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Epidemiological Pattern of Neonatal Calf Diarrhea and a Randomized On-Field Trial to Evaluate Effectiveness of Zinc

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ABSTRACT

Diarrhea is a major cause of mortality in neonatal calves. The objectives of this on-field trial were to study factors responsible for neonatal diarrhea and to evaluate effectiveness of zinc. Cross-bred calves of either sex aged 1 to 45 days were randomized to one of 5 treatments within 1 day of their first diarrhea onset. Calves received a daily dose of zinc @ 2 or 4 mg/kg BW along with zinc-free oral rehydration solution (ORS) either alone or in combination with sulphamethaxozole and trimethoprim @ 20 mg/kg BW till resolution of clinical signs. Fecal and blood samples were collected upon enrolment and exit and analysed for microbiological and parasitological parameters, and trace elements. The study revealed high (80 %) diarrhea occurrence in spring season; more in calves aged less than 30 days (Odds Ratio = 6.000); more in male (63 %) than female (37%) calves. The association between body weight and diarrhea was strong (Odds Ratio = 6.4167). Comparison of epidemiological parameters revealed no significant difference between healthy and diseased calves. *E. coli* was isolated from all enrolled subjects but was not considered causal. *Salmonella* was isolated from 2 cases only. None was found *Cryptosporidium* positive on coprological examination. Diarrheic calves showed relatively low plasma zinc concentration and high fecal coliform count compared to controls. Calves treated with zinc either @ 2 or 4 mg/kg BW alone or together with antimicrobial took significantly ($P<0.05$) less number of days for clinical recovery. The results endorsed zinc as a viable non-antimicrobial alternative.

HIGHLIGHTS

- Study on epidemiology of neonatal diarrhea complex in calves and therapeutic potential of zinc.
- Zinc alone or in combination with antimicrobial was found to have good success in treating calf diarrhea.

Keywords: Calf diarrhea, Epidemiology, Effectiveness of Zinc

Neonatal calf diarrhea (NCD), a common and a serious health problem on dairy farms (Bazeley, 2003) has a significant economic impact directly from calf mortality (Wudu *et al*., 2008; Bartels *et al*., 2010) costs incurred on treatment, veterinary services, diagnostic testing and indirectly from poor growth. In addition, it represents an animal welfare problem from public point of view that needs to be addressed by farmer (Moran, 2002). Increase in livestock productivity enjoins for identification of determinants involved in causation of diseases (Lorini *et al*., 2005) and assist in formulation of effective disease mitigation strategies. The potential farm- and calf-level risk factors for NCD include animal husbandry

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[inadequate colostrum intake (Groutides and Michell, 1990), lack of dam vaccination, importation of calves, winter births, high heifer percentage in the herd, poor sanitary conditions], farm management and infrastructure [type of floors and housing, inadequate shelter/ventilation (Lance *et al*., 1992)], status of calf's immune system, sex and breed, and infection with specific enteropathogens [*Cryptosporidium parvum*, Bovine Rota and Corona virus, K99 and *Salmonella spp.* (Tzipori, 1985; Cho Yong-il, 2012)]. However, it is difficult to generalize risk factors for NCD due to differences in study design and in environments, host and pathogen populations.

