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Haemato-biochemical Changes and Various Risk Factors Associated with Bovine Coronavirus Infection in Cattle Calves

Safdar Abbas[1], Jawaria Ali Khan[1]*, Syed Saleem Ahmed[1] and Aftab Ahmed Anjum[2]

[1]*Department of Veterinary Medicine, University of Veterinary and Animal Sciences, Lahore, Pakistan*
[2]*Institute of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan*

ABSTRACT

Bovine coronavirus (BCoV) is a major viral pathogen linked to respiratory and enteric problems in newborn calves. The goal of this study is to look into the molecular occurrence, haemato-biochemical changes, and risk factors associated with the occurrence of BCoV infection in cattle calves at different dairy farms and small households in the district of Jhelum, Pakistan. From July 2020 to June 2021, 200 faecal samples were collected from newborn cattle calves exhibiting symptoms of diarrhoea and dysentery. S&C Biotech Bovine Coronavirus Antigen Rapid Test Kits were used to screen samples, which were then subjected to RT-PCR for molecular characterization. For the haemato-biochemical analysis, blood was taken from calves infected with BCoV. A questionnaire was used to collect information about the risk factors associated with the occurrence of BCoV infection. BCoV infection was found in 3.5% (7/200) of calves using diagnostic screening kits and 3.0% (6/200) of calves using RT-PCR. Breed, age, sex, vomiting, previous history of diarrhoea, body conditions, food type, colostrum feeding, deworming history, living environment, seasonal variation, and cohabitation with other animals were all assumed risk factors for BCoV infection ($p<0.05$). There was a significant ($p<0.001$) decrease in MCV (fl) and a significant ($p<0.006$) increase in TLC ($1x10^3/cm^3$) on haematological analysis in BCoV-infected calves. TEC ($1x10^6/cm^3$), WBC ($1x10^6/cm^3$), RBC ($1x10^6/cm^3$), and Hct% were significantly ($p<0.05$) higher. Similarly, infected calves had a non-significant ($p<0.090$) increase in K (mEq/L) and a significant ($p<0.000$) decrease in Na (mEq/L), Ca (mmol/L), Cu (mol/L), and Fe (mol/L) on biochemical analysis. It was concluded that the occurrence of BCoV infection was predisposed by assumed risk factors, and haemato-biochemical alterations were observed in BCoV infected calves.

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JAK, SSA and AAA conceptualized the research topic of this manuscript. SA conducted the research, analyzed the data statistically and wrote the manuscript.

Key words
Bovine coronavirus, Cattle calves, Rapid test kits, RT-PCR, Risk factors, Haemato-biochemical analysis

INTRODUCTION

Bovine coronavirus (BCoV) is a significant viral pathogen associated with respiratory and enteric problems in newborn calves. Mebus discovered it by accident in 1972, and it was thought to be the most common cause of calf diarrhoea at the University of Nebraska (Vlasova and Saif, 2021). In 1984, McNulty isolated BCoV strains from the lungs of a calf suffering from respiratory problems, making further progress. As a result, it was determined that BCoV could cause respiratory and enteric problems in cattle (Zhu *et al*., 2022). It is a widespread viral pathogen that causes disease and economic losses in the dairy and beef industries around the world (Oma *et al*., 2016). Bovine coronavirus infections are worldwide reported and cause great economic losses to dairy and beef industry (Yavru *et al*., 2016).

Bovine enteric or enteropathogenic coronaviruses (BECoV) are coronavirus strains isolated from adult cattle with diarrhoea and neonatal diarrheic calves (Lin *et al*., 2002). The BCoV can infect cattle in three ways: calf diarrhoea in calves less than one month old, winter dysentery in adult cattle, and respiratory diseases in both young and adult cattle (Cho and Yoon, 2014). The virus is a major cause of neonatal calf diarrhoea (NCD) in beef and dairy herds during the first three weeks of life (Vlasova and Saif, 2021).

BCoV infection is primarily spread via faecal, oral, and, to a lesser extent, respiratory routes (Thomas *et al*., 2006). Inter-herd transmission can occur either

* Corresponding author: jawaria.khan@uvas.edu.pk
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directly through the transfer of live animals or indirectly through contaminated personnel or equipment on dairy farm premises (Oma *et al*., 2016). BCoV infection is typically transmitted horizontally from carrier dam to offspring after calving. Clinically infected calves spread the infection when housed with healthy calves. There is still no evidence of vertical transmission (Evermann and Benfield, 2001). Dogs are passengers of the BCoV infections on dairy farms and they may play a major role in spreading the BCoV infections (Kaneshima *et al*., 2007).

