PREVALENCE OF ENTEROPATHOGENS ASSOCIATED WITH NEONATAL CALF SCOUR IN CATTLE AND BUFFALO CALVES USING (FASTEST® STRIPS) RAPID FIELD TEST

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ABSTRACT

The prevalence of calf scour-causing enteropathogens was investigated in the current study using rapid field test. Fecal samples were collected from a total of 124 cattle *(Bos indicus)* and water buffalo *(Bubalus bubalis)* calves. Investigated calves were divided into 4 age groups (0–4 days old, 5–14 days old, 15–21 days old and >21 days old). Immuno-chromatographic rapid tests (*FAST*est® Strips) were used for the detection of Bovine coronavirus, rotavirus, *Cryptosporidium parvum* and *E.coli*-K99 (F5) from investigated diarrheic calves. In cattle calves (n= 76), *C. parvum* was the most frequently encountered infection among all age groups followed by rotavirus with an overall rate of 59.2% and 35.5%, respectively. In water buffalo calves (n = 48), the highest infection rate was recorded for *C. parvum* (39.6%) followed by both rotavirus and *E.coli* (20.8% each) in all age groups with exception of the first age group (0-4 days-old), where the highest infection rate was recorded as *E.coli* (57.1%) followed by *C. parvum* (28.6%). In conclusion, *C. parvum* was reported as the most frequently encountered causative agent among both cattle and water buffalo calves whilst coronavirus infection seemed to be of minor importance in the investigated population. The higher rate of *C. parvum* infection was recorded among the second age group (5-14 day old) of cattle calves and the forth age group (> 21 day old) of water buffalo calves, whilst the highest rate of *E.coli*-K99 (F5) infection was recorded among the first age group (0-4 days) of both cattle and water buffalo calves.

Key Words: *Calf scour, Rapid field test, Enteropathogens.*

INTRODUCTION

Neonatal calf diarrhea, also known as calf scours, is a common disease affecting the newborn calf and characterized by diarrhea (scouring), progressive dehydration and death (Radostits *et al.*, 2007). Each year thousands of neonatal calves are lost as a result of calf diarrhea. Calf scours result in losses not only by increasing calf fatality, but also by a decrease in the calf's ability to gain weight. (Frank and Kaneene, 1993).

Infectious agents are the most significant causes of calf scour. The pathogens most commonly incriminated in neonatal calf scour include bacteria as *Escherichia Coli*, rotavirus (RV), bovine corona virus (BCV) or enteric protozoa as *Cryptosporidium parvum*. *E. coli* causes calf diarrhea in the first ten days of life and is characterized as a pasty to a fluid diarrhea. Generally *E. coli* diarrhea affects a small percent of herd unless sanitation is extremely poor. RV and BCV result in scours from 3 to 21 days. And characterized by explosive episodes in a herd and involve a high percentage of the calves. Cryptosporidiosis usually occurs between one and three weeks of age and characterized by bloody and pasty diarrhea (de la Fuente *et al.*, 1998; Reynolds *et al.*, 1986; Waltner-Toews D., 1986).

Several risk factors contribute to the problem of neonatal calf scour including health and nutrition of cows, herd management before and after calving as well as feeding of neonatal calves. However, the most common locally encountered risk factor is having cows and heifers together in the same area they will be calving, which is a common practice among small scale owners as it was the case in the current study. This results in accumulation of manure with the subsequent increase in the number of pathogens in the calf's environment. It was shown that calves born in this environment are 3 times more likely to get scours than calves born in an area that did not have cows or heifers living together in the calving area. (Frank and Kaneene, 1993; Langoni *et al.*, 2004).

Identification of the possible causative agent in outbreaks of diarrhea is important because it allows targeted preventative measures, such as vaccination, and identification of possible risk factors or sources of infection. Conventional diagnostic methods to detect enteropathogens in calves require time,

experience and specialized laboratory equipment. The practicing veterinarian, however, requires rapid, reproducible, sensitive and simple diagnostic tests for quick decisions on therapeutic and prophylactic strategies. Therefore, rapid immune-chromatographic assays have been developed to detect different enteropathogens in calf feces within a few minutes in the field. Advantages of rapid assays are that a large number of samples can be processed quickly with minimum effort and intervention strategies can be implemented immediately and the need for technological expertise or specialized laboratory equipment is minimal (Klein *et al.,* 2009).

