% confidence limit and 5% sampling error according to the following formula.

$$n = \frac{z^2 P_{exp} = (1 - P_{exp})}{d^2} \quad n = \frac{1.96^2 P_{exp}(1 - P_{exp})}{d^2}$$

where, n = sample size, z = statistic for a level of confidence

d = required absolute precision, P_{exp} = expected prevalence

Therefore using the above mentioned formula and the given figures, the sample size was calculated to be 384. However, the actual sample size collected during the study period was 117 since there were no enough amounts of calves in the study area.

Sample Collection and Examination: Fecal samples were collected from calves for identification of pathogenic helminthes parasites and *E. coli*. At the time of sampling, the name of the farm (owner), date of sampling, the age, sex, breed, address and management system was recorded for each calf on a data recording format. Fresh fecal samples were collected from rectum from each calf using sterile disposable plastic gloves. The samples were placed in labeled clean plastic containers (universal bottles) and were transported to the parasitology laboratory of Holeta agricultural research center and Sebeta microbiology laboratory on the same day of collection and were preserved at refrigerator until processing within 48 hr of arrival.

Parasitological Techniques

Floatation Technique: Three grams of feces was placed in a beaker then 50ml of flotation fluid (sodium chloride) was poured to the beaker containing 3 g of feces and then the flotation fluid was mixed with feces thoroughly with stick rod. The resulting fecal suspension was poured through a tea strainer into another beaker. The fecal suspension was poured into a test tube from the second container, then placed in a test tube rack, leaving a convex meniscus at the top of the tube and a cover slip was carefully placed on top of test tube. The tube was left to stand for 20 minutes and finally the cover slip was lifted off from the tube vertically together with the drop of fluid adhering to it and immediately placed on microscope slide and examined the presence of nematode eggs under the microscope [22].

Fecal Cultures for Ostertagia and Cooperia: Fecal samples from calf whenever positive for Strongyies types of eggs were cultured for harvesting third stage larvae (L_3) and identification of the most important genera (Ostertagia and Cooperia) of non-distinguishable nematode eggs in cattle (calves) according to Hansen and Perry [19]. Pooled fecal samples were broken up using stirring device, kept moist and crumbly; the mixtures transferred to Petri-dishes and placed at 27ºC for 7 to 10 days. The samples were kept humid, mixed occasionally and aerated every 1 to 2 days. During this period the larvae hatched from the eggs and developed into L_3. Finally larvae were recovered using the Baermann technique. From each culture, the third-stage larvae (L_3) of Ostertagia and Cooperia were morphologically differentiated and identified according to keys provided by Hansen and Perry [19].

Bacteriological Isolation and Identification of *E. coli*: Isolation of *E. coli* was attempted according to Quinn *et al*. [23] with slight modification. All samples were cultured on blood agar and incubated aerobically at 37°C for 24 to 48hrs. After morphological examination, type of hemolytic and gram staining characteristics pure colonies were further sub-cultured and incubated on nutrient agar and MacConkey agar (MCA), which is a dual purpose (selective and differential) medium, by four flame technique at 37°C for 24 h. Pink colored colonies were considered as presumptive of *E. coli*. Gram's staining was performed to determine the size, shape and arrangement of bacteria. The organisms revealed gram negative, pink colored with rod shaped appearance and arranged in single or in pair were suspected as *E. coli.* A single isolated colony was picked from MCA and streaked on Eosin Methylene Blue Agar (EMB) medium and incubated at 37°C for 24 h. The characteristic green metallic sheen growth of colonies is a presumptive identification for *E. coli.* Colony morphology and colors on MCA and EMB agar plates together with the Gram stain procedure were used as initial identification of *E. coli* colonies. Such colonies were taken from EMB into nutrient broth and agar for further biochemical examination [23].

Biochemical tests were performed to confirm *E. coli* Slide catalase test, Oxidase test, Indole test, Methyl Red (MR) and Vogues Proskauer (VP) tests, Citrate utilization test and Triple Sugar Iron test and Carbohydrate fermentation tests were performed [23].

Data Management and Analysis: The collected data were entered into Microsoft Excel Sheet and analyzed through Statistical Package for Social Sciences (SPSS) Version 20. Accordingly, descriptive statistics such as percentages and frequency distribution were used to determine the prevalence and Pearson's Chi-square (χ^2) test was applied to assess the association between the prevalence of calf scour and different risk factors. A value of $p \leq 0.05$ was considered significant.

RESULTS

Overall Prevalence of Calf Scour: Out of the total 117 examined calves 57 (48.7%) were positive for calf scour. Of the total positive samples 29.8%, 35.1%, 22.8 and 12.3% were found to be positive for *E. coli*, Ostertagia, Cooperia and mixed (Ostertagia and Cooperia), respectively. The chi-square (χ^2) analysis results revealed that among the different risk factors associated with the occurrence of calf scour only colostrum feeding (P=0.001; χ^2=10.191), breed (P=0.001; χ^2=10.191), body condition (P=0.00; χ^2=21.680) and hygiene (P=0.037; χ^2=6.594) had significant difference in the prevalence of calf scour as shown below in Table 1.

