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PREVELANCE AND POTENTIAL RISK FACTORS ANALYSIS OF CRYPTOSPORIDIOSIS IN BUFFALO CALVES IN DISTRICT MARDAN

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Abstract:
Cryptosporidium is a protozoanal infection that affects an ample range of vertebrate including human, animals, birds and fishes and causes acute gastrointestinal infections. The present study was conducted to assess the prevelance of Cryptosporidium infection in a buffalo calves in district Mardan, which causes cryptosporidiosis infections. It is one of the nearly all common infections across the spheroid mainly affecting untamed and prosper nations inclusive of Pakistan. The out-turn of cryptosporidiosis in pass infection to animals were high mortality in neonatal calves and huge economics loss to the dairy farms. Varied risk features such as age, gender, area, season, feeding methods, treatment history, and breeds effects the intensity of cryptosporidiosis. All the specimens were collected in labeled plastic bottles and were stored in refrigerator at 4°C before processing. One hundred samples per tehsil were collected from six tehsil and all the principle information's were entered in pre-designed question sheet at the time of collection from each farm to secure individuals and herd information's. All the samples were stained by Modified Ziehl Nielsen acid fast staining and investigate under microscope at 10X and 40X. As a result, 10% prevelance was noticed (60/600x100) in both diarrhic and non-diarrhic buffalo calves on the basis of various parameters like age, gender and season of the year, so in the present study the highest prevelance was describe at the age of less than six months (12.44%), walk behined by 7-12 months (9.04%), while stunted at the age of more than 12 months (7.73%). Likewise, the highest prevelance was observed in buffalo calves in damp season (monsoon) 22.48% and then in sun shine season (pre-monsoon) 9.72% while lowest prevelance observed in freezing season (post-monsoon) 4.5%. Similarly the prevelance of infection was higher in female buffalo calves (11.87%) as compared to male buffalo calves (5.78%) tested for both diarrhic and non-diarrhic.

Keywords: *Cryptosporidium*, Mardan, Calves, zoonotic, buffalo, prevelance

Introduction:

Overhead the world, humans, cattle, buffalo, sheep, goats, pigs, dogs, cats, and horses are amid the many species that exposure to gastrointestinal illnesses due to cryptosporidiosis, a pop-up protozoan disease that is a major shared health concern (Nasir *et al*., 2009). These varieties of *Cryptosporidium* are apicomplexan protozoan parasites that form oocysts and release sporozoites when the oocyst excystations occurs. Both asexual (merogony) and sexual (gametogony) reproduction are carried out by these excysted parasites, to assemble macrogametocytes and microgametocytes. These gametocytes cross pollinate to create a zygote, which then proceeds through sporogony to create thick-walled oocysts that the host excretes and thin-walled oocysts that are involved in autoinfection (Putignani and Menichella, 2010).

The obligatory parasites that belong to the genus *Cryptosporidium* are eukaryotic and intracellular. Oocysts of *Cryptosporidium* are extremely resilient to environmental conditions; they can survive for long stretches of time and are expelled in faecal samples. More than 150 distinct vertebrate species are known to be susceptible to infection by these coccidian protozoan parasites. Four species of *Cryptosporidium, Cryptosporidium parvum, Cryptosporidium bovis, Cryptosporidium andersoni*, and *Cryptosporidium ryanae*, are regularly seen in cattle. The infection is usually contracted orally, most commonly by eating or drinking contaminated water, coming into close touch with the excrement of sick animals, or both. The condition known as enteritis, which is characterized by acute, watery, and steatorhic diarrhoea, is commonly linked to cryptosporidiosis. “Countries all over the world, including Cuba, Italy, and India, have reported cases of *Cryptosporidium* infections in buffalo populations (Aquino *et al*., 2015).

Since robust oocysts shed by animals can contaminate surface water, the occurrence of Cryptosporidiosis in animals raises worries about its potential to spread to people. Researchers in veterinary science sectors have been interested in cryptosporidiosis because of its potential harm and the difficulties in controlling the disease, which can lead to large financial losses, in addition to its zoonotic implications. The course and result of the infection depend on the immune condition of the host; in immune-competent persons, the infection can be severe and self-limiting, but in immune-compromised individuals, as those with AIDS, it can be life-threatening and persistent (Maurya *et al*., 2013).

About 85% of cases of cryptosporidiosis in pre-weaned calves and just 1% of cases in post-weaned calves are caused by *Cryptosporidium parvum.* The universality of *Cryptosporidium bovis, Cryptosporidium andersoni*, and *Cryptosporidium ryanae* infections is higher in older cattle and post-weaned calves. The only zoonotic species that causes looseness of motion in cattle is *Cryptosporidium parvum*, which is also the main cause of diarrhoea in calves (Heather *et al*., 2006).

The World Health Organization (WHO) separated cryptosporidiosis as a mistreated disease in 2004 and granted it as a worldwide health complication. *Cryptosporidium parvum* was nominated by the National Institute of Allergy and Infectious Diseases as a priority pathogen (NIAID) in 2012, recommended that the pathogen had become visible and had the capability to roll out to other organisms. Since the first set down occurrences of cryptosporidiosis in humans were in people with weakened immune systems, it is currently recognized as a major factor in calf diarrhoea complexes. The year 1993 marked the emergence of *Cryptosporidium* as a public health concern following the world's most extensive recorded outbreak of waterborne illness in Wisconsin, USA, impacting more than 400,000 individuals. In most nations, neonatal diarrhoea is the primary cause of calve mortality and a significant source of financial loss for the cattle sector. Calve scour is a clinical condition linked to multiple diarrhea-causing diseases rather than a single illness. Whatever the reason, altered intestinal fluid absorption might out-come in potentially fatal electrolyte variation (Essa *et al.*, 2014).