Recently, a commercial antigen-capture immune-chromatographic assay (*FAST*est® D4T cattle test kits) has been developed and marketed for rapid detection of the 4 major bovine enteric pathogens (BCV-1, RV, *E. coli* K99$^+$, and *C. parvum*) in feces from diarrheic calves. The principle of the kit, that is designed as a rapid test for field diagnosis, is based on a lateral flow immune-chromatography assay that captures target antigen (s) within a fecal sample (Al-Yousif *et al.,* 2002). The assay was evaluated for detecting infections with BCV, RV-A and *C. parvum* from fecal samples of 180 calves using reverse transcriptase polymerase chain reaction (BCV and RV) and sedimentation-flotation technique (*C. parvum*) as gold standards. High specificity (96.4% and 95.3%) was recorded for the detection of BCV and RV, respectively. However, a relatively low sensitivity (60.0% and 71.9%, respectively) was recorded. On the other hand, sensitivity and specificity for detection of *C. parvum* were high (100% and 94.6%, respectively) (Klein *et al.,* 2009).

Identification of the possible causative agent is crucial for the control of neonatal calf diarrhea. The different infectious agents of neonatal calf scour without specific symptoms make the etiologic differential diagnosis such a difficult process for veterinarians. In this context, scarce information is available about the prevalence and identification of different causative agents associated with diarrhea in neonatal calves in Egypt and particularly in Assiut. Therefore, the aim of the current study was to implement the recently described and recommended rapid field assays to investigate the prevalence of BCV, RV, *C.parvum* and *E.coli* F5 among diarrheic neonatal calves under field conditions at local districts of Assiut, Egypt.

MATERIALS and METHODS

Sample collection:

Fecal samples were collected from a total of 124 diarrheic calves from 2 adjacent districts (Alfateh and Sahel Seleem) of Assiut, Egypt during the period from June to August 2012. This included 76 cattle calves and 48 buffalo calves. Inclusion criteria included diarrheic calves aged from 1-35 days old that were selected from small size herds (10-30 cows) owned by local villagers. All selected calves had signs of systemic disease as pasty–watery feces, poor appetite, reduced suckle reflex and dehydration. Approximately 15-20 mL of fecal material was collected in sterile containers directly from the rectum of diarrheic calves by direct digital stimulation using a disposable latex glove. Samples were then kept in ice box till reach the diagnostic laboratory of the faculty of Veterinary Medicine, Assiut University. Samples were then centrifuged and the resulted supernatant were collected in sterile containers and kept at -20 till time of use.

Pathogen detection and identification:

All samples were tested with *FAST*est® D4T cattle test kits (MegaCor Diagnostik GmbH, LochauerstraBe 2•A - 6912 – Horbranz, AUSTRIA). The test kit included 4 rapid immune-chromatographic test strips for the detection of cattle BVC, RV, *C. parvum* and *E.coli*-K99 (F5). The tests were carried out according to the manufacturer's guidelines. Briefly, one sample tube (P) and one test tube (R) were labeled for each fecal sample. Fecal samples were mixed homogenous and using the spoon on the cap of the sample tube, 2 spoons from each fluid-watery fecal sample were added to the diluents buffer of the corresponding test tube. Test tubes were then closed tightly and rotated in a slight and circular way for homogenous mix of the fecal sample with the diluents buffer. Then the feces-buffer diluents were added vertically to the opened corresponding test tube. Then after adding the sample mix to the test tube, the cap of the test tube was turned until hearing a clicking noise for two times. The test tube was kept on flat surface to allow the absorption of the feces-buffer diluents by the test membranes. Test result was read after 5 minutes and before 10 minutes. Correct test procedure is proved by the occurrence of a second pink/purple CONTROL line. The appearance of a clearly signed weak to strong intensive pink/purple TEST line in the TEST zone and a clearly signed weak to strong intensive pink/purple CONTROL line in the CONTROL zone indicated positive result. On the other hand, the absence of a pink/purple TEST line in the TEST zone with the presence of clearly signed intensive pink/purple CONTROL line in the CONTROL zone indicated negative results.

