

Diagnostic Workup in Relation to Odds of Clinico-Fecal Predictors in Adult Dairy Cattle and Buffalo with Chronic Diarrhea

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Abstract

BACKGROUND: Chronic diarrhea is one of the costly pathologic conditions affecting dairy animals owing to its significant impact on economic returns.

OBJECTIVES: The objective of this study was to evaluate the clinico-fecal alterations associated with underlying disease entities or etiologies in adult dairy cattle and buffaloes with chronic diarrhea.

METHODS: Hundred and two adult dairy cattle/buffaloes with a history of diarrhea (≥ 3 - weeks duration) without any other complications were selected for the study. Quantification of parameters viz; frequency of feces, fecal consistency, abnormalities in feces, and secondary clinical signs such as changes in appetite or weight loss was estimated to assess the clinical severity associated with the chronic diarrheic condition. Etiologies identified were confirmed based on diagnostic techniques and laboratory tests (Fecal microscopy, culture using selective media, PCR, and copper estimation using Atomic Absorption Spectrophotometer).

RESULTS: Different etiologies identified indicate significant variation ($P < 0.05$), with *Balantidium coli* infection diagnosed in 38 of 102 adult dairy animals (37.6%) with chronic diarrhea. The nature of diarrhea was established in dietary origin in 9.9% of animals. Salmonellosis infection was diagnosed in seven animals. Amphistomiasis was established in 7 (6.6 %) adult dairy animals. Johne's disease was diagnosed in 4 animals (3.9%). Chronic diarrhea due to copper deficiency was detected in 6.9 percent of animals. Relation between the fecal odor, fecal consistency, and fecal abnormality showed variation with respect to etiologies. However, out of these parameters, body condition score (BCS) had a significant effect concerning the etiology ($P < 0.05$) shown by the P-value on a log of odd scale. Body condition scores differ significantly ($P < 0.05$) among the etiological groups, with variations in scores ranging between 2 and 3.5 in affected animals. All the animals affected with clinical Johne's disease had the lowest body condition score of 2-2.5.

CONCLUSIONS: The findings of the present study highlight the need for a detailed and elaborative diagnostic workup to identify the underlying causes of the chronic diarrheic condition in bovines to stamp out the possibility of severe economic losses associated with the condition.

KEYWORDS: Balantidiasis, Body condition score, Chronic diarrhea, Dairy animals, Fecal consistency

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Introduction

Diverse studies confirm that chronic diarrhea is a severe pathological condition affecting livestock that poses a great challenge to veterinarians as well as cattle owners (Wyatt *et al.*, 2010). Chronic diarrhea (intermittent or persistent) as a principal clinical feature is the prime manifestation of gastrointestinal or extra-gastrointestinal disorders in animals. Pertinent to Indian conditions, comprehensive information regarding the frequency and distribution of primary and secondary causes of chronic diarrhea in adult bovines is lacking. Clinical experience and feedback from field practitioners show that chronic diarrhea in dairy animals is frequently encountered (Hassan *et al.*, 2019). It has a significant impact on economic returns owing to its effects on animals' general health status, longevity in the herd, losses through premature culling, weight loss, reduced milk production, increased treatment costs, and the possibility of the spread of infection (Stinson *et al.*, 2018). There are umpteen causes that result in the clinical manifestation of diarrhea in dairy animals (Radostits *et al.*, 2010). The multiple etiological factors associated with diarrhea often complicate the diagnosis (Hassan *et al.*, 2018). Inflammatory causes primarily include infectious (bacterial, viral, or parasitic) (Hashemi *et al.*, 2022) and infiltrative disorders with involvement of the gut (Sanjay *et al.*, 2021). Among the protozoan diseases, balantidiasis caused by *Balantidium coli* is associated with chronic diarrhea in cattle and water buffaloes (Randhawa *et al.*, 2010).

Salmonellosis is another bacterial cause of diarrhea in all age groups of dairy cattle and buffalo (Holschbach *et al.*, 2018). Parasitic infections are other major enteric diseases associated with chronic diarrhea and loss of body condition in tropical and subtropical countries (Hassan and Juyal 2006).