In NCD, oral rehydration therapy is still considered as the most viable therapeutic measure if instituted immediately after onset of diarrhea but need to be replaced by intravenous therapy in severe and critical cases (Koch and Kaske, 2008). Antimicrobial use in uncomplicated diarrhea with good therapeutic results is not free from resistance menace but may be indicated in complicated cases. A return to whole milk feeding is recommended within two days of rehydration therapy to avoid a negative energy balance (Grove White, 2004). The wide range of avoidable complications that arise out of the current trends of NCD management like use of high cost antimicrobials, poor owner compliance and immune compromised state of diarrheic calves owing to gastrointestinal zinc loss rightly advocate adoption of novel therapeutic options that ensure better clinical cure. One newer, sustainable and affordable alternative may be incorporation of zinc in therapeutic regimen of diarrheic calves. This may hasten recovery, and reduce morbidity and mortality rates associated with NCD (Glower *et al*., 2013). WHO has long back recommended zinc supplementation to decrease duration and severity of infant diarrhea yet the same hasn't been fully evaluated in animal health. Although the exact mechanism of action of zinc is still unknown; mucosal protective role, enhanced cell-mediated immunity and modification of intra-luminal electrolyte secretion and absorption mechanisms have been proposed (Mazumder *et al*., 2010). Similar benefits of zinc supplementation in neonatal diarrheic calves could significantly impact the economics and sustainability of the production system by reducing antimicrobial use and/or obviating their usage. The hypotheses of this single-blinded randomized on-field study is that multiple calf- and husbandry-related factors are responsible for neonatal diarrhea, and zinc reduces severity and duration of diarrhea and mitigates usage of antimicrobials.

MATERIALS AND METHODS

Epidemiology of NCD

For conducting epidemiological study, 42 cross bred (local x jersey) calves of either sex, aged less than 45 days (calves 28-45 days of age included only to increase the number of cases for study), developing diarrhea for the first time and reared under different managemental conditions were enrolled. Clinically healthy calves (N=10) from the catchment area and/or dairy farm were selected for the study and kept as negative controls. Calves exhibiting signs related to umbilical abscess or pneumonia or previously treated with antimicrobials for other reasons were excluded from the study. The cases presented to Teaching Veterinary Clinical Services Complex, and those observed in SKUAST-Kashmir dairy farm were enrolled. The study parameters included:

- ❒ Age of neonate (days)
- ❒ Body weight (Kgs)
- ❒ Time of occurrence (with respect to season)
- ❒ Managemental practices (Stocking density, Housing)
- ❒ Colostrum intake during first few (two) hours of life (Yes/No)
- ❒ Sanitation and hygiene of animal house
- ❒ Frequency of cleaning of animal house = (Daily/Alternate days/Weekly/Fortnightly/Monthly)
- ❒ Udder hygiene of Dams
- ❒ Frequency of udder cleaning = At the time of milking/Once daily/Alternate days/Weekly
- ❒ Cleaning of udder with = Plain water/Antiseptic. If antiseptic ------------------agent
- ❒ Teat dipping = Yes/No. If yes, by which agent ---------------------------------------

Odds ratio (if any) was calculated to see the strength of association between disease (diarrhea) and causal factors studied.

Clinical trial

Therapeutic effectiveness of zinc was evaluated in diarrheic calves by single-blinded randomized on-field trial (Table 1).

Table 1: Evaluation of therapeutic effectiveness of zinc in NCD

Group	Status of calves (n=6)	Intervention (till resolution of clinical signs)
A	Healthy (Negative control)	Nil
B	Diarrheic	ORS + Zinc (Zinc gluconate) orally @ 2 mg/Kg body wt. once daily
C	Diarrheic	ORS + Zinc (Zinc gluconate) orally @ 4 mg/Kg body wt. once daily
D	Diarrheic	ORS + Sulphamethaxazole & Trimethoprim (Bactrim tab) orally @ 20 mg/kg body wt. 24 hourly
E	Diarrheic	ORS + Sulphamethaxazole & Trimethoprim (Bactrim tab) orally @ 20 mg/kg body wt. 24 hourly. + Zinc (Zinc gluconate) orally @ 2 mg/Kg body wt. once daily
F	Diarrheic	ORS + Sulphamethaxazole & Trimethoprim (Bactrim tab) orally @ 20 mg/kg body wt. 24 hourly. + Zinc (Zinc gluconate) orally @ 4 mg/Kg body wt. once daily

The overall effectiveness of therapeutic regimens was assessed on the basis of recovery from clinical disease as indicated by return to normal rectal temperature, Fecal consistency score of 1 and Attitude score of 1 coupled with changes in fecal colony count. Variables important for understanding the severity of diarrhea were recorded pre- and post- treatment:

