Effect of deworming on milk production in dairy cattle and buffaloes infected with gastrointestinal parasites in the Kavrepalanchowk district of central Nepal

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ABSTRACT
Background In Nepal, knowledge of proper handling, management and causes of cattle diseases is still limited. The main objective of this study was to explore the impact of deworming on milk production and its effect on milk qualities.

Methods A total of 200 faecal samples (100 buffaloes and 100 cows) were collected and analysed for parasitic burden. Half of the infected cattle (buffaloes, Bos bubalis; cow native, B taurus; European, B taurus) were then dewormed with Levamisole Hydrochloride- Oxyclozanide bolus, and the remaining 50 per cent were left untreated. The milk yield from both infected and dewormed cattle was recorded for 30 days and the qualities of milk were analysed.

Results The prevalence of parasitic infection was found to be 22.0 per cent. Fasciola hepatica was the predominant parasite (81.8 per cent), followed by Toxocara vitulorum (34.1 per cent), Strongyloides papillosus (6.8 per cent) and Bunostomum phlebotomum (4.5 per cent). The average milk yield (litre/day/cow) significantly increased, which was 1.22 litres per day for treated cows and 1.06 litres for treated buffaloes. The intervention effect of deworming was 1.22 litres per day for treated cows and 1.06 litres for treated buffaloes. The intervention effect of deworming was 1.22 litres per day for treated cows and 1.06 litres for treated buffaloes.

Conclusion Anti-parasitic treatment in cattle had positive effects on milk qualities such as solid non-fat, lactose, fat percentage and total protein percentage.

BACKGROUND
Dairy farming is a major occupation in Nepal and is growing as entrepreneurs are becoming more involved in the commercial farming of livestock. The major sources of milk in Nepal are cows and buffaloes in the Hill and Terai regions, and yaks in the high altitudes.1 Cow and buffalo supply a significant source of milk to the capital city of Kathmandu. The semi-urban region of the Kathmandu Valley and adjoining districts, including Kavrepalanchowk, Nuwakot and Dhading, provide about 75 per cent of the milk supply in the Kathmandu Valley.1

Gastrointestinal parasitic infection remains a major impediment to the efficient management of cattle. In less developed agricultural systems, parasitic infections may cause severe clinical signs in cattle, such as stunted growth, tissue oedema and diarrhoea.2 Even in efficiently managed herds with no signs of clinical parasitism, the presence of gastrointestinal parasites retards the growth of calves and decreases milk production in adult cattle.3

In South Asia, gastrointestinal parasitic infections are prevalent in cattle and are likely due to the consumption of contaminated grasses and water, and climate conditions conducive to their transmission.4

Parasitic infections negatively affect the economics of raising cattle. The economic impact of parasitism is commonly calculated by comparing production in parasitised cattle with production of those that have had their parasite burden removed with an anthelminthic drug.1,5 Common intestinal parasites associated with cows are Schistosoma species, Dictyocaulus viviparus, Strongylidae, Haemonchus species, Cooperia species, Ostertagia species, Meistocirrus digitatus, Eimeria species, Trichuris species, Buxtonella sulcata, Oesphagostomum, Ostertagia ostertagi, Trichostrongylus axei, Fasciola species, Paramphistomum species and Cryptosporidium species.6 Infection and dissemination of these parasites in herds are affected by various factors. The factors that influence the development, survival, distribution or migratory behaviours of the free-living larvae found on pasture are primarily
dependent on weather. Various other environmental factors such as temperature, moisture, rainfall and the quality of the soil influence the development and survival of the larvae on pasture, as well as their distribution on the herbage.

Gastrointestinal parasites in cattle are a serious problem, affecting the efficient production of both milk and meat throughout the world. Commercial cow farming has been increasing in recent years, yet many farming practices are still based on traditional practices and many farmers are unaware of the potential detrimental effects of gastrointestinal parasites on milk production. Reducing parasitism involves more than just treating cattle after they become infected. For seasonal control, environmental contamination must be minimised to prevent continued reinfection. The existence of infective larvae in the surroundings of cattle is even more damaging than their development within the cattle. Cattle have to give up something in terms of production in order to fight parasitic infections. This study was conducted to characterise the parasites and to compare milk quality parameters between parasitised and non-parasitised cows and buffaloes in the Kavrepalanchowk district of central Nepal.

METHODS

Study sites, study population and sample size

The district of Kavrepalanchowk, which provides more than 60 per cent of milk supply to Kathmandu Valley, was selected as the study site. Both cows and buffaloes supply milk in the region and were included in the study. The cows were mostly crossbred types, cattle either Jersey or Holstein-Friesian crosses, and the buffaloes were Murrah crosses. The mean yield of these crossbred cows is below the breed potential. The average lactation period of a cow is 325 days, ranging from 300 days to 350 days.

