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INVESTIGATION OF ROTAVIRUS INFECTION IN COW CALVES AND ASSOCIATED RISK FACTORS WITH HAEMATO-BIOCHEMICAL ALTERATIONS

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ABSTRACT

The bovine rotavirus (BRoV) is one of the major causes of illness and death in newborn calves. The study's objective was to pinpoint the haemato-biochemical alterations, other risk factors, and molecular manifestations associated with BRoV infections in dairy cow calves in in Jhelum district, Pakistan. From July 2020 to June 2021, a total of 200 faeces samples were taken from neonate cow calves under 28 daysold that had a history of diarrhea and dysentery. Prior to further polymerase chain reaction processing, samples were initially screened using S&C Biotech Bovine Rotavirus Antigen Rapid Test Kits. For the haemato-biochemical study, blood samples were obtained from calves infected with BRoV. On a questionnaire form, information was gathered for the analysis of the various risk factors linked to the occurrence of BRoV infection. The occurrence of BRoV infection while utilizing diagnostic screening kits was 26% (52/200), and when using RT-PCR, it was 21.5% (43/200). BRoV infection was significantly ($p\leq0.05$) influenced by breed, age, sex, vomiting, prior history of diarrhea, bodily conditions, food type, colostrum feeding, deworming history, living environment, interaction with other animals, and season. Hematological and biochemical markers showed significant ($p\leq0.05$) alterations. Mean corpuscular volume, basophils and lymphocytes were decreased significantly ($p\leq0.05$ while mean corpuscular hemoglobin, total leukocyte count, TEC, white blood cells count, red blood cell and Monocytes were increased significantly ($p\leq0.05$). Similarly, among biochemical parameters, Potassium was non-significantly ($p>0.05$) increased, while Sodium, Calcium, copper and iron were significantly ($p\leq0.000$) decreased. Itwas concluded that assumed risk factors were contributed to the BRoV infection, and infected calves showed haemato-biochemical changes**.**

Keywords: neonate calves, rotavirus, diagnostic test, Pakistan

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INTRODUCTION

Because of its high transmission and infectivity, BRoV is a major cause of calf diarrhea among many other infectious agents (Seid U *et al.,* 2021). Rotavirus is a double-stranded RNA virus that encodes six structural viral proteins and six non-structural viral proteins (Matthijnssens and Van Ranst, 2012). Among those etiological agents associated with NCD, bovine rotaviruses (BRoVs) are the most common and major viral enteric pathogens, infecting approximately 27-36% of cattle (Seid U *et al.,* 2020). In India, the incidence rateof bovine rotavirus-associated diarrhea in calves ranged from 7.49% to 43% (Nitur*eet al.,* 2009). Rotavirus is of zoonotic importance and causes gastroenteritis, cerebellitis, and cardiac problems in human beings. Food and water contaminated with bovine rotavirus are the major source of infections in human and animals (Qin *et al.,* 2022).

The faecal oral route is the primary mode of transmission for BRoV infection (Qin *et al.,* 2022). The infection is most common in young calves aged 2 to 8 weeks (Dhama *et al.,*2009). Its infectivity and susceptibility are decreased with age, due to thecalves acquire immunity from previous infections (Miranda *et al.,* 2022). In neonates, the infection is non-viremic, have very short incubation period, and causes profuse diarrhea and severe dehydration (Dhama *et al.,* 2009). It causes mild to severe enteritis, dehydration, dullness, depression and pale yellowish diarrhea in neonatecalves. Sometimes, death occurs in severe cases due to dehydration, electrolytes imbalance and cardiac arrest (Geletu *et al.,* 2021).

BRoV infection can be diagnosed by cell-line culture method or polymerase chain reaction (PCR). These procedures are time consuming and require experienced technician. On other hand, the rapid S&C Biotech Bovine Rotavirus Antigen Rapid Test Kits is very helpful and practical for veterinarians in field as this

method gives quick results and less time consuming (Sakli *et al.,* 2019).

Oral rehydration therapy is a simple and cost-effective management for restoring essential nutrients (Smith, 2009). Good management practices, hygiene, and dam vaccination are critical for the protection of young calves against BRoV infection (Dhama *et al.,* 2009).

Despite advances in animal husbandry practices, diagnostic techniques, and treatment regimens, calf morbidity and mortality remained the BRoV outbreaks a major public hazard (Geletu *et al.,* 2021). Therefore, the rapid diagnosis of BRoV was concerned the main issue of current study in order to improve and protect the country's livestock.

MATERIALS AND METHODS

Study Area and Animals:The cross-sectional 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 (Madina Dairy Farm, Baloch Dairy Farm, NS Dairy Farm, Taqva Dairy Farm, Al Rehman Dairy Farm, Najam Shah Dairy Farm, Kamaly Dairy Farm and Haji Shafique Farm) and small dairy households in the study area. Calves of Holstein Friesian cattle and their crossbreds with local cattle were selected for this study. The duration of this whole study was from July 2020 to June 2021.