- Vital parameters
- Rectal temperature (°F)
- Heart rate (beats /min)
- Respiration rate (breaths/min)
- Attitude score (ATS): ATS was determined as per Glover *et al.* (2013). The scores were assigned as 1 for a calf standing, bright, alert, responsive and with a good suckle reflex; 2 for a calf that stood after stimulation and with moderate suckle reflex; 3 for a calf that was recumbent, didn't stand with stimulation and had weak/absent suckle reflex.
- Fecal consistency score (FCS): FCS was determined as per Glover *et al.* (2013) The scores were assigned as 1 for solid feces; 2 for semi-formed/loose feces and 3 for watery feces.
- Degree of dehydration: Hydration status of the calf was determined on the basis of:

  (i) Cervical skin fold (tent) test (sec): The skin fold test was performed by tenting the skin of the lateral portion of the cervical region of neck and measuring the time (in seconds) required for the skin fold to return to normal. The scale for dehydration used was as per Radostitis *et al.* (2007).

  Mild; Moderate = 2-4 sec; Severe = 6-10 sec; Very severe = 20-45 sec.

Sample collection

Blood was collected from jugular vein using a 20- gauge $1^{1/2}$ inch needle and placed in heparinised tubes. After proper centrifugation the plasma harvested was stored at -20 ^{o}C in capped vials till analysis within a couple of months. The fecal samples collected were immediately processed for committed parameters.

Cultural study

Feces were collected aseptically and processed within 2-4 hours for isolation of *E. coli* and *Salmonella* species using standard microbiological techniques (cultural morphology and biochemical tests). The *E. coli* isolates were also subjected to drug sensitivity tests.

Fecal coliform count

The fecal coliform count was performed by using standard procedure of Pearce *et al.* (2009). One gram (solid)/ one ml (loose) of feces was suspended in sterile normal saline solution (NSS) and serially diluted upto six times by sequentially adding 1 ml of suspension to a further 9 ml of sterile NSS. Then 0.1 ml of suspension was spread on MacConkey agar plate and the series of suspensions were serially plated, incubated at 37°C for 24 hours and counted.

Parasitological study

Detection of *Cryptosporidium* species

The fecal samples were examined as per the method of Henriksen and Pohlenz, (1981), for presence of oocysts of *Cryptosporidium species*. The fecal samples were diluted with 1:5 normal saline and sieved through a strainer to remove coarser particles. Following centrifugation at 1500 rpm for 5 minutes, 15 ml of Sheather's sugar solution was added to the sediment for the separation of oocysts. A thin layer of supernatant was taken on glass slide by platinum loop, and dried at room temperature. The slides were stained by Modified Ziehl-Neelson staining technique (Henriksen and Pohlenz, 1981) and identification of Cryptosporidial oocysts was done under oil immersion by phase contrast microscope.

The detection of *Cryptosporidium* oocysts in faecal samples was also done by the method as follows. Faecal samples collected under sterile measures were air dried and stained with Ziehl-Neelson's carbol-fuchsin solution for 2 minutes, rinsed with tap water followed by acid alcohol and again rinsed with tap water, counterstained with Brilliant green for 2 minutes, once again rinsed with tap water, air dried and finally examined microscopically under oil immersion.

Detection of *Eimeria* species

Faecal sample (1-3 gm) was triturated in mortar and pestle and a uniform solution was made by adding 30 ml of water to it, sieved and centrifuged in two test tubes at 1500 rpm for 5 minutes. Supernatant was poured off and sediment mixed with saturated salt/sugar solution, again centrifuged for same time and at same speed. Due to gravity all oocysts in positive cases floated on the surface of solution in the test tube. A drop of it was taken on clean, grease free slide; cover slip was put on it and examined under different magnifications of microscope. The same sample was examined thrice for confirmation.

Micro-minerals

Plasma trace elements like Cu, Fe, and Zn were estimated as per the method mentioned by Dar *et al*., using Atomic Absorption Spectrophotometry.

STATISTICAL ANALYSIS

The data were analyzed by using SPSS version 20. The quantitative data were analyzed by ANOVA and paired-t test while as qualitative parameters were analyzed using Kruskal-Wallis, and Mann-Whitney tests. Statistical differences were determined at 5% level of significance using intention to treat analysis.