Looking into the intricacy of chronic diarrhea in adult dairy animals, the study was planned to evaluate the clinico-physical predictors associated with underlying disease entities in adult dairy cattle and buffaloes with chronic diarrhea.

Materials and Methods

Hundred and two adult dairy animals (Cattle=65 and buffaloes=37) with a history of diarrhea for 3-

weeks or longer were selected for the study. A detailed history was documented with respect to age and species, duration of diarrhea, dietary history, body condition, and course of diarrhea (recurrent/persistent). Parameters like frequency of feces, fecal consistency, abnormalities (mucus or blood in feces), and secondary clinical signs such as changes in appetite or weight loss were recorded to assess the clinical severity associated with the chronic diarrheic condition. A planned work was framed that included a stepwise approach to identify the etiologies (OIE Terrestrial Manual, 2014). Further, each animal was subjected to general physical examination and rectal examination for lymphadenopathies. Fecal samples and rectal scrapings were collected for microscopy, protozoal or parasitic examination (Radostits *et al.*, 2010). Balantidiasis was diagnosed by demonstrating cysts or trophozoites of *Balantidium coli* in feces (Hussin and Samarai, 2016).

Isolation of Salmonella: Approximately 1 gram of feces was directly inoculated in 9 mL of buffered peptone water, and another 0.1 mL portion was transferred to 10 mL of Rappaport Vassiliadis (RV) broth (HiMedia, India), incubated overnight at 42°C (HiMedia, India). From the selective enrichment, samples were inoculated on Hektoen enteric agar (HE) (HiMedia, India) and incubated at 37°C overnight (18-24 hours). For selective isolation, suspected colonies from the HE agar plate were inoculated on Brilliant Green Agar plates (HiMedia, India) and incubated at 37°C overnight. Characteristic *Salmonella* colonies on a BGA agar plate appear red and impart a pink color to the surrounding. PCR was performed for *Salmonella* spp identification. DNA extraction from the fecal samples was performed using a Qiagen DNA stool mini kit (Qiagen, India) per the manufacturer's instructions ([Figure 3.4](#) for DNA extraction protocol). The suspected isolates were confirmed by PCR using *Salmonella* genus-specific oligonucleotide primers of 25 base pairs (Cohen *et al.*, 1995).

Blood samples were also collected from each animal, and plasma was harvested. One mL of plasma and 9 parts of distilled diluted samples were analyzed using Atomic Absorption Spectrophotometer AAS (Perkin Elmer Analyst 700, USA), to estimate

the total copper in the sample and insoluble copper as per Martos *et al.* (1997).

Feed responsive dietary diarrhea was confirmed as the positive clinical response to the feed or feeding pattern change. Body condition scoring (BCS, 5-integer scale: 1, thin; 2-2.5, underweight; 3-3.5, ideal weight; 4-4.5, overweight; 5, obese) was given to gauge the overall condition of the body in terms of size of the affected animal with respect to diarrhea condition described by Radostits *et al.* (2007).

A One-Way ANOVA was used to test the difference between different etiological groups. The prevalence of *B. coli* was estimated by Pearson's chi-square test for significance. The significant level was set at $\alpha=0.05$ level. The contingency table of etiology concerning factors; body condition, fecal consistency, fecal abnormalities, and body condition score was constructed using R programming odd logistic regression using R (v. 3.2.0) software (Systat 13 software).