Sampling procedure and processing of fecal samples: Four categories were made for samples collection, each category was predefined. Category-1: small households having 1-10 animals, category-2: small livestock farms having 1-50 animals, category-3: medium livestock farms having 51-100 animals and category-4: large livestock farms will be having above 100 animals. The fecal samples were collected from diarrheic calves found in all four categories to perform rapid detection tests and RT-PCR. A total of 200 faecal samples were collected from calves with a history of diarrhoea and dysentery. To screen faecal samples for BRoV infection in cattle calves, the S&C Biotech Bovine Rotavirus Antigen Rapid Test with Sandwich Lateral Flow Immunochromatographic Assay and RT-PCR tests were used. Age wise calves were divided into three groups; namely group 1 (1 to 10 days), group 2 (11 to 20 days), and group 3 (21 to 30 days). By health and body conditions, calves were divided into three groups; normal, emaciated and fatty.

Sandwich Lateral Flow Immunochromatographic Assay and Test Procedure: S&C Biotech Bovine Rotavirus Antigen Rapid Test is based on sandwich lateral flow immunochromatographic assay. All the fecal samples were initially screened out by S&C Biotech Bovine Rotavirus Antigen Rapid (a diagnostic test kit to screen out viruses from feces or vomit with BRoV Ag, commercially available manufactured by China.) The diagnostic kits were used according to the manufacturer instructions (Figure.1). The test procedure was followed as sops set manufacturer and first used in Pakistan by (Sulehria *et al.,* 2020).

Interpretation of the Results: The results were interpreted in three ways in 5 to 10 minutes (Figure.1). After 10 minutes, results were considered as invalid.
Positive (+): The presence of both “C” line and “T” line, no matter T band was clear or vague.
Negative (-): Only a clear C line appeared. No T line.
Invalid: No colored line appeared in C zone. No matter if T line appeared.

Molecular Characterization and PCR Amplification: Following an initial screening with diagnostic kits, faecal samples found positive for BRoV infection were subjected to an RT-PCR assay to compare the efficacy of these two diagnostic tools, screening kits and RT-PCR, in detecting the occurrence of BRoV infection in cattle calves. This phase included RNA extraction, polymerase chain reaction, and Agarose gel electrophoresis. Total RNA Fast Extraction Stool Kit was used to extract RNA from faecal samples (China).The cDNA was synthesized from mRNA or total RNA templates using the Thermo Scientific TM Revert Aid TM First Strand cDNA Synthesis Kit, which is commercially available (Sulehria *et al.,* 2020). To confirm the presence of BRoV in calves' faeces, a portion of BRoV was amplified by RT-PCR according to the protocol described by (Agnihotri *et al.,* 2017). Following the amplification of the virus fragment, electrophoresis was used to visualize the results (Sulehria *et al.,* 2020).

Hemato-biochemical Analysis: Blood samples were collected from diarrheic calves (who tested positive for BRoV infection after preliminary screening tests) for haemato-biochemical analysis. A total of 10 ml of blood was collected from each calf, 5 ml for CBC and 5 ml for serum electrolytes analysis. The CBC was performed using a VET haematology analyzer to estimate the concentrations of packed cell volume, mean corpuscular hemoglobin concentration, mean corpuscular volume, basophils, lymphocytes, hemoglobin, mean corpuscular hemoglobin, total leukocyte count, TEC, white blood cells count, red blood cell and monocytes. The serum was kept at -20°C until further testing. A semi-automated clinical chemistry analyzer machine was used to estimate biochemical parameters like sodium, potassium, calcium, iron and cooperin serum samples. All the tests were performed following the manufacturer’s directions (Sulehria *et al.,* 2020).

Risk Factors Analysis and Husbandry Practices: A questionnaire was constructed to study the assumed risk factors; age (age-based groupings for calves were 1 to 10

days, 11 to 20 days, and 21 to 30 days), sex, breed (Holstein Friesian, crossbred), body conditions (normal, emaciated, and fatty calves), body weight, location, season (winter-December to February, spring-March to April, summer-May to September and autumn-October to November) vomiting, diarrhea, sample source, body size, cohabitation with other animals, living environment, food type, deworming history, contact with the feces of other species, vaccination history, presence of other co-pathogens, any other human infected with BRoV and BCoV. Animal husbandry practices like calf housing either caged or confined or living with other animals, calf housing hygienic conditions, early colostrum feeding and navel cord management etc were observed. The assumed risk factors were also recorded as a husbandry practices like food type either suckling, bucket feeding or milk replacer, location, deworming and vaccination history etc.