Analysis of data:

Investigated calves from both cattle and buffalo species were divided into four age groups for analysis: 0–4 days old, 5–14 days old, 15–21 days old and >21 days old. The age categories were selected

based on the pathophysiology of some of the pathogens and the age groups that have been previously described (de la Fuente *et al.*, 1998; de la Fuente *et al.*, 1999) The age distributions of calves as well as the number and prevalence of causative enteropathogens in different age groups were compared in both cattle and buffalo calves using chi square (SPSS, 16.0).

RESULTS

Age distribution of investigated diarrheic cattle and buffalo calves:

A total of 124 diarrheic calves (76 cattle calves and 48 buffalo calves) were collected from small sized herds of 2 adjacent districts. The mean age of the calves sampled was 13.9 days, with a SD of ± 9.06. The median age was 11 days with an interquartile range of 13 days, a minimum of 1 day and maximum of 35 days. Diarrheic calves from both cattle and buffalo species were divided into four age groups; 0–4 days old, 5–14 days old, 15–21 days old and <21 days old. The majority of calves of both cattle and buffalo herds were in the 5–14-day-old age group followed by the 15-21-day-old age group (Table 1).

Prevalence of enteropathogen infection among diarrheic claves:

Enteropathogens were isolated from 88.7% of investigated diarrheic calves (93.4% of cattle calves and 81.3% of buffalo calves) as shown in figure 1. Out of 124 diarrheic claves, 14 (11.3%) were free of enteropathogens, 78 (62.9%) were infected with single pathogen, 21% with double mixed infection and 4.8% with triple mixed infection (table 2). Significant increases ($P < 0.001$) in the frequency of single infection as compared to mixed infections were recorded among both cattle and buffalo calves. Significant increase ($P < 0.05$) was also recorded in the frequency of single infection among buffalo calves as compared to cattle calves. In addition, a significant increase ($P < 0.05$) in multiple infection rates among cattle calves as compared to buffalo calves was also evident. Regarding type of mixed infection, current results revealed that *C. parvum* infection was more frequently encountered ($P < 0.05$) among single infection group of both cattle and buffalo calves. On the other hand, *C. parvum* and rotavirus mixed infection was the most frequently identified concurrent infection ($P < 0.05$) among the double mixed infection group in cattle calves (Table 3).

Prevalence of different enteropathogens in relation to age of investigated diarrheic calves:

Cryptosporidium parvum was the most common pathogen identified from all investigated calves (51.6%) followed by rotavirus (29.8%), E. coli (21.8%) and coronavirus (16.9%) (Figure 2). With regard to age groups, the obtained results revealed that *C. parvum* was the most frequently identified pathogen from all four age groups except for the first age group (0-4 days old), where E. coli was more frequently identified with an overall rate of 50% of investigated cases (44.45 from cattle calves and 57.1% of buffalo calves). A significant increase ($P < 0.05$) was recorded for *C. parvum* infection in cattle calves as compared to buffalo calves. On the other hand, a significant increase ($P < 0.05$) was recorded for the rate of *E. coli* infection in the first age group of both cattle and buffalo calves as compared to the other three age groups (Table 4).

Table 1: Age distribution of investigated diarrheiccattle and buffalo calves.

Age groups	Cattle calves		Buffalo calves		Total	
	N	%	n	%	n	%
0-4 days old	9	*11.84*	7	*14.58*	16	*12.90*
5-14 days old	34	*44.74*	22	*45.83*	56	*45.16*
15-21 days old	22	*28.95*	14	*29.17*	36	*29.04*
>21 days old	11	*14.47*	5	*10.42*	16	*12.90*
Total	**76**	***61.29***	**48**	***38.71***	**124**	***100***

Table 2: Number of diarrhea-associated enteropathogens from investigated Bovine and buffalo calves.