Results

Out of the total number of 102 clinical cases of dairy cattle (n=65) and buffaloes (n=37) with chronic diarrhea (without any secondary clinical complication), nearly 68.6 percent of adult dairy animals had diarrhea from 1-3 months, 21 percent had intermittent diarrhea from 4-12 months, while as 10 percent of animals had intermittent diarrhea from more than 12 months (Figure 1). The clinical prevalence of chronic diarrhea was recorded more in cattle than buffaloes. Seven inflammatory and non-inflammatory etiologies were established in dairy cattle and

buffaloes with chronic diarrhea. Primary enteropathies were diagnosed in 59 of 102 dairy animals. The percentage of different etiologies of chronic diarrhea indicates significant variation ($P<0.05$), with *Balantidium coli* infection diagnosed in 38 of 102 adult dairy animals (37.6%) (Figure 2). Since *Balantidium coli* infection was recorded more in diarrheic animals, it could be confirmed that *Balantidium coli* infection constitutes the frequent cause of chronic diarrhea in adult bovines under prevalent conditions. Ten animals recovered when a change in feed or feeding pattern was suggested (only after ruling out other causes). The nature of diarrhea was the dietary origin, established in 9.9 % of animals (Figure 3). Salmonellosis infection was diagnosed in 7 cases (by isolation and typing of salmonella serotypes). The main serotypes identified were *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Salmonella enterica* serovars Reading. A parasitic infection, amphistomiasis, was established in 7 (6.6 %) adult dairy animals which clinically improved after giving antiparasitic therapy (Figure 4). Johne's disease was diagnosed in 4 animals (3.9%) (Figure 5). Lymphosarcoma led to chronic diarrhea and was diagnosed in one cross-bred cattle. Secondary enteropathies were diagnosed in 10 animals (9.9%). A copper deficiency was diagnosed as the cause of chronic diarrhea in 7 animals (6.9%), and improvement was seen clinically after giving the intravenous copper therapy in the affected animal. Chronic peritonitis led to chronic diarrhea in 3 dairy animals (2.9%). The mixed or idiopathic nature of infection (mixed infection with amphistomiasis, *B. coli*, and mycobacterium) was confirmed in 25 diarrheic dairy animals (24.5%).



Figure 1. Animal with chronic diarrhea



Figure 2. Fecal smear with *B.coli* cysts

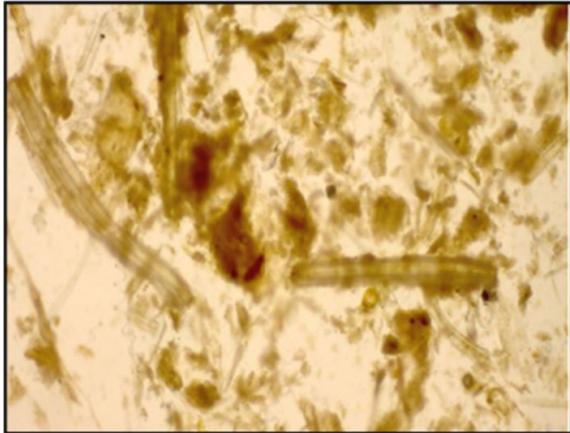


Figure 3. Fecal smear with undigested feed



Figure 4. Fecal smear showing Amphistome egg

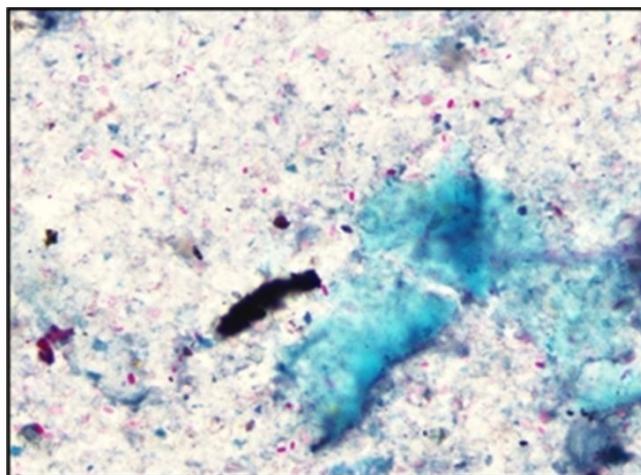


Figure 5. Fecal smear scoring with clumps of MAP

Effect of body weight in relation to respective etiologies: No change in body weight was recorded in 68.4 percent of animals affected with balantidiasis. Animals with responsive dietary diarrhea had a mild weight loss of 20 percent. No weight loss was reported in 42.5 percent of animals affected with Salmonellosis. Moderate weight loss was observed in 28.5 percent of copper-deficient animals. Dairy animals with clinical Johne's disease had mild (25%) to moderate weight loss (75%). Moderate weight loss was observed in 66.6 percent of affected animals with chronic peritonitis.