Animal-borne cryptosporidiosis is classified as a zoonotic parasite and is additionally divided into four subtypes: direct zoonotic, met zoonotic, cyclozoonotic, and saprozoonotic. Under the direct zoonotic category, *Cryptosporidium* can spread as a direct route from animals to people (Ranjbar *et al.*, 2011), infecting both humans and animals with intestinal and extra intestinal diseases.

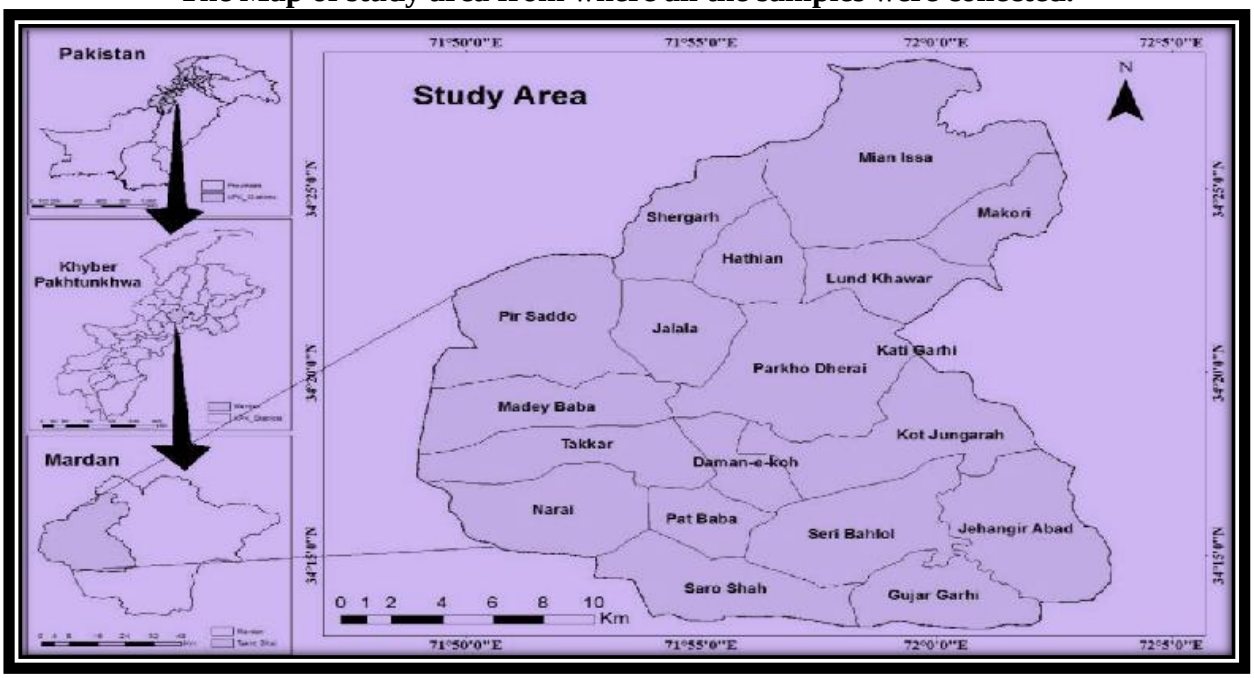
The majority of ulceration with *Cryptosporidium* takes place in the lower small intestine, but injury

can also be found in the caecum and colon (Jarvie *et al*., 2005) where they cause villous atrophy and the onset of diarrhoea. Additionally, infections with *Cryptosporidium* may affect other segments of the respiratory or digestive systems. People with compromised immune systems are more liable to serious illness.

Material and methods

Study Area : The study was conducted in district Mardan region at Khyber Pakhtunkhwa(KP) ,Pakistan which stretch down in 34◦12’22.0428’ to the north and 72◦1’47.2800’ to the east , Mardan region elevation is about 352 meters/1155 feets and barometric pressure measure to be 97 Kpa. District Mardan was mainly cleave up into two parts, the north eastern hilly areas and south western plain. The whole northern side of district Mardan is bounded by mountain range. The highest point in that mountain is Sakkra or Pajja which is 2056 meters high. The mountains in this region are mostly vegetative and contain an immeasurable number of faunas. Chiefly the population in that area are living in rural areas about (79.78%) where people were mostly connected with agriculture practices, livestocks and farmings mean their life is depend upon these resources.

The Map of study area from where all the samples were collected.



Sample size: A total of 600, faecal representative were collected from buffalo calves from differenth zones.

Sample Collection: There are many varieties of sample collections like random sample collection, systematic sample collection, Stratified sample collection, Cluster sample collection. In this study preference was given to random sampling, so a total of 600 faecal trial were collected randomly from numerous dairy farms or from herds of buffalo calves from ground immediately after excretion in area around district Mardan and around six tehsil of district Mardan and in various villages of this district like Takht Bhai, Qasmi, Mazdor Abad, Garhi Kapora, kot, Babozai , Pipal, Lund Khwar, Kohi Barmol, Taza Gram, Aloo, Alum Jung , Shah dund baba, and various other villages in the form of questionnaire which were applied in order to obtain the following statistics such as age and housing of calves of buffalo at below the age of approximately one year. At the time of collection of samples, each animal were examined clinically and also studied their age which was further divided into three categories 1. Less than six months, 2. Age between 6-12 months, and 3. Age more than 12 months, sex of the calve (male and female), various seasons of the year like monsoon (rainy), pre-monsoon (summer) post-monsoon (winter) and faecal consistency

like diarrhic (liquid or semi-liquid) or non-diarrhic (solid or semi-solid) were recorded. After collection of samples, the samples were immediately brought to the laboratory and further processes were performed when immediate process were not possible, then the samples were kept in refrigerator below 4◦C before processing or apply the applications of formalin to the faecal sample. Further, more the location of sample collection need to be shielded, satisfactory and accessible.