Statistical Analysis: SPSS version 20 was used for statistical analysis. The t-test was used to analyse data on hemato-biochemical parameters, and Chi Square was used to analyse data on risk factors. The degree of association of risk factors with the occurrence of BRoV infection in cattle calves was determined using an odds ratio, and a *p*-value of (≤0.05) was considered significant.

RESULTS AND DISCUSSION

Occurrence of bovine rotavirus infection and associated risk factors:The S&C Biotech Bovine Rotavirus Antigen Rapid Test was used to screen the fecal samples, and the results showed that out of 200 fecal samples, 26% (52/200) were positive through diagnostic kits, and 21.5% (43/200) were positive for BRoV infection in diarrheal cattle calves after confirmation through RT-PCR (Figures.2, Figures.3, and Figure.4). Statistical analysis revealed that using diagnostic kits increased the likelihood of BRoV infection 1.2833 times (OR=1.283; 95% CI=0.808-2.037) more frequently than using RT-PCR (Table 1). When examined utilizing kits and RT-PCR, the percentage of infected calves was non-significant (p≤0.290). The findings of (Uddin Ahmed *et al.,* 2022; Ammar *et al.,* 2014; Wei *et al.,* 2021) supported the current study's findings. These differences in seroprevalence in different countries may be due to differences in cattle population age, cattle density, herd size, housing systems, biosecurity and management practices, which in general could be important risk factors for rotavirus transmission and persistence (Stahl., 2007).

Each variable's (risk factor) association with the occurrence of bovine BRoV infection had been computed and given against each variable (Table.1). Each risk factor was found to have a significant (p≤0.05) relationship with the occurrence rate of BRoV infection in diarrheic cattle calves (Table.1). When data on calves' breeds was statistically analysed, it was found that breed had the highest potential for disease dynamics, implying that breeds were highly significantly (p≤0.000) associated with the occurrence of BRoV infection in calves. The odds ratio (OR=10.628; 95% CI=4.291-26.323) suggested that Holstein Friesian breed was the highest potential risk factor for disease dynamics in the study area, followed by crossbreds. The age of the calves, which were separated into three groups depending on their age in days, namely group 1, group 2 and group 3, was another significant risk factor. When the data were evaluated between three groups, calves 1 to 10 days old had a substantially (p≤0.001) higher chance of having BRoV infection than calves 11 to 20 days old. According to the odds ratio (OR=43.8, 95% CI=5.78-332.08), calves between the ages of 1 and 10 days were more probable to have BRoV infection than calves between the ages of 11 and 20 days. Calves between age 21 to 30 days were less affected as compared to two other groups. Similar to the previous example, sex was another risk factor connected to the incidence of BRoV infection in calves (p≤0.034). Male calves had a two-fold (OR=2.011, 95% CI=1.050-3.852) higher chance of contracting BRoV infection than female calves. The findings of (Seid *et al.,* 2020; Bertoni *et al.,* 2021) regarding breed, age, and sex supported the current study's findings. The possible reason for this could be due to immune system of female calves with high antirotavirus IgG concentrations compared to male calves. It could be due to the management practices, as in most of the dairy farms, female calves are better cared than male calves.

When data among winter, autumn, spring and summer was statistically analysed, it was found that that in comparison to winter (reference value=1); autumn (OR=1.82, 95% CI=0.21-16.14) and spring (OR=1.93, 95% CI=0.20-19.00) did not significantly affect the calves while summer season was harsh enough to affect the calves significantly (p≤0.001). According to statistical analysis (OR=11.98, 95% CI=1.51-95.15), the summer season affected the calves with BRoV 11.98 times more than winter season. According to figure.5, the highest occurrence of BRoV (48.0%) was observed during the summer season. The winter season had the lowest percentage occurrence of BRoV infection (7.1%). Figure.5 depicts the seasonal variation in the occurrence ofBRoV infection round the year. The most important risk factor that contributes to the spread of disease caused by BRoV infections is the weather (Boileau and Kapil, 2010). The current study's findings were very similar to those of (Trotz-Williams *et al.,* 2007).

When data from normal, emaciated, and fatty calves were compared, the emaciated calves had significantly (p≤0.065) higher BRoV infection rates than the normal calves. The results also showed that emaciated calves had an 8.79 times increased risk of BRoV

infection compared to normal calves (OR=8.79; 95% CI=1.0-77.10). Statistically, in comparison to normal and emaciated calves, fatty calves were not significantly ($p \leq 0.065$) influenced by BRoV infection (OR=3.09; 95% Cl=0.19-50.80). This finding was also consistent with the findings of a study conducted in North West Ethiopia by (Tamrat *et al*., 2020).