No. of pathogens	Total calves (n=124)	Bovine calves (n=76)	Buffalo calves (n=48)
0	14 (11.3%)	5 (6.6%)	9 (18.8%)
1	78 (62.9%)[a]	43 (56.6%)	35 (72.9%)[b]
2	26 (21.0%)	22 (28.9%)[c]	4 (8.3%)
3	6 (4.8%)	6 (7.9%)[c]	0 (0.0%)

[a] Significant increase ($P < 0.001$) in the frequency of single infection as compared to mixed infections; [b] significant increase ($P < 0.05$) in the frequency of single infection among buffalo calves as compared to Bovine calves; [c] significant increase ($P < 0.05$) in multiple infection rates among Bovine calves as compared to buffalo calves.

Table 3: Types of diarrhea-associated enteropathogens in relation to single and mixed infections among investigated Bovine and buffalo calves.

	Enteropathogen infection rate / type	Total infected calves (110)	Infected Bovine calves (71)	Infected buffalo calves (39)
Single Infection	*C. parvum*	37 (33.6%)[a]	20 (28.2%)	17 (43.6%)
	BCV	8 (7.3%)	6 (8.5%)	2 (5.1%)
	RV	15 (13.6%)	8 (11.3%)	7 (17.9%)
	E. coli	18 (16.4%)	9 (12.7%)	9 (23.1%)
Double mixed infection	*C. parvum* + *RV*	14 (12.7%)	13 (18.3%)[b]	1 (2.6%)
	C. parvum + *BCV*	3 (2.7%)	2 (2.8%)	1 (2.6%)
	C. parvum + *E. coli*	2 (1.8%)	2 (2.8%)	0 (0.0%)
	RV + *BCV*	4 (3.6%)	2 (2.8%)	2 (5.1%)
	E. coli + *RV*	1 (0.9%)	1 (1.4%)	0 (0.0%)
	E. coli + *BCV*	2 (1.8%)	2 (2.8%)	0 (0.0%)
Triple mixed infection	*C. parvum* + *RV* + *BCV*	3 (2.7%)	3 (4.2%)	0 (0.0%)
	C. parvum + *RV* + *E. coli*	2 (1.8%)	2 (2.8%)	0 (0.0%)
	C. parvum + *E. coli* + *BCV*	1 (0.9%)	1 (1.4%)	0 (0.0%)

[a] Significant increase ($P < 0.05$) in the frequency of *C. parvum* infection among single infection group of both Bovine and buffalo calves as compared to other types of infection.[b] significant increase ($P < 0.001$) in the frequency of *C. parvum* and rotavirus infection as compared to other types of double mixed infections in Bovine calves. Rotavirus (RV), Bovine coronavirus (BCV).

Table 4: Frequency of different enteropathogens among diarrheic calves in relation to age groups.

Age groups (n)		*BCV* n *(%)*	*C. parvum*n *(%)*	*RV* n *(%)*	*E.coli* n *(%)*
(0-4) days old	**cattle calves (9)**	1 *(11.1)*	5 *(55.6)*[a]	2 *(22.2)*	4 *(44.4)*
	Buffalo calves (7)	0 *(0.0)*	2 *(28.6)*	1 *(14.3)*	4 *(57.1)*
	Overall group (16)	1 *(6.3)*	7 *(43.8)*	3 *(18.8)*	8 *(50.0)*[b]
(5-14) days old	**Cattle calves (34)**	10 *(29.4)*	21*(61.8)*[a]	15 *(44.1)*	9 *(26.5)*
	Buffalo calves (22)	3 *(13.6)*	8*(36.4)*	5*(22.7)*	5 *(22.7)*
	Overall group (56)	**13 *(23.2)***	**29 *(51.8)***	**20 *(35.7)***	**14 *(25.0)***
(15-21) days old	**cattle calves (22)**	4 *(18.2)*	13 *(59.1)*[a]	7 *(31.8)*	1 *(4.5)*
	Buffalo calves (14)	2 *(14.3)*	6 *(42.9)*	3 *(21.4)*	1 *(7.1)*
	Overall group (36)	**6 *(16.7)***	**19 *(52.8)***	**10 *(27.8)***	**2 *(5.6)***
(>21) days old	**cattle calves (11)**	1 *(9.1)*	6 *(54.5)*[a]	3 *(27.3)*	3 *(27.3)*
	Buffalo calves (5)	0 *(0.0)*	3 *(60.0)*	1 *(20.0)*	0 *(0.0)*
	Overall group (16)	**1 *(6.3)***	**9 *(56.3)***	**4 *(25.0)***	**3 *(18.8)***

[a]A significant increase ($P < 0.05$) for the rate *C. parvum* infection between Bovine calves and buffalo calves in all age groups.[b]A significant increase ($P < 0.05$)for the rate of *E. coli* infection in the first age group of both Bovine and calves as compared to the other three age groups.