The sample size was calculated using OpenEpi software. Estimating a group difference of 0.85 and a power of 80 per cent, the sample size necessary to detect a significant difference between groups was 44, with 22 animals in each group. Both faecal samples and milk samples were included in the study.

Faecal sample collection, transportation and processing

To end up with 44 infected cattle, faecal samples from 200 cows and buffaloes were collected from local farms at villages in Kavrepalanchowk and were transported to the Parasitology Laboratory of the Kantipur College of Medical Science, Sitapaila, Kathmandu, following standard parasitological procedures with preservatives using cool iceboxes. No commercial farms were considered for sampling. Briefly, each sample was tested for presence of parasite eggs. A modified Wisconsin sugar faecal worm egg flotation technique was used to determine worm egg counts in parasite-positive faecal samples. In this method, 3 g of faecal material was added to 15-ml sugar solution (454 g of sugar in 355 ml of water). The solution and faecal matter were stirred until materials had even consistency and was poured through a tea strainer. The strained mixture was transferred into 15-ml centrifuge tube and centrifuged at 112g for 10minutes. The centrifuge tube was placed in rack and topped off with sugar solution forming a meniscus. The tube was then covered with 22 x 22 mm cover slip and set aside for five minutes. The cover slip was lifted directly upwards and immediately placed on microscope slide. The entire cover slip region was scanned by microscope and eggs were counted. The data were recorded using standard data sheet and then entered in SPSS software for data analysis.

Deworming of cattle

Out of 44 infected cattle, 22 randomly selected livestock (11 cows and 11 buffaloes) were treated with Levamisole Hydrochloride-Oxydolanide bolus (Adzanide-L, Medivet Pharmaceuticals, Nepal) on the second day, and the remaining 22 (8 cows and 14 buffaloes) were left untreated. However, all of the cattle were given a mineral mixture (Vetmix, Nepal).

Quantification of daily milk yield

The volume of milk yield was recorded daily for 30 days using a measuring cylinder during milking. The daily measurement of milk volume was recorded using a standard data sheet.

Milk sample collection, transportation and analysis

Milk samples were collected in sterile and leak-proof vials from all cattle twice during the study period. The samples were collected on day 0 and day 15. The samples were transported to the Himalayan Chilling Centre, Panauti, in cool iceboxes. Ten different milk qualities, including fat percentage, solid non-fat (SNF) percentage, density, lactose percentage, solid percentage, total protein percentage, conductivity, temperature, freezing point and added water, were measured using Lactoscan MCC50 (Bulgaria). The four milk qualities of conductivity, temperature, freezing point and added water were not deemed to have any significance in this study; hence, the results were not included.

Data collection and analysis

The data concerning milk yield, qualities of milk and number of eggs per gram (EPG) count were recorded in Microsoft Excel sheet and further analysed using SPSS V.21. The prevalence of parasitic infection, frequency of parasitic species and parasite burden in the cow and buffalo dung, milk yield from treated and untreated animals, and milk quality were calculated. Milk production was used as an outcome variable and all other variables were treated as independent variables. The paired t test was used to compare milk quality parameters. Linear regression analysis was performed using differences in milk yield after antiparasitic treatment as the outcome variable, and parasite burden, infection with parasites, type of parasites, types of dairy animal and antiparasitic treatment as predictor variables.
Table 1  Monoparasitic and polyparasitic infections among dairy cattle

| Dairy cattle | Infected cattle, n (%) | Monoparasitic infection (%) | Polyparasitic infection (%) | Non-infected cattle, n (%) | Total, n (%) |
|--------------|------------------------|----------------------------|----------------------------|---------------------------|-------------|
| Cows         | 19 (9.5)               | 15 (7.5)                   | 4 (2.0)                    | 81 (40.5)                 | 100 (50.0)  |
| Buffaloes    | 25 (12.5)              | 18 (9.0)                   | 7 (3.5)                    | 75 (37.5)                 | 100 (50.0)  |
| Total, n (%) | 44 (22.0)              | 33 (16.5)                  | 11 (5.5)                   | 156 (78.0)                | 200 (100.0) |

RESULTS
Prevalence of parasitic infection
Out of the 200 dairy cattle screened, the prevalence of parasitic infection in the sampled livestock in the district of Kavrepalanchowk was found to be 22.0 per cent. Among them, 16.5 per cent were infected with a single parasite and 5.5 per cent were infected with multiple parasites, excluding protozoal infections. Higher proportions of cattle were infected with a single parasite in both cows and buffaloes (table 1).