Food was another very likely risk factor that contributed to the development of BRoV infection in calves. Milk-fed calves significantly ($p \leq 0.005$) more likely to contract the virus than calves fed milk replacer feed. According to the findings, calves on milk were 3.188 times more likely than those on milk replacer to get BRoV infection (OR=3.188; 95% CI =1.399-7.262).These findings were similar to those of (Kayasaki *et al.,* 2021).

When data from calves with a history of diarrhoea were statistically analysed (OR=0.322; 95% CI =0.107-0.967), calves with no previous history of diarrhoea were significantly ($p \leq 0.035$) more likely to be infected with BRoV than calves with a previous history of diarrhoea. The current study also found that calves with a history of diarrhoea had a higher infection rate (13.3%) than those without a history of diarrhoea (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 diarrhea recovered well. The minor discrepancies and variations in the results could be attributed to the inability of screening diagnostic kits to provide accurate results. The variations in results could be attributed to the virus's inability to remain intact in the faeces due to activities of the endogenous RNase enzyme, and the lack of partially degraded RNA may affect the sensitivity of RT-PCR or intermittent virus shedding in faecal materials (Vermeulen *et al.,* 2011).

Another risk factor that contributed to the spread of the infection was housing. In this study, open housing had a significantly greater ($p \leq 0.000$) impact on calves with BRoV infection than confined housing. According to the findings (OR=8.313; 95% CI =2.467-26.006), open housing affected calves with BRoV infection 8.313 times more than confined housing. Further investigation into the cohabitation of calves living with other animals revealed that those calves significantly ($p \leq 0.000$) affected with BRoV infection who had a history of living with other animals rather than being housed in an open housing system. The findings of (Knauer, 2021) 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 BRoV than calves being raised in open housing. The disparity in results could be attributed to the selection of calves for sampling from less congested confined housing in small households with only one or two calves being raised. These findings may differ due to differences in study design, or seasonal variation. When developing an experimental design, it is critical to keep all major variables in mind and control them as much as possible. A change in the variation of different variables is another common mechanism influencing study results (National Research Council, 2011).

Hematological parameters analysis: Packed cell volume (PCV %) and mean corpuscular hemoglobin concentration (MCHC g/dl) did not decrease significantly ($p > 0.05$) while Mean corpuscular volume (MCV fl), basophils (%) and lymphocytes (%) were decreased significantly ($p \leq 0.05$) in infected calves. Hemoglobin (Hb g/dL) did not increase significantly ($p \leq 0.269$) while mean corpuscular hemoglobin (MCH pg), total leukocyte count (TLC thousands/cm3),TEC (million/cm3), white blood cells count (WBC m/mm3), red blood cell (RBC m/mm3) and Monocytes (%) were increased significantly ($p \leq 0.05$) (Table.2). An increase in hemoglobin in calves with diarrhea may be due to hemoconcentration associated with dehydration. The findings of previous studies (Barua *et al.,* 2018; Song *et al.,* 2020) supported the current study's findings.

An increase in PCV was observed in diarrheal calves compared to non-diarrheal calves. PCV elevation is an indicator of dehydration (Al-Robaiee and Al-Farwachi, 2012). Thus, the PCV estimate is of the utmost importance to monitor the animal's hydration status and is a sensitive indicator for assessing the severity of dehydration. Increase in PCV in calves with diarrhea was apparently due to hemo-concentration associated with dehydration and hypovolemic. The TLC increased in calves infected with diarrhea and also reported significant leukocytosis due to normal reaction of the body's defense mechanism against the infectious cause of diarrhea (Brar *et al.,* 2015).

Biochemical parameters analysis: Biochemical analysis results from the laboratory study revealed that Potassium (P mEq/L) was not significantly ($p \leq 0.090$) increased, while Sodium (Na mEq/L), Calcium (Ca mmol/L), copper (Cu mol/L) and iron (Fe mol/L) were significantly ($p \leq 0.000$) decreased in diarrheic calves infected with BRoV infection (Table.3). The current study's findings were similar to those reported by (Barua *et al.,* 2018; Tajik *et al.,* 2012). The slight variation in results could be attributed to differences in study design, study area, target animals, seasonal variation, and environmental conditions.

Sodium is the most important cation in the extracellular fluid because it is responsible for maintaining osmotic pressure (Klinkon and Ježek, 2012). Together cooperates with chlorine (Cl) in water metabolism and regulation of the acid-base balance in the cell. The current study revealed significant reduction in Na values in calves with diarrhea. These findings were consistent with observations (Shekhar *et al.,* 2017).

Similarly, Singh *et al.,* (2006) also reported hypernatremia in calves with diarrhea due to excessive secretion of sodium along with water into the intestinal lumen.