The BCoV first replicates on the villi of the small intestine, then spreads to the large intestine up to the end of the colon and rectum, causing malabsorptive diarrhoea (Boileau and Kapil, 2010). This virus causes mild to severe clinical symptoms such as fever, respiratory problems, gastrointestinal symptoms, dysentery, and yellowish watery diarrhoea (Boileau and Kapil, 2010). During the research, it was discovered that respiratory distress was linked to BCoV infection in calves and adults (Lathrop *et al*., 2000).

These viruses can be identified using the cell-line culture method or the polymerase chain reaction or rapid detection kits tests. Rapid disease diagnosis using a Rapid Diagnosis kit is very useful and practical for veterinarians in the field because it provides quick results and takes less time (Sakli *et al*., 2019). There is no cure for viral infections; instead, supportive care is provided to correct electrolyte imbalances, metabolic acidosis, and dehydration (Fox, 2015). Oral electrolyte and rehydration therapy is a simple and cost-effective method for correcting the diarrhea (Smith, 2009). Vaccinating dams in their final trimester of pregnancy is the primary strategy for preventing BCoV-induced enteritis in calves and providing passive immunity (Boileau and Kapil, 2010). Infection prevention is based on prepartum dam vaccination, biosecurity of farm premises, good colostral management protocols, and hygiene practices (Hodnik *et al*., 2020).

They are the most commonly found pathogens, causing acute and chronic diarrhea in bovine calves and posing a significant threat to the dairy and beef industries (Alfieri *et al*., 2018). It results in massive economic losses for the livestock industry (Boileau and Kapil, 2010). Keeping in mind the importance of concerns about morbidity and mortality linked to BCoV infection among newborn cattle calves, this is the first study on the occurrence of BCoV infection in cattle calves in Pakistan. It focuses on various risk factors associated with the occurrence of BCoV infection and its effects on various haemato-biochemical parameters of cattle calves.

MATERIALS AND METHODS

Study area and animals

The study was carried out in district Jhelum of Province Punjab, Pakistan (Latitude: 32.940548° N and Longitude: 73.727631° E) on cattle calves less than one month of age exhibiting symptoms of diarrhea and dysentery at different livestock farms and small dairy households in the study area. The duration of this whole study was 12 months (July 2020 to Jun 2021). Holstein Friesian and crossbred cattle calves were selected in this study.

Sampling procedure and processing of fecal samples

A total of 200 fecal samples were collected from the rectum of calves with the history of diarrhea and dysentery. The S & C Biotech Bovine Coronavirus Antigen Rapid Test with principle of Sandwich Lateral Flow Immunochromatographic Assay and RT-PCR tests were performed to screen the fecal samples for BCoV infection in cattle calves.

Molecular characterization and PCR amplification

After initial screening with diagnostic kits, fecal samples found positive for BCoV infection were subjected to RT-PCR assay to compare the efficacy of these two diagnostic tools i.e diagnostic screening kits and RT-PCR for the occurrence of BCoV infection in cattle calves. In this phase, RNA extraction from fecal sample, polymerase chain reaction and Agarose gel electrophoresis were performed. RNA was extracted from fecal samples by Total RNA Fast Extraction Stool Kit (China). The cDNA was prepared from mRNA or total RNA templates using commercially available Thermo Scientific™ RevertAid™ First Strand cDNA Synthesis Kit (Sulehria *et al*., 2020). To confirm the presence of bovine coronavirus in the fecal sample of diarrheic cattle calves, a portion of BCoV was amplified by RT-PCR following the protocol described by (Agnihotri *et al*., 2017). After amplification of the virus fragment, the electrophoresis was performed to visualize the results (Sulehria *et al*., 2020).

Hemato-biochemical analysis

The blood samples of the diarrheic calves (who were found positive for BCoV after preliminary screening rapid detection tests) were collected for haemato-biochemical analysis. From each calf, a total of 10 ml of blood was collected, 5 ml for CBC and 5 ml for serum electrolytes analysis. The VET hematology analyzer was used for performing the CBC (red blood cells, packed cell volume (PCV), hemoglobin (Hb), mean corpuscle volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells

(WBC), lymphocytes, basophils, total leukocytes count (TLC), total erythrocyte count (TEC), hematocrit (Hct) and monocytes). The serum was stored at -20°C till further analysis (Sulehria *et al*., 2020). The serum samples were analyzed for estimation of biochemical parameters (sodium, potassium, calcium, copper and iron) using a semi-automated clinical chemistry analyzer machine. All the tests were performed following the manufacturer's directions. The results were obtained and recorded for later interpretations.