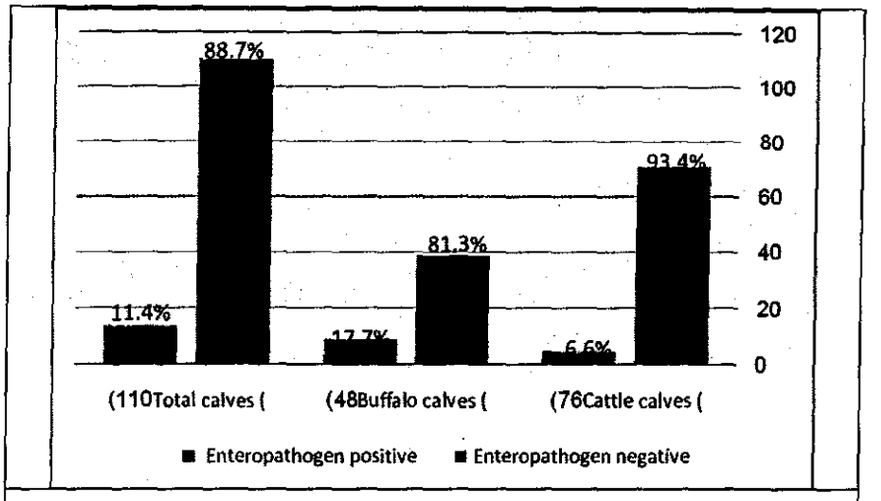

Figure 1: Overall prevalence of enteropathogen infection among diarrheic Bovine and buffalo claves.

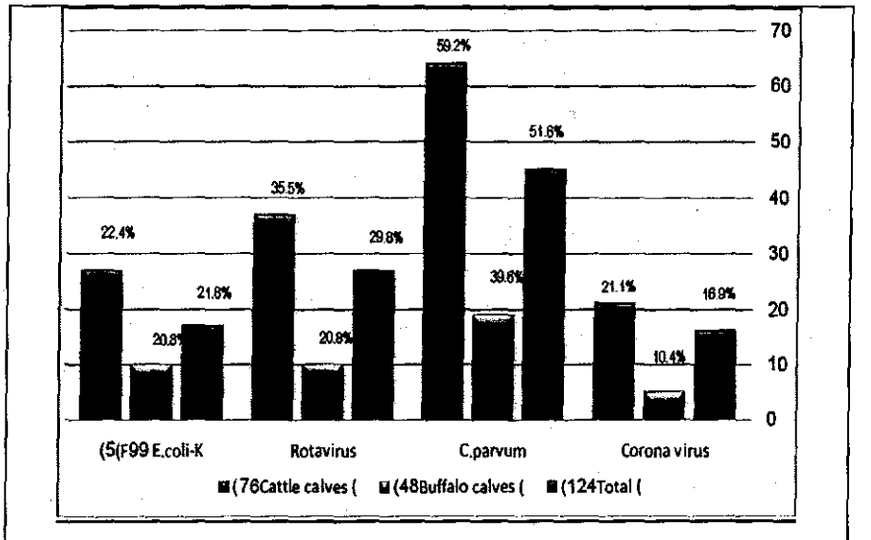

Figure 2: Prevalence of different enteropathogens associated with diarrhea in cattle and buffalo calves.

DISCUSSION

Diarrhea is a leading cause of economic losses to the beef industry and major cause of calf mortality and morbidity during the first few weeks of life in most countries (Radostits *et al.*, 2007). Defining the map of calve diarrhea-causing enteropathogens allows for application of effective and targeted preventative measures. The objective of the current study was to determine the prevalence of enteric pathogens associated with neonatal calf diarrhea among both cattle and buffalo calves. Immuno-chromatographic rapid tests (FASTest® Strips) were used for the detection of BCV, RV, *C. parvum* and *E.coli*-K99 (F5) from diarrheic cattle and buffalo calves.