Haematological and serological values changed in neonatal diarrheal calves infected with BRoV. These findings contradict those of Kaur *et al.,* (2006) who reported hypernatremia in calves infected with diarrhea compared to healthy calves. Most microorganisms that cause diarrhea disrupt intestinal function and dehydrate the body either by increasing the activity of chloride excretion crypt cells or disruption of villus cell by sodium absorption or both. Potassium is important for the generation of electrical potential for the transport of nerve impulses and for maintaining muscle tonicity. Potassium is also important for regulating the acid-base balance in the body (Klinkon and Ježek, 2012). Hypokalemia increases the membrane potential and cause hyperpolarization block, which affects lower muscle tone and paralysis. The current study revealed the altitude potassium levels in all calves with diarrhea. Hyperkalemia is caused by increased retention of potassium by the kidneys as well as cellular damage (Sobiech *et al.,* 2013). The hyperkalemia observed in this study may be due to enterocyte damage by microorganisms. Similar observations were reported by other researchers (Brar *et al.,* 2015). In this study, serum chloride values were elevated in calves with diarrhea.

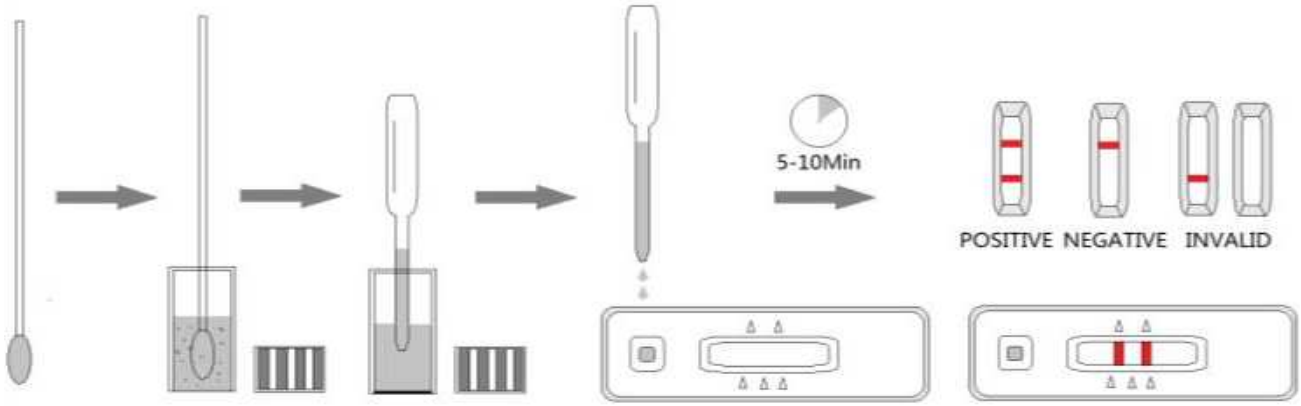

Figure.1: Depicting the whole procedure of S&C Biotech Bovine Rotavirus Antigen Rapid Test and interpretation of results (Figure was developed by manufacturers 'Shanghai S&C Biotechnology Co. Ltd, China').