Statistical analysis

All statistical analysis was done on SPSS version 20. Data regarding hemato-biochemical parameters was analyzed by t-test. And data regarding various assumed risk factors was analyzed by Chi Square. Odds ratio was determined to know the degree of association of risk factors with the occurrence % of bovine coronavirus infection, *p*-value (<0.05) was considered significant.

RESULTS AND DISCUSSION

Occurrence of bovine coronavirus infection and associated risk factors

With the development and advancement in the best farm practices, diagnostic techniques and treatment regimes, the mortality of calves have been remained a major problem even in developed countries. Pakistan is an agricultural country with huge population of livestock in it, facing the neonatal calf diarrhea associated with BCoV outbreaks.

The current study demonstrated that out of 200 fecal samples, 3.5% (7/200) were found positive through diagnostic kits and after confirmation through RT-PCR, 3.0% (6/200) were found positive for BCoV infection in diarrheic cattle calves. When data was statistically analysed, occurrence of BCoV infection through diagnostic screening kits was 1.173 times more than checked through RT PCR. Similarly, results of percentage occurrence when checked through kits and PCR were non-significant ($p<0.778$). The graphical representation of percentage occurrence of the BCoV infection through diagnostic kits (3.5%) and PCR (3.0%), respectively is shown in (Fig. 1). The findings of Lotfollahzadeh *et al*. (2020), Brunauer *et al*. (2021), Barkley *et al*. (2021) and Singh *et al*. (2020) corroborated the current study's findings. There are some differences in the results, but these differences could be due to a different study design, a different target animal, or seasonal variation. The incidence rate of BCoV infection varies throughout the year due to seasonal variations. The most important risk factor that contributes to the spread of disease caused by BCoV infections is the weather (Boileau and Kapil, 2010). The variations in results might be due to the incapability of the bovine coronavirus to remain intact in the feces due to activities of endogenous RNase enzyme and unavailability of partially degraded RNA may affect the sensitivity of RT-PCR or intermittent shedding of the viruses in fecal materials (Vermeulen *et al*., 2011).

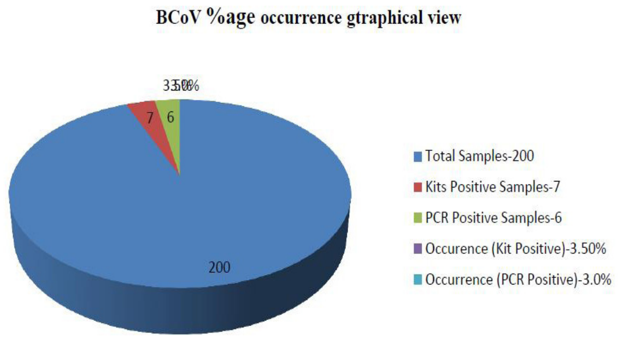

Fig. 1. Pie chart showing the percentage occurrence of BCoV infection after performing diagnostic Screening kits tests and RT-PCR.

The occurrence rate of each variable had been calculated and recorded for each variable. Each risk factor had a significant ($p<0.05$) association with the occurrence of BCoV infection in diarrheic cattle calves. According to the current study's findings, exotic Holstein Friesian and crossbred calves were significantly ($p<0.000$) more likely to be infected with BCoV than indigenous crossbred calves in the country. Among three age groups of calves, 1 to 10 days, 11 to 20 days, and 21 to 30 days, calves 1 to 10 days were significantly ($p<0.001$) affected with BCoV infection when compared to the other two age groups. Male calves were significantly ($p<0.034$) more affected by BCoV infection than female calves. Male calves were two (OR=2.011) times more affected than female calves. The findings of Seid *et al*. (2020) and Bertoni *et al*. (2021) supported the current study's findings.

The season was found to be significantly ($p<0.001$) related to the occurrence of BCoV infection in calves. The seasonal association between winter and monsoon had no significant ($p<0.586$) effect on the occurrence rate in calves, whereas spring and summer had a highly significant ($p<0.001$) effect on calves infected with BCoV. The highest occurrence (5.10%) of BCoV infection was observed during the summer season, while zero occurrences were observed during the winter season. The current study's findings are very similar to those of Trotz-Williams *et al*. (2007) and Boileau and Kapil (2010).