RESULTS AND DISCUSSION

Epidemiological study

Forty two (42) calves enrolled for the study consisted of healthy (N=10) and diseased/diarrheic (N=32) calves. The study outline is depicted in Fig. 1. The observations made are indicated in Table 2 and Fig. 2.

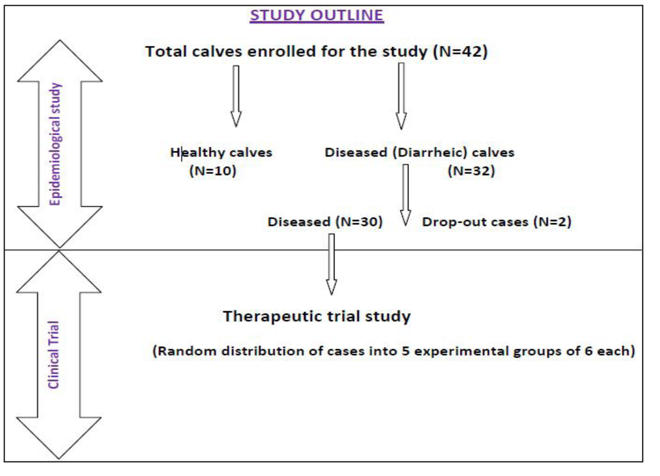

Fig. 1: Study Outline

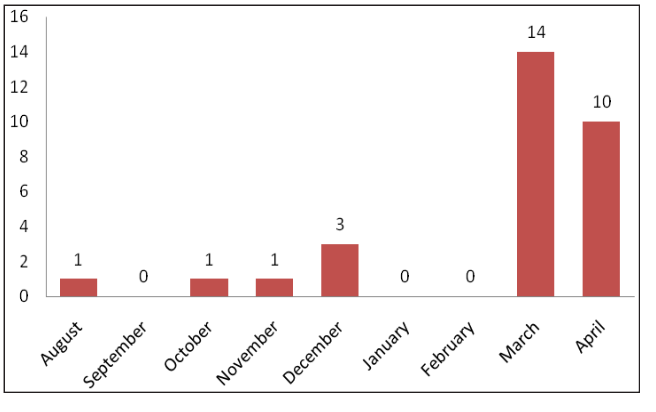

Fig. 2: Month-wise distribution of cases

Table 2: Age- and sex-wise distribution of diarrhea in calves

Parameter	Days/Sex	Number of calves affected	Percent
Age	1-15	12	40.00
	15-30	12	40.00
	30-45	06	20.00
Sex	Male	19	63.33
	Female	11	36.67

Table 3: Epidemiological study

Sl. No.	Variable	Healthy (N=10)	Diseased (N=30)
1	Colostral intake	Yes	Yes
2	Frequency of cleaning of animal house	Daily	Daily
3	Frequency of udder cleaning	At the time of milking	At the time of milking
4	Cleaning of udder with	Plain water	Plain water
5	Teat dipping (yes/no)	No	No
6	Stocking density	3.50 ± 0.76	3.60 ± 0.52
7	Body weight	25.70 ± 2.39	19.30 ± 1.46*

*P<0.05.

Table 4: Strength of Association between diarrhea, and body weight and age of calves

Cases (Diarrheic) n=30		Control (Healthy) n=10	p value	Odds Ratio	CI
Body weight (<25 kgs)					
Yes	22	3	0.0208	6.4167	1.3268 -31.0320
No	8	7			
Age (< 30 days)					
Yes	24	4	0.0234	6.0000	1.2741 -28.2551
No	6	6			