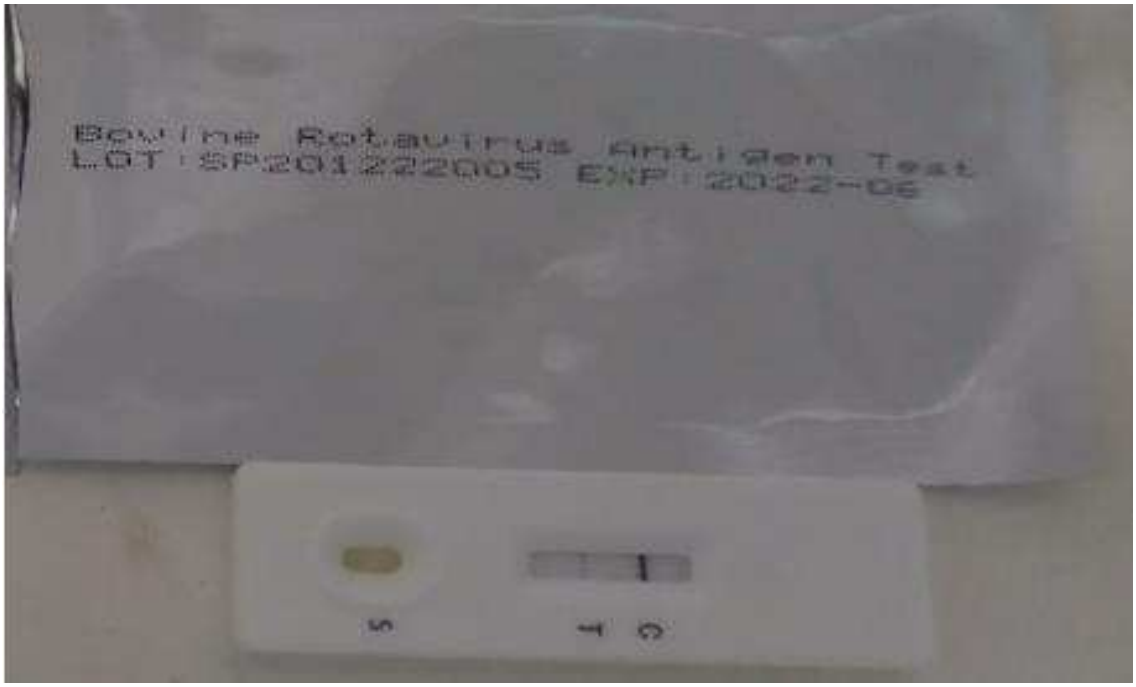

Figure.2: Showing the positive results through diagnostic kits

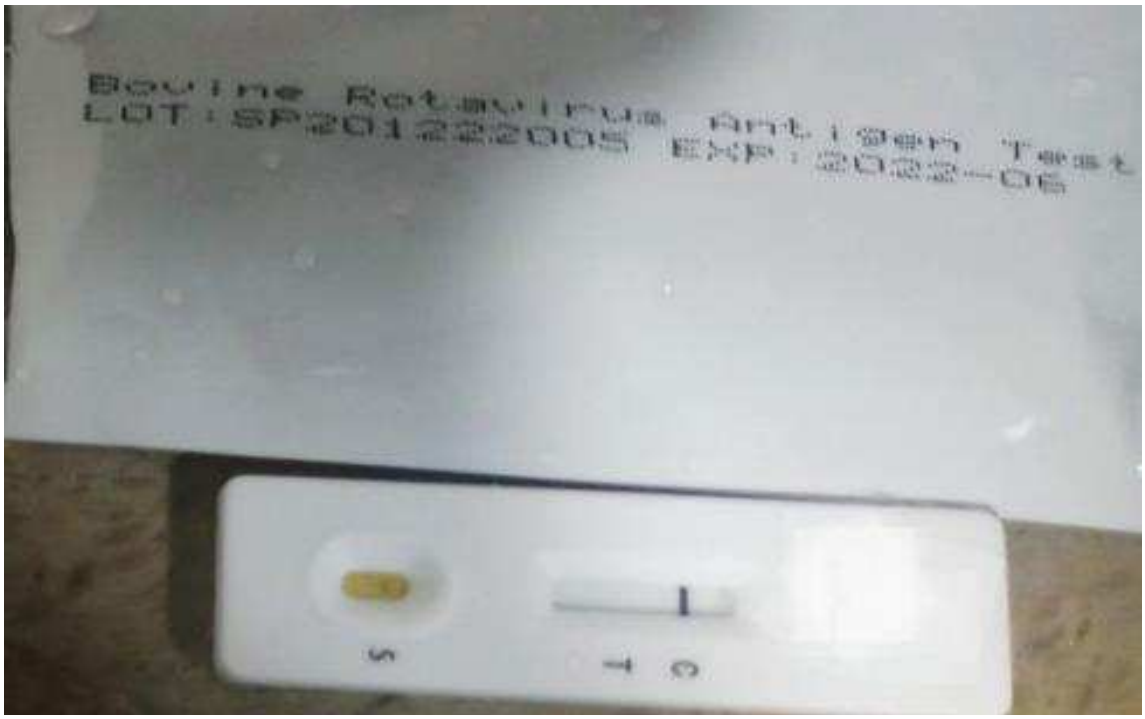

Figure.3: Showing the negative results through diagnostic kits

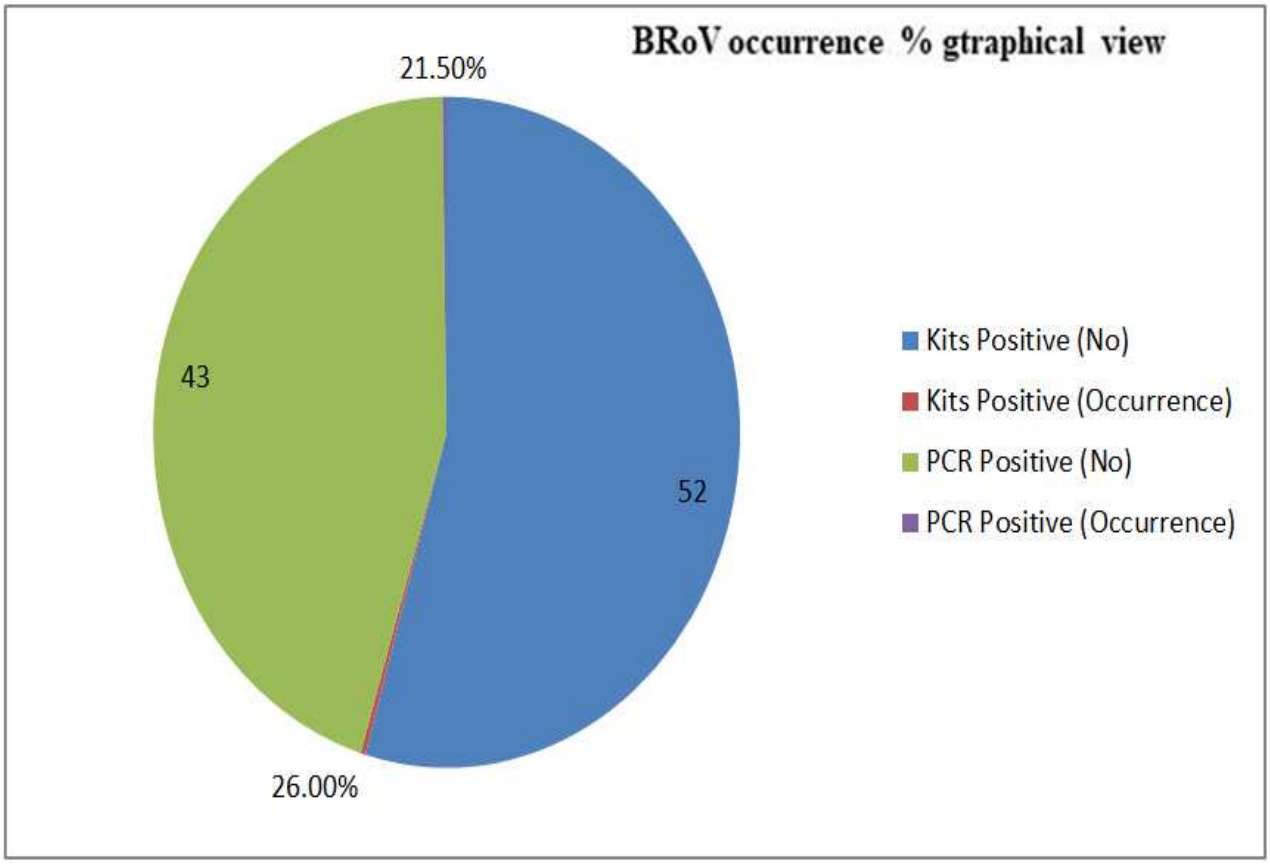