According to body condition, when data from normal, emaciated, and fatty calves was compared, emaciated calves (7.9%) were significantly ($p<0.02$) more affected by BCoV infection than fatty calves (2.9%) and normal (1.0%) calves. The findings also revealed that emaciated calves were 8.793 times more likely to be affected by BCoV infection than fatty and normal calves. This finding is also consistent with the findings of a study conducted in North West Ethiopia by Tamrat *et al*. (2020).

Food was also a high risk factor in calves; milk-fed calves were significantly ($p<0.005$) more likely to be infected with BCoV than calves on milk replacer. According to the findings, milking calves were 3.188 times more likely to be infected with BCoV than calves on milk replacer. The current study also found that calves raised on milk had a higher rate of infection (35.2%) than calves being raised on milk replacer (14.5%). These findings are similar to those of Kayasaki *et al*. (2021).

Table I. Risk factors associated with the occurrence of BCoV infection in cattle calves.

Risk factors	No of samples	Occurrence	Odd ratio	95 %CI	P-value	Chi square
Breed						
Holstein Friesian	117	45.3%	10.63	4.29- 26.32	0.000**	33.837
Crossbred	83	7.2%				
Age						
Less <10 days	91	51.6%	0.32	0.15 - 065	0.001**	10.103
11-20 days	67	16.4%				
11-20 days	67	16.4%	8.05	1.00 - 64.88	0.023*	5.192
21 - 30 days	42	2.4%				
Sex						
Male	57	40.4%	2.01	1.05 - 3.85	0.034*	4.513
Female	143	25.2%				
Season						
Winter	14	7.1%	0.55	0.06 - 4.87	0.586	0.297
Monsoon	57	12.3%				
Spring	31	12.9%	0.16	0.052 - 0.49	0.001**	12.107
Summer	98	48.0%				
Body condition						
Fatty	34	2.9%	0.35	0.04 - 3.14	0.33	0.95
Emaciated	63	7.9%				
Emaciated	63	7.9%	8.79	1.003-77.09	0.02*	5.444
Normal	103	1.0%				
Food						
Milk	145	35.2%	3.19	1.39 - 7.26	0.005**	8.158
Milk replacer	55	14.5%				
Previous history of diarrhea						
Yes	30	13.3%	0.32	0.11 - 0.97	0.035*	4.435
No	170	32.4%				
Housing						
Open	150	34.7%	8.31	2.47- 26.006	0.000**	15.457
Confined	50	6.0%				
Living with other animals						
Yes	50	6.0%	0.12	0.04 - 0.40	0.000**	15.457
No	150	34.7%				

Data are indicated as (Occurrence%, odd ratio, 95% confidence interval, P-value, chi square) and ** shows the values are highly significant ($p<0.01$), while * shows values are significant ($p<0.05$).

The current study also found that calves with a history of diarrhea had a higher infection rate (13.3%) than those without a history of diarrhea (32.4%). The findings of this study differed from the findings of Tamrat *et al.* (2020). The disparity in results could be explained by the fact that the calves with a history of diarrhoea recovered well.

According to this study, open housed calves, i.e. calves in cages, had 34.7% infection compared to confined and living with other animals (6.0%). The findings of Waltner-Toews *et al.* (1986), Quigley *et al.* (1995) and Bazeley (2003) corroborated the current study's findings. Bertoni *et al.* (2021) discovered that calves raised in grouped housing with other animals had a higher risk of infection with BCoV than calves being raised in open housing. The disparity in results is due to calves being separated for sampling from less congested confined housing in small households where only one or two calves were raised. Calves that live with other animals are more likely to get diarrhea. However, the results of this study differ because the samples were mostly taken from calves living in cages. The calves living with other animals were chosen from small households that were raising one or two calves at a time and had clean housing, but the calves were confined to a fixed location with a rope. As a result, the infection occurrence ratio was lower when compared to calves placed in cages. The study design, animal selection, location, and seasonal variations may all have an impact on the results. When developing an experimental design, it is critical to keep all major variables in mind and control them as much as possible. Another common mechanism influencing study results is a change in variable variation (National Research Council, 2011).

Further investigation into the cohabitation of calves living with other animals revealed that those calves who had a history of living with other animals were significantly (p<0.000) affected with BCoV infection (Table I).