Visiting of dairy farms for faecal collection : I went to saw various dairy farms in district Mardan and also visiting into those dairy farms which were included in various tehsil of district Mardan and especially concentrating into that dairy farms which are nearby into our area of tehsil Katlang like Iqbal dairy farm located in tehsil Katlang in Miankhan Sanghao road, and also visiting into Zahoor dairy farm near Sheikh Kara baba for sampling collection and also the sample collected in that area from buffalo herds behind to these I were also visiting into Khan dairy farm in university road Mardan, also visiting into Ayaz dairy farm Canal road Mardan and visiting into so many other farms for further learning purpose.

Faecal Examination: After collection of samples, the samples were shift into clean, dry, leak proof and label plastic bottles, by using latex sterile gloves, facts about living location were introduced. Each faecal sample was processed through Faecal-floatation technique. An electric balance was used to weigh a two-gram sample of faeces. Then, faeces were added to 3 millilitres of faecal flotation substances in a 10-milliliter test tube, which was gradually filled up to 10 millilitres while being unceasingly mixed to create a positive meniscus. After letting the solution rest for ten to fifteen minutes, the test tube's cover was slipped on, and a tiny drop was placed to a spotlessly washed, clearly marked glass slide to create a smear. Modified Ziehl-Neelsen (mZN) acid fast staining was used to stain the smears.

Study Design: A cross sectional study was managed to approximate, the widespread presence of *Cryptosporidium* infection in calves and its associated risk element related to the prevelance of this infection around district Mardan and all tehsils of district Mardan it also comprises asking of interview from herd or farm owner, and also divided the calves on the basis of age like 3months, 6 and 9 month and one year calves correspondingly.

Parasitological technique: A modified Ziehl-Neelsen (mZN) acid-fast staining methodology was applied to stain fecal smears in order to found *Cryptosporidium* oocysts. The oocysts discharged by animals were quantified using a method similar to that used for cattle, with a minor adjustment: 2 grams of feces were employed instead of the usual 5 grams of feces.

Ziehl-Neelsen staining method: The modified Ziehl-Neelsen (mZN) staining method is based on the recognition of oocysts through their morphological features. The quantity of chemical that can be used in acid fast staining are Carbol fuchsin, acid alcohol, methanol, methylene blue and immersion oil which were applicable for 100x objective lense of microscope. Positive samples have oocysts that look like pink-red granules with a diameter of around 4 to 5 mm. The mZN staining technique is believed to have a sensitivity of roughly 70% when compared to other procedures like lateral flow immunological tests. Human and animal infections caused by *Cryptosporidium* have obtained prominence in recent years. Countless techniques for colouring and concentrating stool have been developed such that oocysts in faeces can be directly noticed. Microscopy is still the best method for determining many diseases, however because of its limited sensitivity and subject to observer interpretation; its reliability may be called into question. Most *Cryptosporidium* parasites were found in stool samples by a combination of techniques such as immuno-fluorescent assays, direct fluorescent assays, and acid-fast staining. I therefore concentrated mostly on the acid quick staining method.

Procedure of acid fast staining

1. Two grams of faeces samples were massed.
2. Putting 60 ml of ZnSO4 solution in a beaker and vortexing it to make it comparable.
3. Flow through a filter into a different beaker.
4. Spout the filtrate into a sterile 15 ml tube, and cover the tube with a cover slip for a while.
5. Gently push the cover glass slip into a glass slide and examine it using 10X and 40X objective lenses under a microscope.

Microscopic Examination of faecal smear: The faecal smear were examined by ordinary direct smear method and saline smear method, and then it can be examined under the microscope by using 10X and 40X objective lens of microscope.

Cryptosporidium oocysts detected under microscope at 10X and 40X objective lense of a microscope. The appearance of *Cryptosporidium* oocysts after staining with Modified Ziehl-Neelsen staining as bright red spherical oocysts with a typical diameter of 5.2ˣ4.4 micrometers (Al-alousi *et al*., 2012*).*The *Cryptosporidium* oocysts appear as ring or ovoid in shape. *Cryptosporidium* oocysts typically stain reddish-brown in color, and the surrounding may become revealed uniformly green when using certain staining techniques.

Statistical Analysis: The documentation obtained during the prevelance of cryptosporidiosis was calculated in the form of Chi-square test, and all the statistics can be evaluated in the form of percentage (%) by using SPSS software.

Results and Discussion

The clinical manifestations of diarrhoea in calves comprises symptoms like mucoid diarrhoea that fluctuate from green to yellowish, sporadic episodes of bloody stool, lethargic behavior, decreased appetite, mild fever, and dehydration. These signs were habitually observed in calves that had cryptosporidiosis, especially in the premature stages of their lives. It is noteworthy that these clinical indications tended to become less serious as the animals grew mature. Several risk factors can influence the prevalence of *Cryptosporidium* infection in calves, including factors such as bedding type, hygiene, colostrum feedings, management practices, feed, and climate and water standards.