Figure.4: Pie Chart showing the occurrence % of BRoV infection after performing diagnostic test and PCR.

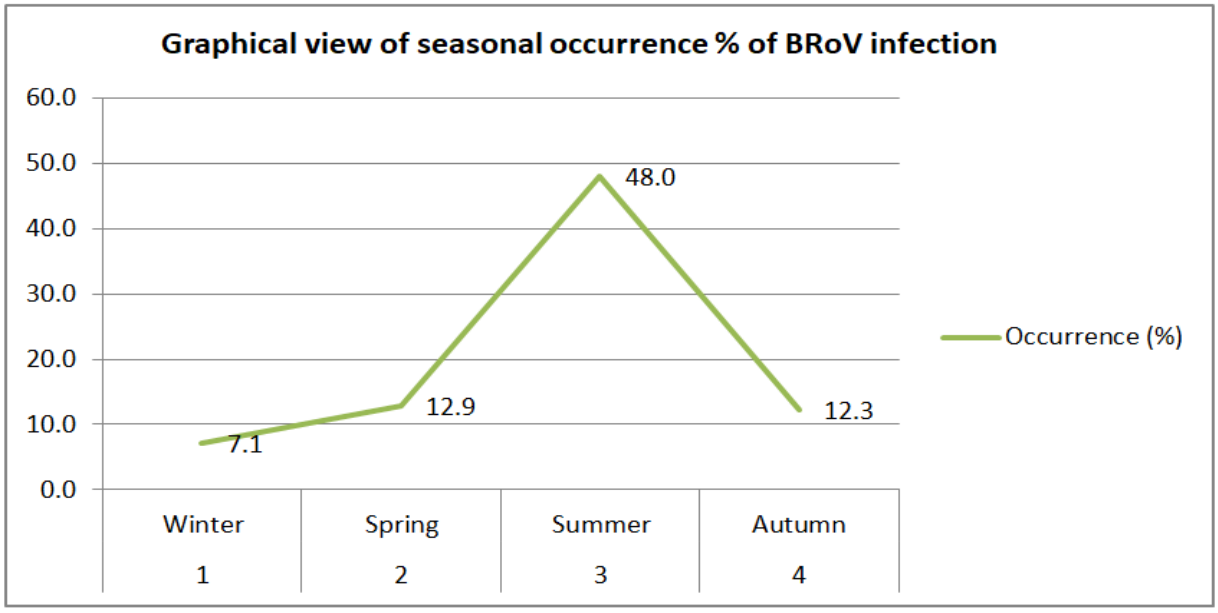

Figure.5:Graphical presentation of seasonal occurrence % of BRoV infection round the year.