Haematological parameters analysis

Complete blood count (CBC) and serum electrolytes analysis were performed using an atomic absorption spectrophotometer for haematological and serum biochemistry analyses, respectively. Statistical analysis revealed that MCV, TLC, TEC, WBC, RBC and Hct were significantly (p<0.05) increased while MCH, basophil and monocytes were significantly decreased in diarrheic calves infected with BCoV infection. Other parameters such as Hb, MCHC, lymphocytes, and PCV were not affected significantly with the occurrence of BCoV infection (Table II). The findings of previous studies (Barua *et al*., 2018; Song *et al*., 2020) supported the current study's findings.

Biochemical parameters analysis

Biochemical analysis revealed that there was no significant (p<0.09) increase in potassium ions (mEq/L) in calves infected with BCoV. Calves infected with BCoV had significantly lower levels of sodium ions (mEq/L), calcium ions (mmol/L), copper ions (mol/L), and iron ions (mol/L) (Table III). The current study's findings were similar to those reported by Barua *et al.* (2018) and Tajik *et al.* (2012). The minor variations in results could be attributed to different study designs, different target animal locations, or seasonal variations.

Table II. Hematological parameters of diarrheic cattle calves infected with BCoV infection.

Parameters	Mean±SD	SEM	t statistics	P-value	95% C.I
PCV (%)	43.14 ± 1.60	.72	.88	.425	41.15-45.13
Hb (g/dL)	10.39 ± 1.92	.86	-1.28	.269	8.01-12.78
MCV (fl)	38.00 ± 3.39	1.51	-7.91	.001**	33.79-42.21
MCH (pg)	12.93 ± 0.54	.24	-4.43	.011*	12.26-13.60
MCHC (g/dl)	33.36 ± 2.68	1.20	-1.37	.243	30.02-36.69
Monocytes (%)	2.43 ± 0.45	.20	-2.79	.049*	1.87-3.00
Lymphocytes (%)	53.65 ± 5.98	2.67	-2.37	.076	46.22-61.07
Basophils (%)	0.56 ± 0.09	.04	-10.25	.001**	0.44-0.68
TLC ($1x10^3/cm^3$)	13.12 ± 1.44	.64	5.31	.006**	11.33-14.91
TEC ($1x10^6/cm^3$)	8.72 ± 0.28	.12	4.91	.008**	8.37-9.06
WBC ($1x10^6/mm^3$)	21.37 ± 2.76	1.23	10.82	.000**	17.94-24.81
RBC ($1x10^6/mm^3$)	12.09 ± 1.15	.51	6.96	.002**	10.66-13.52
Hct (%)	56.63 ± 1.55	.69	27.65	.000**	54.71-58.55

Data are indicated as (Mean±SD, SEM, t statistic, P-value, 95% confidence interval) and ** shows that the values are highly significant (p<0.01), while * shows values are significant (p<0.05). BCoV stands for bovine coronavirus.

Table III. Biochemical parameters of diarrheic cattle calves infected with BCoV infection.

Parameters	Mean±SD	SEM	t statistic	P-value	95% C.I
Na (mEq/L)	127.892 ± 0.957	.42804	-37.632	.000**	126.70-129.08
K (mEq/L)	6.242 ± 0.293	.13113	2.227	.090*	5.88-6.61
Ca (mmol/L)	1.324 ± 0.238	.10647	-11.985	.000**	1.03-1.62
Cu (µmol/L)	7.554 ± 0.426	.19072	-31.176	.000**	7.02-8.08
Fe (µmol/L)	8.382 ± 0.350	.15682	-77.813	.000**	7.95-8.82

Data are indicated as (Mean±SD, SEM, t statistic, P-value, 95% confidence interval) and ** shows that the values are highly significant ($p<0.01$), while * shows values are significant ($p<0.05$). BCoV stands for bovine coronavirus.

CONCLUSION

It was concluded that BCoV infection was present in cattle calves in Punjab, Pakistan. Assumed risk factors like breed, age, sex, season, housing type, food type, hygiene, environmental conditions, body conditions, and contact with animals were all found to be less or more significantly associated with the occurrence of BCoV infection. The variability in haematological and biochemical findings can aid in the diagnosis of BCoV infection in animals.

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IRB approval

This study was approved by Advanced Studies and Research Board, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan on 09-10-2019. (Approval no: DAS/:8250 Dated: 28/10/2019).

Ethical statement

The study design was presented to the Ethics Committee, University of Veterinary and Animal Sciences, Lahore, for ethical approval.

Statement of conflict of interest

All authors have declared no conflict of interest.

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