Frequency of different parasites
The predominant parasite found in dairy cattle was *Fasciola hepatica*, accounting for 81.8 per cent, followed by *Toxocara vitulorum* (34.1 per cent), *Strongyloides papillosus* (6.8 per cent) and *Bunostomum phlebotomum* (4.5 per cent). *F hepatica* was reported in most of the monoparasitic and polyparasitic infections.

Parasitic burden
The EPG count among 44 infected cattle showed that a higher number (17, 38.6 per cent) of dairy cattle had 1 EPG count, followed by 2, 3 and 5 EPG count among 12 (27.3 per cent), 5 (11.4 per cent) and 4 (9.1 per cent) dairy cattle, respectively.

Comparison of milk yield in treated and untreated cows
The infected cattle were in different phases of lactation. The mean differences in milk production on day 0 and day 30 in treated and untreated cows were 1.22 litres and 0.43 litres, respectively. A significant increase in milk production was found among treated cows in comparison with untreated ones (P=0.008), and the increase in milk production due to intervention was found to be 14.1 per cent (figure 1).

Milk yield comparison in treated and untreated buffaloes
Similarly, the mean differences in milk production on day 0 and day 30 in treated and untreated buffaloes were 1.06 litres and 0.39 litres, respectively. A statistically significant increase in milk production was found in treated buffaloes in comparison with untreated ones (P<0.001). The increase in milk due to intervention was found to be 8.3 per cent (figure 2).

Milk quality analysis in treated and untreated dairy cattle
In dairy cows, various milk quality parameters such as SNF percentage, lactose percentage, solid percentage and total protein percentage were increased, while fat percentage and density decreased after treatment. The protein percentage showed a statistically significant increase after treatment with antiparasitic drugs (P=0.035). Similarly, the lactose percentage and solid percentage showed statistically significant increases in buffaloes after deworming (P=0.002 and P=0.028) (table 2).

The milk quality parameters fat percentage, SNF percentage, density, lactose percentage, solid percentage and total protein percentage decreased in the infected cows over time. Solid percentage was significantly decreased (P=0.021). Similarly, density and solid percentage were significantly decreased (P=0.036 and P=0.008) among the infected buffaloes (table 2).
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**Figure 2** Milk yield comparison in treated and untreated buffaloes.

![Milk yield comparison in treated and untreated buffaloes.](image)

analysis of various independent variables for prediction of milk production in dairy cattle revealed that antiparasitic drug treatment of cattle increased milk production significantly (P<0.001) (table 3).

**DISCUSSION**

Parasitological inspection of cattle dung of 200 dairy cattle (cows and buffaloes) showed that 22 per cent of cattle had parasitic infection. This could be due to the development of antihelminthic resistance rather than lack of treatment. There are no comprehensive studies on antihelminthic resistance in Nepal. Many researchers have suggested that dairy cattle are infected with parasites at a higher rate than buffaloes, although the present study found a higher rate among buffaloes. The higher rate of parasitic infection in buffaloes was in agreement with one study in Pakistan. Similarly, two studies from rural Bangladesh showed a more than 50 per cent prevalence of gastrointestinal parasites in Red Chittagong cattle. The low rate of prevalence in this study might be due to exclusion of protozoal infections. In the present study, the higher incidence in buffaloes as compared with cows might be due to differences in feeding habits and environmental hygiene.

The present study reveals a higher percentage of monoparasitism in comparison with polyparasitism. The higher rate of monoparasitic infection was also reported in Bangladesh with triple types of endoparasitic infections. The highest rate of fascioliasis (81.8 per cent infection by *F hepatica*) in the present study echoes with a previous study which showed a high prevalence of fascioliasis (more than 50 per cent). However, research

| Parameters | Treated | Untreated |
|------------|---------|-----------|
|            | Day 1   | Day 15−   | P value* | Day 1   | Day 15−   | P value* |
| Cows (n=11)|         | Day 1      |         |         | Day 1      |         |
| Fat %      | 5.308   | 4.941      | −0.367  | 1.851   | 0.525      |         |
| SNF %      | 8.016   | 8.231      | +0.215  | 0.526   | 0.206      |         |
| Density    | 28.615  | 27.674     | −0.941  | 6.056   | 0.618      |         |
| Lactose %  | 4.249   | 4.453      | +0.204  | 0.495   | 0.202      |         |
| Solid %    | 0.640   | 0.654      | +0.014  | 0.088   | 0.617      |         |
| Total protein % | 3.180 | 3.731      | +0.551  | 0.748   | 0.035      |         |
| Buffaloes (n=11) |         |            |         |         |            |         |
| Fat %      | 7.337   | 8.282      | +0.945  | 1.508   | 0.064      |         |
| SNF %      | 7.732   | 7.437      | −0.294  | 0.968   | 0.337      |         |
| Density    | 26.904  | 27.029     | +0.125  | 1.620   | 0.804      |         |
| Lactose %  | 3.384   | 3.982      | +0.598  | 0.463   | 0.002      |         |
| Solid %    | 0.535   | 0.582      | +0.047  | 0.060   | 0.028      |         |
| Total protein % | 3.388 | 3.464      | +0.076  | 0.618   | 0.694      |         |