Table.1:Analysis of risk factors associated with the occurrence of BRoV infection in cattle calves

Risk Factors	Parameters	No of negative samples	No of positive samples	Occurrence	Odds Ratio	95 % CI	P-Value
Breed	H. Friesian	64	53	45.3%	10.628	4.291 - 26.323	0.000
	Crossbred	77	6	7.2%			
Age	21 - 30 days	41	1	2.4%	reference value=1		0.001
	Less ≤ 10 days	44	47	51.6%	43.8	5.78 - 332.08	
	11 - 20 days	56	11	16.4%	8.05	1.00 - 64.98	
Sex	Male	34	23	40.4%	2.011	1.050 - 3.852	0.034
	Female	107	36	25.2%			
Season	Winter	13	1	7.1%	reference value=1		0.001
	Autumn	50	7	12.3%	1.82	0.21 - 16.14	
	Spring	27	4	12.9%	1.93	0.20 -19.00	
	Summer	51	47	48.0%	11.98	1.51 - 95.15	
Body conditions	Normal	102	1	1.0%	reference value=1		0.065
	Fatty	33	1	2.9%	3.09	0.19 - 50.80	
	Emaciated	58	5	7.9%	8.79	1.0 - 77.10	
Food	Milk	94	51	35.2%	3.188	1.399 - 7.262	0.005
	Milk replacer	47	8	14.5%			
Previous history of diarrhea	Yes	26	4	13.3%	0.322	0.107 - 0.967	0.035
	No	115	55	32.4%			
Housing	Open	98	52	34.7%	8.313	2.467 - 26.006	0.000
	Confined	47	3	6.0%			
Living with other Animals	Yes	47	3	6.0%	0.12	0.036 - 0.405	0.000
	No	98	52	34.7%			

Data are indicated as No of negative samples, No of positive samples, Occurrence, Odds Ratio, 95% confidence interval and P-Value. BRoV stands for bovine rotavirus.

Table.2:Hematological parameters of diarrheic cattle calves infected with BRoV infection.

Parameters	Mean ±SD (Infected)	Mean ±SD (Non-infected)	P-value
PCV (%)	43.136 ± 1.602	42.5 ± 9.19	0.425
Hb (g/dL)	10.396 ± 1.922	11.5 ± 4.95	0.269
MCV (fl)	38.00 ± 3.391	50 ± 14.14	0.001
MCH (pg)	12.932 ± 0.539	14 ± 4.24	0.011
MCHC (g/dl)	33.358 ± 2.684	35 ± 7.07	0.243
Monocytes (%)	2.434 ± 0.452	3 ± 2.83	0.049
Lymphocytes (%)	53.648 ± 5.978	60 ± 21.21	0.076
Basophils (%)	0.556 ± 0.096	1 ± 1.41	0.001
TLC(thousand/cm3)	13.124 ± 1.441	9.7 ± 1.41	0.006
TEC (million/cm3	8.716 ± 0.280	8.1 ± 1.41	0.008
WBC (m/mm3)	21.374 ± 2.764	8 ± 5.66	0.000
RBC (m/mm3)	12.086 ± 1.152	8.5 ± 3.54	0.002
Hct (%)	56.626 ± 1.546	37.5 ± 17.68	0.000

Data are indicated as Mean±SD (Infected),Mean±SD (Non infected) and p-value. BRoV stands for bovine rotavirus.

Table.3: Biochemical parameters of diarrheic cattle calves infected with BRoV infection

Parameters	Mean ±SD (Infected)	Mean ±SD (Non infected)	P-value
Na (mEq/L)	127.892 ± 0.957	144 ± 2.83	0.000
K (mEq/L)	6.242 ± 0.293	5.95 ± 1.41	0.090
Ca (mmol/L)	1.324 ± 0.238	2.6 ± 0.57	0.000
Cu (µmol/L)	7.554 ± 0.426	13.5 ± 7.78	0.000
Fe (µmol/L)	8.382 ± 0.350	20.585 ± 13.92	0.000

Data are indicated as Mean±SD (Infected),Mean±SD (Non infected) and p-value. BRoV stands for bovine rotavirus.

Conclusion: It was concluded that BRoV was present in cattle calves in Punjab, Pakistan. Assumed risk factors like breed, age, season, housing type, food type and contact with animals were all found to be more significantly associated with the occurrence of BRoV infection. Rapid diagnostic kits tests were proved to the best diagnostic tool for the immediate diagnosis of BRoV. Furthermore; it was concluded that haemato-biochemical alterations can aid in the diagnosis of BRoV and BCoV infections in calves.

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Author's Contribution: JAK, SSA and AAA conceptualized the research topic of this manuscript. SA conducted the research, analyzed the data statistically and wrote the manuscript.

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