In this component, a total of 600 fecal samples were meticulously scrutinized under a microscope. Among these samples, *Cryptosporidium* oocysts were detected in 60 buffalo calves out of 600 sample sizes, these cases were recorded in (diarrhic and non-diarrhic) buffalo calves 403 for non-diarrhic and 197 for diarrhic consistency according to the age, gender, season, and level of contaminations in each dairy farm etc. Indicating an approximate prevalence of 10%. A reasonably inspection of *Cryptosporidium* oocyst occurrence was conducted between dairy farms and buffalo herds using the Chi-square test as shown in Table 1 and Figures 1&2

It was proof that the infection's prevalence in calve was significantly determined by the age of the calves. When considering the age of the calves, those within the age range of 1 to 30 days exhibited a notably higher prevalence of infection compared to older age groups. As the age of the calves increases, the prevalence rate decreases, and vice versa. Infection with *Cryptosporidium* is more common in younger age group than in older ones. Animals are less likely to get sick as they get older. The main origin of this is the regular finding of the highest oocyst counts in the first few months of life, which recommend that newborn calves get infected early (Noordeen *et al.*, 2001).

As the animals' ages increased, the infection rate dropped. The evolution of immunity against the virus may be the cause of this. Therefore, immunological opposition that develops with age and the movement of animals from heavily polluted to lean environments can lessenl the parasite load that these animals carry consistent results have been published by (Masoud *et al.*, 2013).

Table 1. The prevelance of *Cryptosporidium* oocysts in diarrhic and non-diarrhic buffalo calves with respect to age group.

Diarrhic buffalo calve				Non-diarrhic buffalo calve			
Age	No of animal examined	No of positive cases	Prevelance	No of animal examined	No of positive cases	Prevelance	Total prevelance (%)
Less than six month	85	18	21.17%	148	11	7.43%	12.44%
7-12 months	66	12	18.18%	133	6	4.51%	9.04%
More than 12months	46	8	17.39%	122	5	4.09%	7.73%
Total	197	36	18.27%	403	24	5.95%	10%

p-value =0.05; this value is considered as significant.

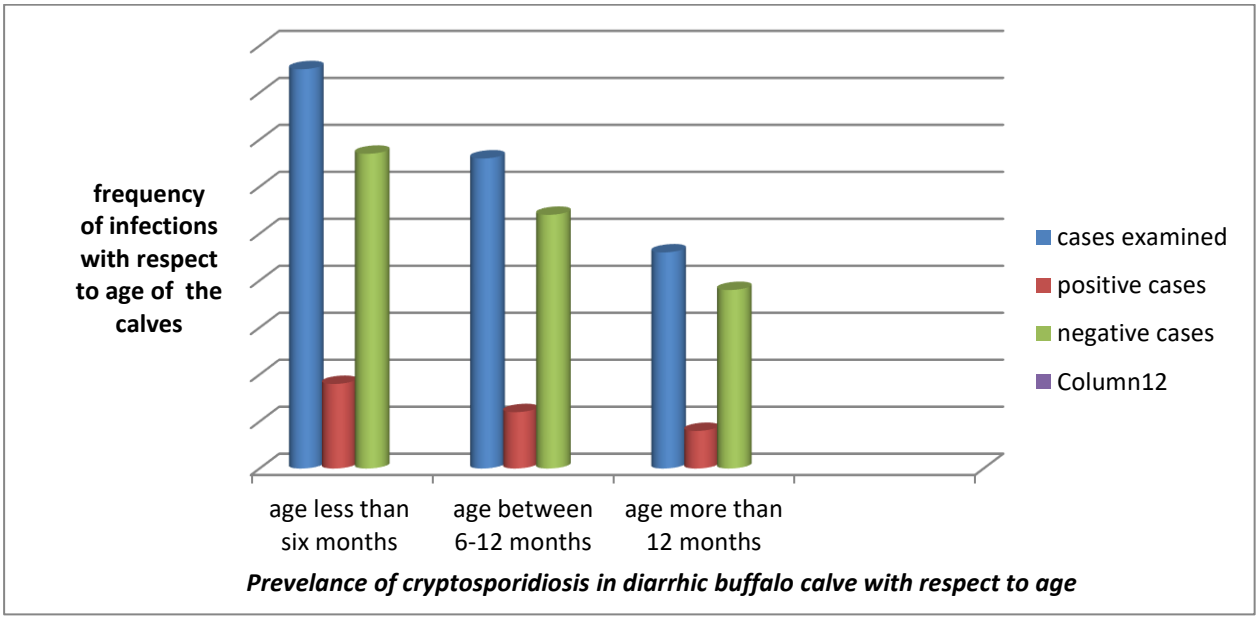


Figure 1.