Relationship of fecal odor with respect to etiologies: Diarrheic feces were not foul smelling in animals affected with clinical Johne's disease. Whereas 13.1% and 57.1% of animals with balantidiasis and amphistomiasis infections had foul-smelling feces. However, 71.4 percent of Salmonellosis-affected animals had foul-smelling feces.

Relationship between fecal abnormalities and etiologies: Partly digested fecal material was found in 36.8 percent of balantidiasis-affected animals. Undigested feed particles were reported in the feces of 60 percent of animals with the dietary origin of diarrhea. Fecal abnormalities mixed with mucoid and blood-tinged feces were reported in 57.1 percent of salmonellosis-affected diarrheic animals. In copper deficient diarrheic dairy animals, 42.8 percent of animals had undigested fecal matter in feces.

Relationship between the consistency of feces and respective etiologies: Balantidiasis-affected animals (47.3%) and animals with dietary diarrhea (20%) had watery feces. Watery and mucoid feces were recorded in 28.5% of Salmonellosis-affected dairy animals. Moderately loose fecal consistency was recorded in 57.1% of animals with copper deficiency. Significantly, fecal consistency was watery (pea soup) in all animals with Johne's disease. Fecal

consistency in amphistomiasis-affected animals was moderately loose in 71.4 percent of animals, whereas fecal consistency was watery in 40 percent of diarrheic animals diagnosed with mixed infection.

Relationship between body condition score (BCS) and etiologies: Animals affected with balantidiasis had body condition scores of 3-3.5 (55.2%). Among Salmonellosis, copper deficiency, and amphistomiasis-affected animals, 57.14 percent of animals, had BCS ranging between 2-2.5, respectively. Dairy animals with clinical Johne's disease had BCS from 2-2.5 in all the affected animals (100%). Body condition score in cattle and buffaloes with chronic

diarrhea was found to be significantly associated with the occurrence of clinical Johne's disease.

Relation between the fecal odor, fecal consistency, and fecal abnormality showed insignificant variation with respect to etiologies. However, out of these parameters, body condition score (BCS) had a significant effect in relation to the etiology ($P < 0.05$) shown by the P-value on the log of odd scale (Table 1). The odds of body condition score in animals with weak body condition increases by a factor of 3.423 unit compared to animals whose body condition was average or good.

Table 1. Logistic Regression Analysis of etiologies with respect to various factors

	S. Error	t-value	P-value
Fecal consistency	0.296	0.818	0.413
Fecal odor	0.296	0.818	0.413
Fecal abnormality	0.5491	1.332	0.182
Body condition score	0.548	2.244	0.024**

The odds ratio (OR) for the levels is as follows:

	OR	2.5 %	95 %
Fecal consistency	1.274	0.713	2.294
Fecal odor	0.481	0.162	1.412
Fecal abnormality	0.560	0.277	1.108
Body condition score	3.423**	1.175	10.194

* Indicates significant ($P < 0.01$)

** Indicates significant ($P < 0.05$)

Discussion

The findings of the present study are attributed to the inclusion criteria that dairy cattle and buffaloes with chronic diarrhea without any secondary clinical signs had been included in the study. Since *Balantidium coli* infection was recorded more in diarrheic cases, it could be confirmed that *Balantidium coli* infection constitutes the frequent cause of chronic diarrhea in adult bovines in this study. Among the protozoan diseases, balantidiasis caused by *Balantidium coli* is associated with chronic diarrhea in cattle and water buffaloes (Randhawa *et al.*, 2010). The findings of the present study, chronic diarrhea and moderate loss of body weight in cattle with *B. coli* infection are substantiated by findings in cattle by Sudan *et al.*

(2012). Palanivel *et al.* (2005) also reported *Balantidium coli* diarrhea in cattle and buffaloes. When feed is not properly fermented in the rumen, some undigested nutrients may reach the small intestine resulting in diarrhea. Loose feces also may result from high levels of rumen degradable protein. (Blood *et al.*, 2007).