The results revealed high percentage (80%) of diarrhea in calves of less than 30 days age compared to 20% in calves aged 30-45 days (Fig. 1). Diarrheic calves (cases) had 6.000 times higher odds than controls of having had the history of less than 30 days age (Table 4). The occurrence of diarrhea was more in male calves (63.33%) compared to female calves (36.67%) (Fig. 2). The body weight (Kgs) of healthy calves (25.70 ± 2.39) was significantly (P<0.05) higher compared to diseased calves (19.30 ± 1.46). Diarrheic calves (cases) had 6.4167 times higher odds than controls of having had the history of less than 25 Kg body weight (Table 4). Most of the cases of NCD were observed during spring season (24 cases) followed equally (03) by autumn and winter (Fig. 1). Comparison of epidemiological parameters like colostral intake (within 1st 2 hours), housing (semi-intensive), frequency of cleaning of animal house (daily), udder cleaning (at the time of milking, with plain water) and teat dipping (not practiced) in both categories (control versus cases) revealed no significant difference. Stocking density was comparable in both the groups [control (3.50 ± 0.76) versus cases (3.60 ± 0.52)]. The pathogen-wise occurrence of diarrhea is depicted in Table 4, indicating an absence of Cryptosporidium-associated diarrhea in our study.

Clinical trial

Vital parameters

The rectal temperature of infected calves was near normal. The respiration rate and heart rate was non-significantly higher in cases compared to controls. The treatment intervention had variable effect on these vital parameters (Table 6).

Table 5: Cultural and parasitological observations

Status at enrolment	Enrolments (No. of calves)	Status by day 3 (No. of calves)
Salmonella spp.	2	Negative
Eimeria spp.	14	10 Negative
		4 Positive
Cryptosporidium spp.	All Negative	All Negative
*E. coli spp.**	All Positive	All Positive

Pre-treatment ATS in cases was significantly different from control animals. Appreciable improvement in attitude was recorded in treated calves excepting those treated with zinc @ 2 mg/kg BW and antimicrobial. FCS improved in all treated cases and returned to near normal by day 3 especially those treated with antimicrobial alone probably due to insignificant difference at enrolment. Duration of skin tent didn't differ significantly either pre- or post-treatment, reason could be exclusion of calves requiring intravenous fluid therapy (Table 7, 8, 9). Calves treated with zinc either @ 2 or 4 mg/kg BW alone or together with antimicrobial took significantly less number of days for cure compared to calves treated with antimicrobial alone (Table 10).

Cultural and parasitological parameters

The experimental findings observed with respect to cultural and parasitological parameters at enrolment and exit are presented in table 5. Zinc @ 4 mg/kg BW produced antimicrobial like reduction in fecal coliform (*E. coli*) count by day 3. However, zinc @2 mg/kg BW alone or with antimicrobial showed similar growth inhibition potential (Table 11).

Micro-minerals

The micro-mineral estimation revealed relatively low Cu and Zn concentration in diarrheic calves compared to healthy calves. Zn supplementation showed variable effects on plasma Zn concentration. However, double dose of Zn either alone or in combination with antibiotic reduced the Cu concentration. Non-significant ($P<0.05$) alterations in Fe concentration were noticed in the study (Table 12).

Data collected from our study indicated a definite pattern of occurrence and age-related incidence of diarrhea in neonatal calves with maximum number of cases occurring in first month of life with a decline as the age progressed. More frequent observance of diarrhea in young calves is consistent with results of Garcia *et al*. (2000) and Bekele *et al*. (2012) who reported 76 % *E. coli*-associated diarrhea in young calves. The seasonal variation observed in number of cases could be related to prevalent (spring) calving period in our conditions. However, harsh weather conditions such as low temperature, rain, heavy snow, wind and high levels of moisture act as stress factors to young calves and increase their susceptibility to diarrhea (Larson *et al*., 2005). Neonatal calves are not able to effectively regulate their body temperature, when exposed to extreme weather conditions; this may induce hypothermia or hyperthermia resulting in immune system impairment. Special care is required to reduce environmental risk factors closely associated with calving season including the provision of dry and free shelter. Further, the calving season can be adjusted to a time when environmental conditions are more favourable by implementing a controlled breeding program. Isolation of *E. coli* from all diarrheic calves was

Table 6: Mean±SE of Vital parameters in healthy and diarrheic calves pre- and post treatment