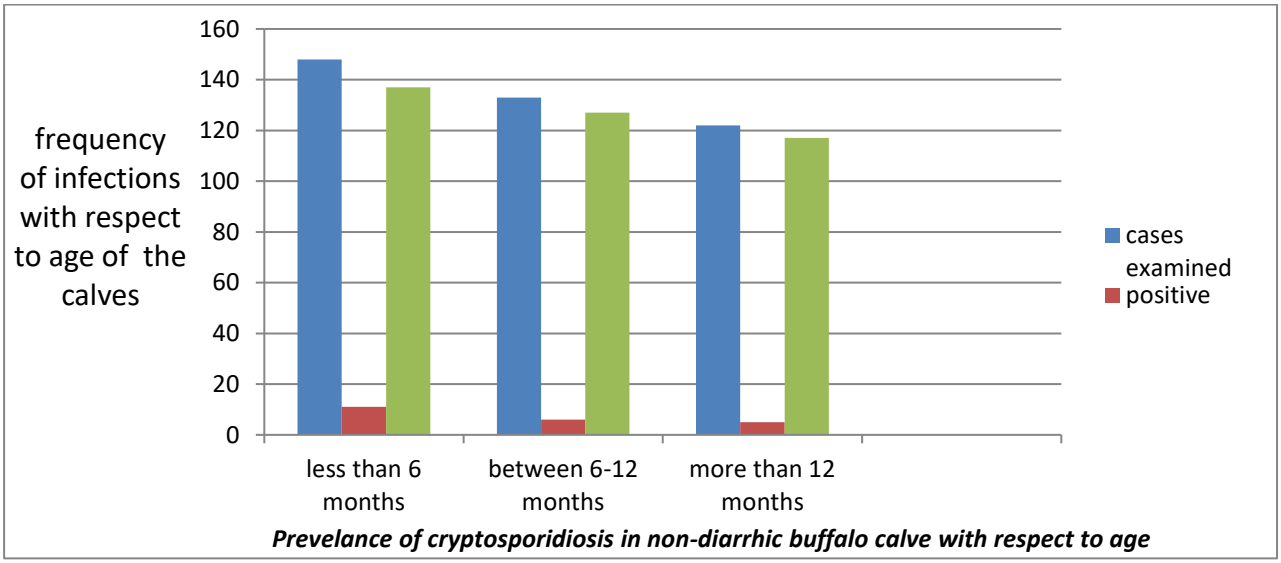


Figure 2.

In calves younger than three months old, diarrhoea is most commonly linked to an infection with *Cryptosporidium* (Lassen *et al.*, 2009). Diarrhoea in young calves up to six weeks of age, significantly increased worldwide by cryptosporidiosis. Animals that do not have diarrhoea have a significant infection rate and are crucial to the poisning of the environment (Robertson *et al.,* 2014). The frequency of *Cryptosporidium* cases in cattle is closely related to the hygiene practices implemented on farms (Khelef *et al*., 2007).

These 600 samples tested for positive cases in them according to the age of the calve, for this purpose these samples were divided into two categories first diarrhic buffalo calves with 197 and second is non-diarrhic calves with 403 samples respectively for each age group. The prevelance of *Cryptosporidium* infection according to age wise in a buffalo calves was non-significants, calve with age less than six months show 12.44% chances to *Cryptosporidium* infection, while calve with age between 7-12 months shows 9.04% chances to infection similarly calve with age more than 12 months shows 7.73% rate to the infection occurance as shown in the above table and Figures.

The greater vulnerability to infection in various age groups can likely be attributed to the compromised immune systems of infected organisms or perhaps to the higher rate of *Cryptosporidium* oocyst contamination (Castro-Hermida *et al*., 2005).The findings of this study conclusively established the presence of cryptosporidial infection in buffalo calves with respect to age within both rural and urban areas of District Mardan.

Additionally, female calves exhibited a higher prevalence of infection compared to their male counterparts.

Table 2. **The prevelance of *Cryptosporidium* infections in male and female buffalo calves.**

Diarrhic buffalo calve				**Non-diarrhic buffalo calve**			
Gender	No of animal examined	No of positive cases	Prevelance	No of animal examined	No of positive cases	Prevelance	Total prevelance (%)
Male	87	14	16.09%	193	8	4.14%	5.78%
Female	110	22	20%	210	16	7.61%	11.87%
Total	197	36	18.27%	403	24	5.95	10%

The p-value = 0.05, this value is significant.

This table 2 shows the prevelance of cryptosporidiosis in a buffalo calve in the current study area in district Mardan with respect to sex of the calve, for this purpose the total number of samples observed under the microscope in female and male in both diarrhic and non-diarrhic buffalo calves which was 600 samples each of 197 for diarrhic buffalo calves and 403 for non-diarrhic buffalo calves, the number of positive cases examined in diarrhic and non-diarrhic male buffalo calves a prevelance rate of 5.78%, while the total number of positive cases observed in diarrhic and non-diarrhic female buffalo calves that is 11.87% , From this It was observed that the number of positive cases observed in female diarrhic and non-diarrhic calves have higher number than male diarrhic and non-diarrhic buffalo calves as shown in Figures 3 and 4.

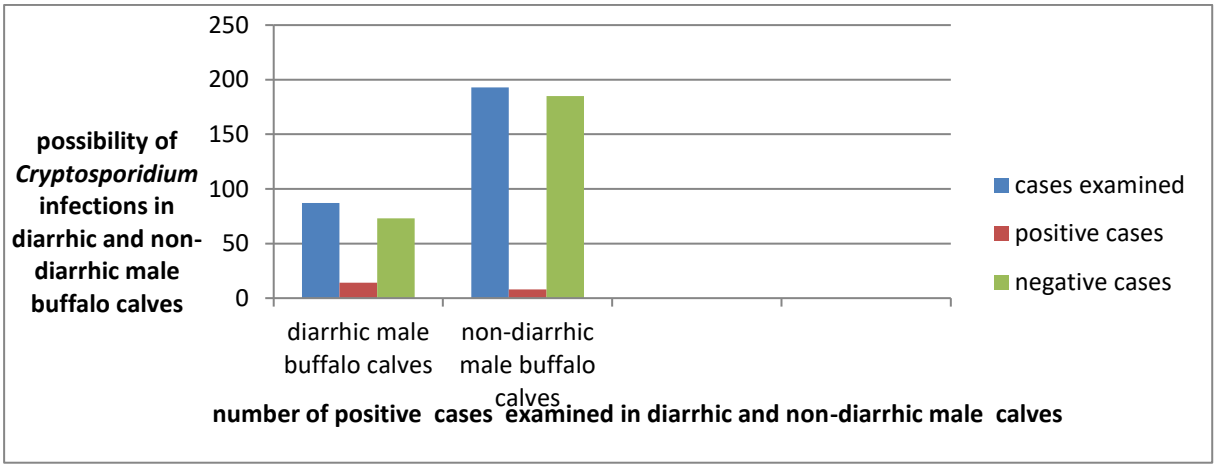


Figure 3. Prevalence of cryptosporidiosis in diarrhic buffalo calve with respect to age.