Salmonellosis is the bacterial cause of diarrhea in all age groups of buffalo and dairy cattle (Aziz *et al.*, 2018). Veling *et al.* (2002) reported diarrhea as the most frequently observed clinical sign in adult dairy cattle due to Salmonellosis. Warnick *et al.* (2003) also reported diarrhea (persistent or recurrent) in 91% of adult bovines that experienced clinical Salmonellosis

at the time of diagnosis. Several studies have reported the prevalence of fecal shedding of *Salmonella* in dairy farms; however, very little information is available regarding the occurrence of chronic diarrhea associated with *Salmonella* infections in adult cattle (Hanson *et al.*, 2016; Fanta *et al.*, 2019). In the present study, the percentage of animals infected with amphistomiasis was low, which is comparable to earlier reports of 7.3 percent infection in cows and 10 percent in buffaloes (Harid *et al.*, 2006). However, this could even be attributed to the fact that older animals develop resistance to re-infection.

Chronic diarrhea with unthriftiness and wasting is seen as a sporadic disease most commonly due to paratuberculosis (Singh *et al.*, 2008). Bhutediya *et al.* (2017) emphasized that clinical paratuberculosis is merely the tip of the iceberg in terms of the total number of infected animals. In line with our study, Radostitis *et al.* (2010) stated that the common presenting histories for lymphosarcoma cases include chronic wasting and chronic diarrhea. Millar *et al.* (1988) reported that Copper deficiency could lead to diarrhea and reduced growth. The most frequently observed clinical findings of copper deficiency in cattle were poor weight gain and diarrhea that could be detected when it is constant and profuse, as Davis (1995) reported. Hajighahramani and Ghane (2010) observed chronic diarrhea associated with acute diffuse TRP. Another study reported chronic diarrhea and chronic weight loss in cows and buffaloes due to peritonitis. Chronic diarrhea associated with peritonitis may be due to enteritis leading to diarrhea. (Tharwat *et al.*, 2012). The results of this study are consistent with findings that chronic balantidiasis is characterized by the signs of alternating watery diarrhea of prolonged duration (Ahmed *et al.*, 2019). In the chronic form, due to protozoal and parasitic infection, there are episodes of intermittent diarrhea, weight loss, or muscular weakness (Hassan *et al.*, 2017). The present study's findings demonstrated that cows with clinical disease due to GI form of salmonella infection had watery consistency of the feces and were following the findings of Van Kessel *et al.* (2007). Nikpay *et al.*

(2019) have also reported chronic foul-smelling diarrhea due to amphistomiasis in dairy animals. Abdullah *et al.* (2013) reported positive bovines were experiencing intermittent diarrhea and chronic weight loss. To date, physical predictors of chronic diarrhea have not been described in bovines with respect to the etiological study. However, the physical characteristics of feces and clinical signs like weight loss might help diagnose the underlying cause. Although GI conditions often appear in dairy bovines with chronic intermittent or persistent diarrhea, they might become unmanageable and therefore must not be ignored during the diagnostic workup.

Conclusion

The findings of the present study highlight the need for a detailed and stepwise diagnostic workup to identify the causes of the chronic diarrheic condition in bovines to eliminate the possibility of severe economic losses associated with the condition. The clinical prevalence of chronic diarrhea is more in cattle than in buffaloes. As per our study, balantidiasis is the major cause of chronic diarrhea in adult dairy animals. Among all the odds, body condition score is significantly related to the etiology, whereas fecal odor, fecal consistency, and fecal abnormalities showed non-significant association related to particular etiology.

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Conflict of Interest

The authors declared no conflict of interest.

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