Previous evaluation studies of the use of rapid tests for field diagnosis of calve diarrhea-causing agents revealed high sensitivity and specificity for detection of *C. parvum* and E. coli-K99 (Cho *et al.*, 2012; Klein *et al.*, 2009). However, regarding the detection of BCV and RV, rapid tests showed high specificity but a relatively low sensitivity (Klein *et al.*, 2009; Luginbuhl *et al.*, 2005). Despite of that, the use of rapid tests was recommended with standard methods in calves with acute diarrhea where the specificity

might be of higher importance than the sensitivity, which could be increased by testing on a herd basis (Luginbuhl *et al.,* 2005). In general, previous evaluation studies suggested that rapid field test could be considered as a helpful tool for fast and effective diagnosis of common enteropathogens associated with neonatal calve diarrhea (Cho *et al.,* 2012; Klein *et al.,* 2009; Luginbuhl *et al.,* 2005).

In spite of the complex etiology of calf diarrhea, microbial infections represent the main cause especially in neonatal calves (Bazeley, 2003; Boyed *et al.,* 1974). This notion was confirmed in the current study, where 88.7% of investigated cases of calve diarrhea was proved due to microbial agents. The majority of those microbial infections were recorded as single-pathogen infections (78 out of 110). This could be attributed to the age factor as in current study most enteropathogen infections were recorded among early age groups where previous study recorded the presence of a significant age-associated decrease in the detection rate of mixed infections (de la Fuente *et al.,* 1999). Although there was a significant increase in the overall rate of single infection as compared to mixed infection as revealed in the current study, this rate was significantly higher among buffalo calves as compared to cattle calves, or in other word the rate of mixed infection was significantly higher among cattle calves as compared to that in buffalo calves. This finding could be attributed to the lower susceptibility of buffalo calves to attract mixed infection as compared to cattle calves due to different susceptibilities (Malik *et al.,* 2012). In addition, it could be also related to the diversity of the natural immunity of the two different species as manifested in other diseases (Yang *et al.,* 2012).

The overall results from all diarrheic calves revealed that *C. parvum* was the most frequently isolated pathogen (51.6%), followed by rotavirus, E. coli-K99 (F5) and coronavirus with an overall rate of 29.8%, 21.8% and 16.9%, respectively. Although similar findings were reported in central Spain (de la Fuente *et al.,* 1999), the currently reported prevalence of *C. parvum* was higher than has been reported earlier in Europe (Bordas *et al.,* 1985; Reynolds *et al.,* 1986; Snodgrass *et al.,* 1986). Moreover, the currently recorded higher prevalence of *C. parvum* as compared to rotavirus contrasts findings of recent study in Australia, which reported higher prevalence of RV than that of *C. parvum* (Izzo *et al.,* 2011). This discrepancy may in part reflect the lower sensitivity of the rapid assay used in the current study as compared to the more sensitive qRTPCR (Gutierrez-Aguirre *et al.,* 2008) used in the Australian study for detection of rotavirus. However, one disadvantage of the qRTPCR higher sensitivity is the ability to detect very low viral numbers that may not be of clinical significance. The different geographic areas and the different conditions of the studies may represent another possible explanation of this discrepancy especially with regard to *C. parvum* for which the sensitivity and specificity were proved high using the rapid assay *(Cho et al., 2012; Klein et al.,* 2009; Trotz-Williams *et al.,* 2005). In general, the high rate of *C. parvum* infection recorded in the current study could have attributed to several factors. The resistance of the oocysts and the great proliferative capacity of the parasite (Trotz-Williams *et al.,* 2007), in addition to the poor hygiene and sanitary conditions found on many ruminant exploitations contribute to the important presence of the parasite on such farms, particularly those that raise sheep and goats, which serve as asymptomatic carriers of the disease (O'Donoghue, 1995; Robertson *et al.,* 1992).