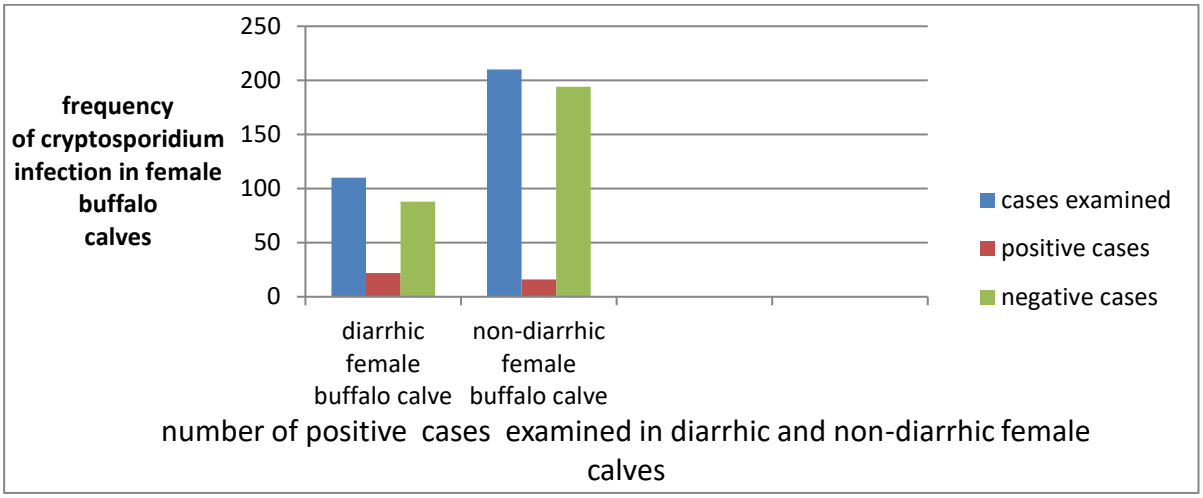


Figure 4. prevelance of Cryptosporidium infections in diarrhic and non-diarrhic female buffalo calves

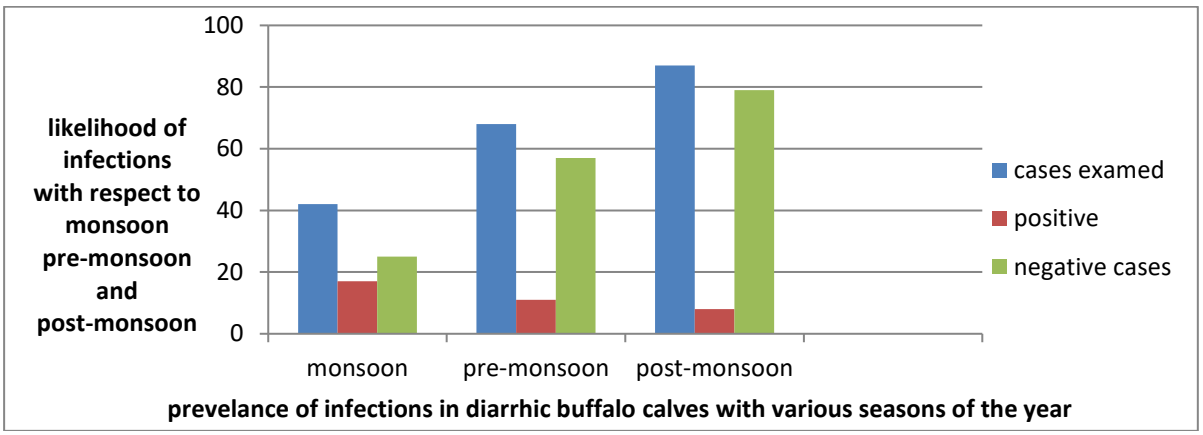
In female calves, diarrhoea has an especially negative effect, resulting in less milk production and an earlier onset of foraging. It's important to remember, though, that diarrhoea is most common in female calves up to two months of age. This might be related to adjustments made to their feeding schedule, which could stress the animals and increase their susceptibility to illnesses (Coklin *et al*., 2009).From this it was confirmed that infection chances were high in female as compared to male, similarly the chances of infection was higher in lower age group as compared to the buffalo calves with age more than six months or one year.

Seasonal fluctuations can also impact the prevalence of *Cryptosporidium* infection; in large ruminants, the prevalence peaked during the monsoon (rainy), pre-monsoon (summer), and post-monsoon (winter). The ideal temperature and high humidity of the monsoon allow *Cryptosporidium* species to spread quickly through fecal-oral routes (Caccio *et al*., 2005). Additionally, the prolonged wet season increases the likelihood of animal congestion and hinders the drying of damp animal shed walls and floors. From this it will shows that, the higher prevelance of cryptosporidiosis were recorded in monsoon (rainy) month this report were shown by (Yadav *et al.*, 2008).The heightened occurrence of cryptosporidiosis in the monsoon season can be ascribed to the excessive density of animals in shelters within systems of free-range farming. The prevelance of *Cryptosporidium infection* in monsoon was 22.48%, followed by 9.72% in pre-monsoon, while less in post-monsoon which was 4.5%, with respect to season of the year.