Group	Status	Temperature (ºF)		Respiratory rate (bpm)		Heart rate (bpm)	
		Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
A	Healthy	$102.53±0.57^{Aa}$	$102.60±0.34^{Ba}$	$23.00± 2.36^{Ba}$	$25.66±2.40^{Ca}$	$109.00±5.25^{Aa}$	$109.66±4.80^{Aa}$
B	Diarrheic	$102.68±0.33^{Aa}$	$101.17±0.32^{Ab}$	$24.66 ±1.84^{Aa}$	$22.33±2.22^{Ab}$	$111.33±5.98^{Aa}$	$115.00±2.68^{Aa}$
C	Diarrheic	$102.43±1.09^{Aa}$	$101.46±0.49^{Aa}$	$27.00±2.86^{ABa}$	$24.16±2.42^{ABb}$	$117.66±6.97^{Aa}$	$109.66±5.54^{Ab}$
D	Diarrheic	$101.20±0.49^{Aa}$	$100.93±0.33^{Aa}$	$33.66±3.55^{ABa}$	$31.66±3.02^{BCa}$	$110.00±7.30^{Aa}$	$105.66±4.11^{Aa}$
E	Diarrheic	$101.20±0.49^{Aa}$	$100.86±0.20^{Aa}$	$30.33±4.66^{ABa}$	$27.00±3.24^{ABa}$	$112.66±7.08^{Aa}$	$103.50±2.36^{Aa}$
F	Diarrheic	$101.40±0.57^{Aa}$	$100.70±0.25^{Aa}$	$26.66±1.60^{ABa}$	$24.33±1.82^{ABb}$	$123.66±3.10^{Aa}$	$114.66±1.83^{Ab}$

Values with different superscript differ significantly ($P<0.05$); capital alphabets represent column-wise and small alphabets represent row-wise.

not considered causal in our study owing to inability to perform molecular characterization. Yet the significant and appreciable reduction in coliform count endorses the inclusion of Zn in therapeutic regimen of diarrheic calves; with double dose (4 mg/kg) of Zn producing antibiotic like reduction in *E. coli* count. Absence of *Cryptosporidium* claim warrants molecular epidemiological tools to be used for its diagnosis.

Table 7: Kruskal-wallis test results on clinical cure in healthy and diarrheic neonatal calves

Parameter	Pre-treatment	Post-treatment
ATS	0.014^{S}	0.801^{S}
FCS	0.003^{S}	0.025^{S}
Skin tent (Sec)	0.086^{NS}	0.573^{NS}

S-Significant, NS- Non-Significant based on Kruskal-wallis test.

Table 8: Mann-Whitney test results on clinical cure in healthy and diarrheic neonatal calves

ATS	Pre-treatment	Post-treatment
Group A and E	0.002^{S}	0.699^{NS}
FCS		
Group A and D	0.006^{NS}	0.007^{NS}
Group A and B	0.002^{S}	0.317^{NS}
Group A and E	0.002^{S}	0.138^{NS}
Group A and F	0.002^{S}	0.138^{NS}
Group A and C	0.001^{S}	0.317^{NS}

S-Significant, NS-Non Significant based on Mann-Whitney test.

Table 9: Chi-square test results of ATS, FCS and skin tent from a clinical trial in healthy and diarrheic neonatal calves

Groups	ATS	FCS	Skin tent
B	0.444^{S}	8.800^{NS}	5.667^{S}
C	3.286^{S}	8.667^{NS}	9.000^{S}
D	1.867^{S}	1.667^{S}	6.800^{S}
E	8.667^{NS}	8.000^{NS}	6.000^{S}
F	0.444^{S}	7.200^{NS}	6.286^{S}

S-Significant, NS-Non Significant based on Pearson Chi-Square values.

Table 10: Mean±SE of number of days taken for recovery in therapeutic trial study in diarrheic neonatal calves

Treatment groups	Enrolments/ group	Clinical cure by day 3	Number of days taken for recovery
B	6	6	2.17^{a}±0.17
C	6	5	3.00^{a} ±0.63
D	6	2	5.00^{b} ±0.37
E	6	6	3.00^{a} ±0.00
F	6	6	2.83^{a} ±0.17

Values with different superscript column-wise differ significantly (P<0.05).