In relation to age of investigated diarrheic calves, current study showed that, with exception of the first age group (0-4 days old) in buffalo calves where *E. coli* was more frequently encountered, *C.parvum* was the most frequently detected enteropathogen in all age groups of both cattle and buffalo calves. This finding could be attributed to the limited ability of *E.coli* to affect large percent of herds unless sanitation is extremely poor and its tendency to cause its characteristic pasty to fluid diarrhea during the first ten days of life and not in later ages on the contrary of to *C. parvum*, which has the ability to produce progressive infection at all ages (de la Fuente *et al.,* 1998; Reynolds *et al.,* 1986; Waltner-Toews D., 1986). Although *C.parvum* showed the higher infection frequency approximately in all age groups, the rate of infection was highest at the 2nd age group (4-15 day old) and reduced as the age of investigated calves get older. This observation was in agreement with many studies that reported the highest prevalence of *C. parvum* in animals of less than a month of age (Huetink *et al.,* 2001; Santin *et al.,* 2004; Sturdee *et al.,* 2003).

In conclusion, *C. parvum* was reported as the most frequently encountered causative agent among diarrheic cattle and buffalo calves whilst coronavirus infection seemed to be of minor importance in the investigated population. The higher rate of *C. parvum* infection was recorded among the second age group (5-14 day old) of cattle calves and the forth age group (> 21 day old) of buffalo calves, whilst the highest rate of *E.coli* infection was recorded among the first age group (0-4 days) of both cattle and buffalo calves. Cattle calves were more likely to have mixed multiple infections than buffalo calves.

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دراسة مدى انتشارية العوامل المعدية المسببة لاسهال العجول حديثة الولادة في العجول البقري والجاموس باستخدام الاختبار الحقلي السريع (*FAST*est® Strips)

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قامت الدراسة الحالية بالتحقيق في مدى انتشار العوامل المعدية المسببة للاسهال في العجول حديثة الولادة باستخدام الاختبار الحقلي السريع. تم تجميع عينات البراز من اجمالي عدد ١٢٤ من العجول البقري والجاموسي حديثي الولادة والمصابين بالاسهال. قسمت العجول محل الدراسة إلى ٤ مجموعات عمرية تشمل (٠-٤) أيام ، (٥-١٤) يوم، (١٥–٢١) يوم و >٢١ يوم. تم استخدام الاختبار المناعي الكروماتوغرافي السريع (*FAST*est® Strips) للكشف عن فيروس الكورونا، فيروس الروتا، الكريبتوسبوريديوم بارفام والايشيريشيا كولاي من العجول محل الدراسة. أظهرت نتائج الدراسة ان طفيل الكريبتوسبوريديوم بارفام كان الاكثر انتشارا بين جميع الفئات العمرية للعجول البقري (n = 76)، يتبعه فيروس الروتا وذلك بنسبة منوية تبلغ ٥٩.٢% & ٣٥.٥% على التوالي. وفي عجول الجاموس (n = 48)، وسجلت الدراسة أعلى معدل للإصابة بطفيل الكريبتوسبوريديوم بارفام بنسبة منوية اجمالية بلغت ٣٩.٦ % يليها كل من فيروس الروتا وميكروب الايشيريشيا كولاي (٢٠.٨% لكل منهما) وذلك في جميع الفئات العمرية باستثناء الفئة العمرية الأولى (٠-٤ أيام)، حيث سجلت الايشيريشيا كولاي أعلى معدل للإصابة (٥٧.١ %) تليها الكريبتوسبوريديوم بارفام (٢٨.٦ ٪). في المحصلة أظهرت الدراسة ان طفيل الكريبتوسبوريديوم بارفام هو الاكثر انتشارا بين جميع المسببات المعدية للاسهال في العجول حديثة الولادة سواء بين العجول البقري او عجول الجاموس. من ناحية اخري، اظهرت الدراسة ان فيروس الكورونا كان الاقل انتشارا بين الحيوانات المصابة محل الدراسة. كذلك سجلت الدراسة أعلى معدل إصابة بالكريبتوسبوريديوم بارفام بين الفئة العمرية الثانية (٥-١٤ يوم) في العجول البقري والمجموعة العمرية الرابعة (< ٢١ يوم) في عجول الجاموس ، بينما سجلت الدراسة أعلى معدل للإصابة بميكروب الايشيريشيا كولاي بين الفئة العمرية الأولى (٠-٤ أيام) من كلا من العجول البقري والجاموس.