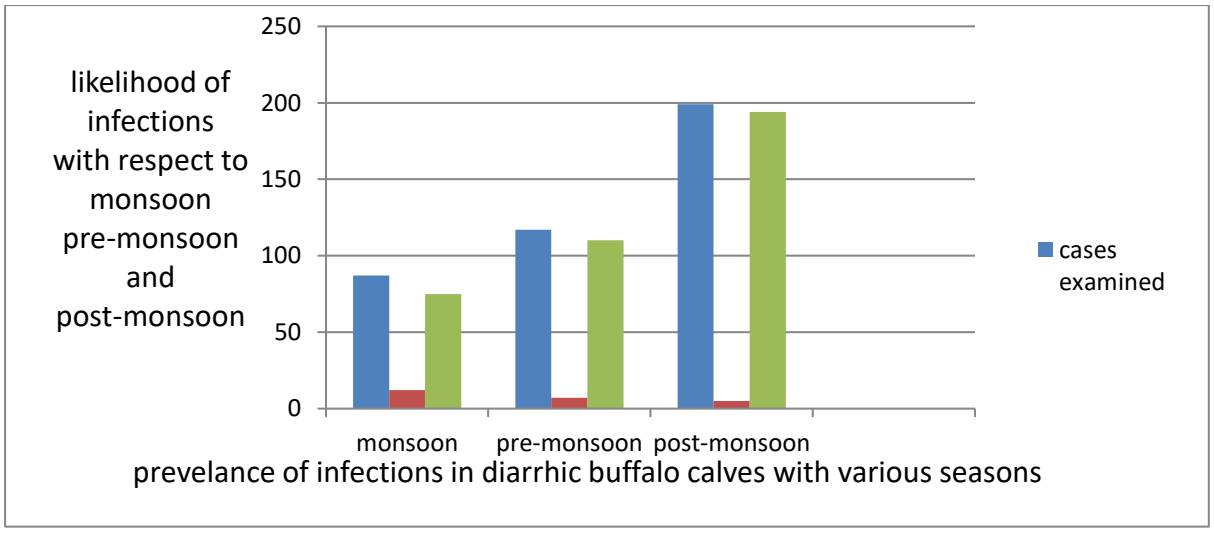
Table 4.7. **Likelihood of diarrhic and non-diarrhic buffalo calves excreting *Cryptosporidium* oocysts in various seasons like winter (post-monsoon), summer (pre-monsoon) and rainy (monsoon) seasons of the year.**

Diarrhic buffalo calve				Non-diarrhic buffalo calve			
Season	No of animal examined	No of positive cases	Prevelance	No of animal examined	No of positive cases	Prevelance	Total prevelance (%)
Winter season (post-monsoon)	87	8	9.19%	199	5	2.5%	4.5%
Summer (pre-monsoon)	68	11	16.17%	117	7	5.98%	9.72%
Rainy (monsoon)	42	17	40.47%	87	12	13.79%	22.48%
Total	197	36	18.27%	403	24	5.95%	10%

The p-value is <0.00001. This value is significant.



Graph 4.8. The prevelance of Cryptosporidium infection in a diarrhic buffalo calve with respect to monsoon (rainy), pre-monsoon (summer), and then in post-monsoon (winter) is shown in the following graph.



Graph 4.9. The prevelance of Cryptosporidium infection in non-diarrhic buffalo calve with respect to monsoon (rainy), pre-monsoon (summer), and then in post-monsoon (winter) is shown in the following graph.

From this chart it was consider that the rate of infection was higher in monsoon (rainy), which is then high in pre-monsoon (summer), and then in post-monsoon (winter) season in the current study area.

The percentage of *Cryptosporidium* oocyst excretions in district Mardan and its numerous tehsils were impacted by the rainy (monsoon), cold winter (post-monsoon), and dry summer (pre-monsoon) seasons. Similar to this, it was observed that temperature and precipitation were strong indicators of *Cryptosporidium* oocyst excretions in moist Trophical locations, where precipitation was a strong seasonal factor. In the current research region, the average temperature was 22.3◦C/72.1◦F, with annual precipitation ranging from 817mm to 32.2 inches. Additionally, the province's climate changes with elevation. Additionally, a lot of rain helps the oocysts survive in their surroundings. Seasonal variations causes cryptosporidiosis in buffalo calves in the current area, seasonal variations may be caused by temperature, rainfall or cycle of the seasons, also the variations may be hourly, weekly or annually. These variations can be reduced by using immunization or get vaccinated.

Buffalo calves in the rainy season have a more chances of infection as compared to the summer and winter season because in the rainy season the large number of oocysts were shed which can increase the chances of infection in a calve. Rainfall and high relative humidity are necessary for the survival of *Cryptosporidium* oocysts. Environmental factors can also be linked to changes in illness occurrence; higher humidity levels raise the risk of infection. Furthermore, a host with a compromised immune system may be more susceptible to infection (Samir *et al*., 2014).The geographic location of the farm, farm management practices, agricultural practices, and herd sizes are a few examples of the variables that might affect the prevalence of various pathogens and disease incidence. Diarrhoea has been linked to high death rates in unweaned dairy calves in many areas across the world (Hur *et al*., 2013).