There is high risk for calves to develop *E. coli* diarrhea during first month of life (Scott *et al.*, 2004) and low risk to develop *Cryptosporidium*-associated diarrhea reasonably due to their milk feeding upto 3 months of age (Shahardar *et al.*, 2009). However, due to high stocking rate indoor management may also facilitate transmission

Table 11: Mean±SE of alterations in fecal coliform count from therapeutic trial study in healthy and diarrheic neonatal calves

Group	Status	***Fecal Coli form (E. coli) count (x 10^7)***	
		Pre-treatment	**Post-treatment**
A	Healthy	1.54^{Aa} ± 0.29	1.23^{Ba} ± 0.24
B	Diarrheic	1.66^{Aa} ± 0.33	0.89^{ABb} ± 0.33
C	Diarrheic	1.95^{Aa} ± 0.44	0.48^{Ab} ± 0.14
D	Diarrheic	2.01^{Aa} ± 0.38	0.42^{Ab} ± 0.08
E	Diarrheic	1.96^{Aa} ± 0.42	0.92^{ABb} ± 0.26
F	Diarrheic	2.01^{Aa} ± 0.30	0.48^{Ab} ± 0.09

Values with different superscript differ significantly (P<0.05); capital alphabets represent column-wise and small alphabets represent row-wise.

Table 12: Mean±SE of Plasma Copper, Zinc, and Iron in healthy and diarrheic calves pre- and post- treatment

Group	Status	Copper (ppm)		Zinc (ppm)		Iron (ppm)	
		Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
A	Healthy	0.81 ± 0.24^{Aa}	0.82 ± 0.24^{ABa}	3.73 ± 0.40^{Ba}	3.65 ± 0.39^{Ba}	15.37 ± 2.18^{Aa}	15.20 ± 2.20^{Aa}
B	Diarrheic	0.62 ± 0.51^{Aa}	0.75 ± 0.20^{ABa}	2.86 ± 0.24^{ABa}	2.50 ± 0.19^{Aba}	14.92 ± 5.27^{Aa}	12.20 ± 1.87^{Aa}
C	Diarrheic	0.65 ± 0.19^{Aa}	0.30 ± 0.19^{Aa}	2.10 ± 0.48^{Aa}	2.50 ± 0.30^{Aba}	12.65 ± 1.95^{Aa}	12.13 ± 1.20^{Aa}
D	Diarrheic	0.44 ± 0.13^{Aa}	0.75 ± 0.20^{ABa}	2.97 ± 0.53^{ABa}	3.33 ± 0.54^{Aba}	14.89 ± 2.42^{Aa}	12.91 ± 1.36^{Aa}
E	Diarrheic	1.01 ± 0.55^{Aa}	0.87 ± 0.06^{ABa}	3.20 ± 0.82^{ABa}	2.79 ± 0.60^{Aba}	13.32 ± 1.19^{Aa}	13.30 ± 1.79^{Aa}
F	Diarrheic	0.82 ± 0.25^{Aa}	0.52 ± 0.13^{ABa}	2.63 ± 0.15^{ABa}	2.36 ± 0.13^{Aa}	14.20 ± 2.91^{Aa}	10.80 ± 0.56^{Aa}

Values with different superscript differ significantly (P<0.05); capital alphabets represent column-wise and small alphabets represent row-wise.