*P value calculated using paired t test.
SNF, solid non-fat.
higher milk fat, protein and overall milk solids.27 The milk was superior from treated cattle, with consistently and trichuriasis.16 Coccidia and loiasis but higher rates of toxocariasis, strongyloidiasis study from Bangladesh also reported a low rate of fascioliasis.28

The pre-emptive regular deworming of milking cattle is critical rather than just for treating clinical diseases. The treatment of parasitism is a prerequisite for reducing the threat of economic loss as well as for decreasing the number of parasites in the livestock environment. An effective deworming programme could contribute to an economic gain among farmers preventing unseen production losses.

| Table 3 | Predictors of milk yield |
|---------|--------------------------|
| Predictors | Regression coefficient | P value | 95% confidence interval |
| Parastic burden | 0.182 | 0.269 | Lower: -0.041  Upper: 0.144 |
| Infection with parasites | -0.338 | 0.333 | Lower: -1.392  Upper: 0.483 |
| Parasites isolated | 0.160 | 0.641 | Lower: -0.143  Upper: 0.229 |
| Types of dairy animal | -0.071 | 0.573 | Lower: -0.378  Upper: 0.212 |
| Antiparasitic drugs treatment | -0.595 | 0.000 | Lower: -0.992  Upper: -0.393 |

In the present study, the authors found significant increase in milk production in cattle after antiparasitic drug (Levamisole Hydrochloride-Oxyclozanide) treatment. Similar results were reported from studies conducted in Bangladesh.8 15 21–23 Eprinomectin treatment (up to 274 days) in calving cattle resulted in increased daily milk yields.12 A study from Bangladesh also reported increased milk yield following antihelminthic treatment in cows.24 In this study, the untreated groups of cows and buffaloes were shown to have higher production of milk, which may be due to higher stress factor in treated groups and thus should be considered as a placebo.

Among various parameters of milk qualities, SNF percentage, lactose percentage, solid percentage and total protein percentage were found to be increased, while fat percentage and density were decreased after deworming. The protein percentage was found to be significantly increased after treatment with antiparasitic drugs. A study from the Netherlands reported an increase in milk yield similar to this study but no effect on the percentage fat and protein in two trials using albendazole.3 25 and one trial using ivermectin.26 A majority of the studies showed that antihelminthic treatment increased milk production, with yield of milk fat higher than in the controls in 26 of the 35 experiments.22 The quality of the milk was superior from treated cattle, with consistently higher milk fat, protein and overall milk solids.27 The present study also reported significant increment in milk protein among treated cows in comparison with control ones. However, the milk composition was not found to be affected in cows after treatment with eprinomectin and trichlorfon. The pre-emptive regular deworming of milking cattle is critical rather than just for treating clinical diseases.28

Acknowledgements The authors express their special thanks to Dr Diana Fahrenbruck for technical support; Dr Mani Prasad Sapkota, Veterinary Officer, Kavrepalanchowk District, for helping on the fieldwork throughout the project, training the farmers and guidance on feeding antiparasitic drugs for infected dairy cattle; the Director, Himalayan Chilling Centre, Kusadevi, Panauti, Kavre for providing facility for lactoscanner; all farmers who were directly involved in this project by providing faecal and milk samples; Professor Dr Vishwanath P Agrawal, Executive Director, Research Laboratory for Biotechnology and Biochemistry (RLABB); and Late Professor Dr Shital Raj Basnyat, Head, Department of Microbiology, Kantipur College of Medical Science (KCMS), Sitapaila, Kathmandu, Nepal.

Contributors UTS and NA developed the proposal for the study, which was finalised with the suggestions of KS and RB. UTS, NA and SK worked on fieldwork, milk yield analysis and quality analysis using lactoscanner. NS technically supported the fieldwork. KS and RB helped design the research, and edited and proof-read the manuscript. MRS drafted and reviewed the manuscript and analysed the data. KRR and BA helped in editing and reviewing the subsequent version of the manuscript. PG reviewed the manuscript and supervised the work overall. All authors reviewed and approved the final version of the manuscript.

Funding The study was funded by TIRI (Targeted Investment for Research Impact) Scholar Award 2015 from Colorado State University, USAID. Colorado State University Feed the Future Innovation Labs, USA also provided financial support to this work.

Competing interests None declared.

Provenance and peer review Not commissioned; internally peer reviewed.

Data availability statement All data relevant to the study are included in the article.

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