The results of this study shows that *Cryptosporidium* infection was present in buffalo calve in district Mardan, Pakistan. This study was carried out in buffalo calve in province Khyber Pakhtunkhwa, Pakistan in area of district Mardan have a reported prevelance of 10%. The rate of infection was 14.33% in buffalo calve with diarrhea; similarly this rate of infection was 5.66 % without diarrhea which were confirmed by using microscopy. In another study, which involved young buffalo calves in Turkey with a prevalence of 22.8%, the likelihood of contracting diarrhoea was found to be 30.3%, whereas the incidence of infection without diarrhoea was 10%. PCR instruments can be used to detect this infection, which was found to be 28% prevalent in young buffalo calves in New Zealand. In Argentina, the prevalence of *Cryptosporidium* infection in a buffalo calf was 17% for the first time (Bahadori *et al.,* 2010). According to data from various research sources, the prevalence of *Cryptosporidium* infection was 17.9% in France and 20% in Canada (Lefay *et al.*, 2000). In Poland, calves have reported a 25 percent frequency (Pilarczyk and Balicka-Ramisz, 2000). Infection with *Cryptosporidium* was prevalent in 40% of Germany, 45.5% of the USA, 20% of Canada, 19% of Spain, and 27% of Hungary (Kumar *et al*., 2005). In Central Ethiopia, the total prevalence of *Cryptosporidium* infection in calves was determined to be 18.6% (Abebe *et al*., 2008).In the Mandalay region of Myanmar were reported to have an overall prevalence of 14% in the country. The breed of the calves, their age and nursing conditions, the farming and organizational scheme, the time frame for sample collection, and the hygienic conditions of the surroundings both inside and outside the farms could all be contributing factors to the variations in prevalence between the current trials and the previously mentioned reports. These variables may work alone or in concert to increase the risk associated with the occurrence and spread of *Cryptosporidium parvum* in calves (Duranti *et al*., 2009). The likelihood of the disease surviving in the area under consideration is increased by additional factors such as inadequate nutrition, unsanitary conditions, and overpopulation of farm animals. Similarly, because the ground is not cemented and is difficult to clean entirely, feeding calves on it raises the risk of contamination for the particular animal that was grazing there.

In several Iraqi provinces, the prevalence of cryptosporidiosis in buffalo calves was estimated to be

10% in Babylon Province (Al-Amery,2022), which is coincide with our value done in district Mardan, Khyber Pakhtunkhwa, Pakistan the resulted prevelance value was (10%). However, the prevelance of *Cryptosporidium* infection in western Iran was 5% (Gao *et al*., 2023), which is about half of our prevelance carried out in Pakistan.

The overall infection rate of cryptosporidiosis in a buffalo calves China was 11.9% (Gong *et al.*, 2017) which is little high as compared to our data done in Khyber Pakhtunkhwa, Pakistan.

In Galicia (Spain), the prevalence of infection was 17.72% in calves that had diarrhoea and 7.54% in calves that did not have a diarrhea (Castro-Hermida *et al*., 2002). Conversely, a small number of researchers in Egypt discovered that, because of the parasite's extremely superficial location, the infection was not linked to bloody diarrhoea (El-Khodery and Osman, 2008).

There was a discernible increase in prevalence between the sexes, with females being more infected than males (Laatamna *et al*., 2018), Nevertheless, this outcome did not agree with the earlier research conducted in Eastern Nigeria by (Adamu *et al*., 2015).

The second most common species found in pre-weaned buffalo calves with severe diarrhoea was *Cryptosporidium bovis* (Lichtmannsperger *et al*., 2020). That being said, a number of researches have provided evidence that *Cryptosporidium bovis* does not induce diarrhoea (Cai *et al*., 2017).

By considering, age of the calves the *Cryptosporidium* infections in Romania was (10.5%) in a buffalo calves (Barburas *et al*., 2021) this prevalence is comparable to earlier studies that was done in Egypt from buffalo calves (Ibrahim *et al*.,2016).

Watery diarrhoea was strongly linked to the shedding of *Cryptosporidium parvum* oocysts, primarily in calves younger than two weeks, who had a higher probability of being positive than other animals. These findings are consistent with earlier research conducted in Argentina by (Garro *et al*., 2016).There were no significant correlations found between the consistency of the faeces at the time of sample collection and the presence of diarrhoea throughout the first 14 days of life ($P>0.05$). This lack of importance could be explained by the existence of bacteria, some of which might be enteropathogenic or enterotoxic or it could be brought on by external variables that affect alterations in the consistency of the faeces. According to the findings of the study, animals with diarrhoea had a greater prevalence of *Cryptosporidium* infection (94%) than animals without diarrhoea (51%) by (Rajkhowa *et al.*, in 2006).

Conclusion

The results of this study revealed that *Cryptosporidium parvum* was the most prevalent species found in the fecal samples of buffalo calves in the Mardan district. These infected animals can act as potential reservoirs for zoonotic *Cryptosporidium*, which can be transmitted to family members and farm workers if proper hygiene and calve management practices are not observed. Furthermore, the oocysts can contaminate milk and milk products. Therefore, veterinarians, butchers, breeders, and farm owners should be aware of the disease in animals to prevent significant economic losses and to avoid transmission to humans.

Microscopic technique is the most widely used method for the detections of cryptosporidiosis in a faecal samples, the microscopic accuracy of these methods is largely dependent on the experience of the microscopist. Since early diagnosis is the best way to fight against infections to minimize the effects of this lethal infections in animals which adversely effects the economy of a farmers.

Conflict of interest

The authors declared that present study was performed in absence of any conflict of interest.

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