of protozoan parasites with subsequent occurrence of diarrhea and nutritional deficiency (El-Khodery *et al*., 2008). Housing of calves with their mothers and without contact with other calves tended to decrease the prevalence of Cryptosporidium infections, and infection risk decreased when calves were individually housed in pens with a cement floor previously disinfected and daily washed with water under pressure (Kvac *et al*., 2006). Salmonella positive cases (2 only) got completely cured by day 3 using zinc @ 2 mg/kg BW and antibiotic, hence the study remains inconclusive regarding individual effect of zinc on *Salmonella*-associated diarrhea. Out of 14 pre-treatment *Eimeria* positive cases 10 were declared negative post-treatment but rest 4 cases still showed mild-moderate infection. Zinc either @ 2 mg/kg BW or 4 mg/kg BW produced effects comparable to antibiotic alone or in combination suggesting *in-vitro* studies to be carried out regarding effect of Zn on oocyst count. In all the calves (healthy or infected) enrolled, normal colostrum intake and cleaning of udder before milking with lukewarm water was revealed by the owners. Housing was semi-intensive and cleaning of animal house was observed daily in all cases. Thus, there was no significant impact of these parameters on the occurrence of calf diarrhea in our study. However, large scale epidemiological study is needed to arrive at a conclusion.

Diarrheic calves showed variable clinical manifestations - soiling of perineum and tail, depression, varying degree of dehydration, tachycardia, tachypnea, rough body coat, dry muzzle and weakness. The feces were semi-formed to watery with yellowish-white in color and sometimes blood stained. In diarrheic calves profuse watery to pasty, usually pale yellow and occasionally streaked with blood flecks and very foul smelling feces (Ahmad, 1990) coupled with tachycardia and tachypnea were observed (Kumar *et al.*, 2002). Yellowish white nature might be due to high content of salt particularly bicarbonates (Ward, 1976) with heavy secretion of water in intestinal lumen (Szancer, 1980). These findings were similar to earlier reports (Radostitis *et al*., 2007; Bhat, 2013). Increased cervical skin tent duration (seconds) in diarrheic calves indicated decreased skin elasticity owing to extracellular fluid loss (Radostitis *et al.*, 2007).

Relative decrease in rectal temperature though non-significant and well within the normal range as observed in diarrheic calves might be due to dehydration and consequent reduced peripheral perfusion (Bhat, 2013). Colibacillotic calves may have subnormal body temperature (Fernandes *et al*., 2009) or can be pyrexic (Samad *et al*., 2003). Dehydration and metabolic upsets could be a reason for tachypnea and tachycardia (Kumar *et al.*, 2002).

Reduced intestinal absorption of food nutrients and consequent fecal loss in diarrheic calves could justify the relatively low concentration of Cu and Zn (Ghanem *et al*., 2003). Plasma Zn fluctuates with age, stress, infections and feed restrictions. It is very high (2.3 µg/ml) in newborn calves and drops to 1.3 µg/ml by 12 weeks of age (Kincaid *et al*., 1989). Plasma Zn as part of an acute phase response is initially reduced by infection (Wellinghausen and Rink, 1998); only to become elevated within a few days. Reduction in Cu concentration due to 4 mg/kg BW Zn supplementation could be due to their antagonistic nature. Hence, addition of 1 mg of Cu/10 mg of Zn supplemented as reported in literature is justified and imperative in such studies.

The treatment intervention showed significant ($p<0.05$) decrease in coliform count in all the groups. Microbial killing effect of the antibiotic and/or dose-dependent reduction in microbial proliferation by Zn could be considered for decrease in coliform count (Crane *et al*., 2007). Significant reduction in number of days taken for clinical recovery in Zn supplemented cases could be attributed to mucosal protective role, enhanced cell-mediated immunity and modification of intra-luminal electrolyte secretion and absorption mechanisms of zinc (Turner *et al*., 2000). Higher rate of clinical cure in calves treated with zinc oxide compared to placebo-treated calves has been reported (Glower *et al*., 2013). Zinc citrate or zinc gluconate clinically were equally effective in treatment of diarrhea in human recipients (Wegmuller *et al*., 2014). Other clinical trials with zinc reported similar benefits in addition to reduction in number of diarrhea episodes (Bhutta *et al*., 2000) but the same couldn't be evaluated given the on-field nature of this study.

CONCLUSION

The present study identified and strengthened the clinical importance of zinc in neonatal calf diarrhea. It provides Veterinary Practitioners' a viable alternative for checking the gratuitous use of antibiotics in neonates.

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