Prevalence of Colibacillosis: Of the total 117 examined calves 17 (14.5%) were positive for *E. coli*. The chi-square (χ^2) analysis results revealed that among the different risk

Table 1: Overall prevalence of calf scour with different associated risk factors

Risk factors	No. of examined	No. of positive (%)	χ^2	P-value
Age				
≤ one month	11	7 (63.6)	1.082	0.298
one month-12month	106	50 (47.2)		
Sex				
Female	93	45 (52.7)	2.860	0.091
Male	24	8 (33.3)		
Colostrum feeding				
Bucket	80	47 (58.8)	10.191	0.001
Natural	37	10 (27.0)		
Breed				
Cross	80	47 (58.8)	10.191	0.001
Jersey	37	10 (27.0)		
Body condition				
Good	22	2 (9.1)	21.680	0.00
Moderate	67	34 (50.7)		
Poor	28	21 (75.0)		
Hygiene				
Good	12	7 (58.3)	6.594	0.037
Moderate	75	30 (40.0)		
Poor	30	20 (66.7)		
Overall	117	57	(48.7)	

Table 2: Prevalence of *E. coli* with different associated risk factors.

Risk factors	No. of examined	No. of positive (%)	χ2	p-value
Age				
≤ one month	11	7 (63.6)	23.576	0.000
one month-12month	106	10 (9.4)		
Sex				
Female	93	14 (15.1)	0.100	0.752
Male	24	3 (12.5)		
Colostrum feeding				
Bucket	80	17 (21.2)	9.199	0.002
Natural	37	0 (0.0)		
Breed				
Cross	80	17 (21.2)	9.199	0.002
Jersey	37	0 (0.0)		
Body condition				
Good	22	2 (9.1)	1.469	0.480
Moderate	67	12 (17.9)		
Poor	28	3 (10.7)		
Hygiene				
Good	12	0 (0.0)	16.453	0.000
Moderate	75	6 (8.0)		
Poor	30	11 (36.7)		
Overall	117	17 (14.5)		

Table 3: Prevalence of helminths infection with different associated risk factors.

Risk factors	No. of examined	No. of positive (%)	χ^2	P-value
Age				
≤ one month	11	0 (0.0)	6.307	0.012
One month-12 months	106	40 (37.7)		
Sex				
Female	93	35 (37.6)	2.393	0.122
Male	24	5 (20.8)		
Colostrum feeding				
Bucket	80	30 (37.5)	1.233	0.267
Natural	37	10 (27.0)		
Breed				
Cross	80	30 (37.5)	1.233	0.267
Jersey	37	10 (27.0)		
Body condition				
Good	22	0 (0.0)	22.756	0.00
Moderate	67	22 (32.8)		
Poor	28	18 (64.3)		
Hygiene				
Good	12	7 (58.3)	3.503	0.174
Moderate	75	24 (32.0)		
Poor	30	9 (30.0)		
Overall	117	40 (34.2)		

factors associated with the occurrence of colibacillosis age (P=0.000; χ^2=23.576), colostrum feeding (P=0.002; χ^2=9.199), breed (P=0.002; χ^2=9.199) and hygiene (P=0.000; χ^2=16.453) had significant difference in the prevalence of colibacillosis as shown below in Table 2.

Prevalence of Helminthiasis: Of the total 117 examined calves 40 (34.2%) were positive for helminth parasites. Of the helminth positive samples 50%, 32.5% and 17.5% were positive for Ostertagia, Cooperia and mixed (Ostertagia and Cooperia), respectively. The chi-square (χ^2) analysis results revealed that among the different risk factors associated with the occurrence of helminthiais age (P=0.012; χ^2=6.307) and body condition (P=0.00; χ^2=22.756) had significant difference in the prevalence of helminthiais as shown below in Table 3.

DISCUSSION

The overall prevalence of calf scour in the study was 48.7%. Of the total positive samples 29.8%, 35.1%, 22.8 and 12.3% were found to be positive for *E. coli*, Ostertagia, Cooperia and mixed (Ostertagia and Cooperia), respectively. Among the different risk factors associated with the occurrence of calf scour only colostrum feeding, breed, body condition and hygiene had significant difference in the prevalence of calf scour.

The prevalence of *E. coli* in the present study was 14.5%. The current finding is lower than reports Dereje [24] (43.1%), Masud *et al.* [25] (44%), Paul *et al.* [26] (76%), Haggag and Khaliel [27] (82%), El-Shehedi *et al.* [28] (35.83%), Osman *et al.* [29] (63.6%) and Hassan [30] (50%). However, this finding is higher than the findings of Viring *et al.* [31] (11.5%) and Azzam *et al.* [32] (5.4%). This high and low occurrence of *E. coli* may be due to the difference in study area, age of calves, farm size and sample size, managements and hygiene measurements.

The prevalence of helminth parasites was 34.2%. Of the helminth positive samples 50%, 32.5% and 17.5% were positive for Ostertagia, Cooperia and mixed (Ostertagia and Cooperia), respectively. The present finding for ostertagiosis was higher than previous finding by Refiullah *et al.* [33] (6.7%), Elele [34] (5.2%) and Addisu and Berihu [35] (1.8%). However, it was less than reports of Slocombe [36] (60.2%) and Enyiew *et al.* [37] (52.6%). The difference the occurrence of ostertagiosis may due to seasonal variation among the different study periods, housing system, age of calves, hygienic conditions of the farms, appropriate timing for colustrum feeding and other managemental practices in the different study areas.

CONCLUSION AND RECOMMENDATIONS

The current study revealed that there is alarmingly high prevalence of calf scour in the study areas with high level of prevalence of helminth parasitic infections. However, the occurrence of collibacillosis is lower than

the helminth infections. Among the different associated risk factors, colostrum feeding type, breed, body condition and hygiene, have significance differences in the occurrence of calf scour. Moreover, the age of the claves has also a significant association with the occurrence of colibacillosis. Therefore, improved calf management practices should be implemented so as to minimize the occurrence of calf scour and appropriate amount of colostrum and feeding time should be practiced uniformly in the different farms so as to boost the immune systems. Further advanced investigations should be conducted in order to render more detail information related to the mentioned etiological agents of calf scour in the study area, so as to put appropriate control and prevention measures in place.

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