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RAPID-TEST AND ELISA BASED IDENTIFICATION OF *ROTAVIRUS* IN CAMEL CALVES IN MIDDLE AND SOUTH OF IRAQ

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Abstract

The current study was intended to study the rotavirus infection occurring in camels in south of Iraq. In the beginning, 470 camel calves (CCs) were general-health-based investigated. The current work included 175 diarrheic and non-diarrheic CCs (DCCs) and (NDCCs), respectively, located in middle and south Iraq. Fifty fecal samples from DCCs and NDCCs were collected from different animal farms and sales locations of some provinces. One-step Rotavirus rapid test (OSRRT) and enzyme-linked immunosorbent assay (ELISA) were performed. The results for the rapid test showed higher positive rates of the diarrheic camel calves (DCCs), 35% and 37.1%, in both males and females, respectively, than those in non-diarrheic camel calves (NDCCs), 9.6% and 8.3%, in both males and females, respectively. The results indicated higher positive rates of the DCCs of age at >30 days, 22 (56.4%), than those in the NDCCs of age at >30 days and DCCs and NDCCs of age at d"30 days, 7 (14%), 5 (13.8%) and 2 (4%), respectively. For the highest infection rates, the results in March revealed 90% and 44.44% for both DCCs and NDCCs, respectively. For the highest infection rates, the results Mothana revealed 66.67% and 25% for both DCCs and NDCCs, respectively. For the ELISA, the results indicated higher positive rates 31.42% and 4.17%, of DCCs and NDCCs, respectively, in females than those from male calves 30% and 3.85%, respectively. The results indicated a higher positive rate 51.28% of the DCCs of age at >30 days than those in the NDCCs of age at >30 days and DCCs and NDCCs of age at d"30 days, 8%, 8.83% and 0%, respectively. For the highest infection rates, the results in March uncovered 80% and 33.33% for both DCCs and NDCCs, respectively. For the highest infection rates, the results Mothana revealed, 50% and 16.67%, for both DCCs and NDCCs, respectively. The study improves the presence of the *rotavirus* in the camel calves in the tested cities from Iraq.

Key words: Camel calves, *Rotavirus*, Rapid-test, ELISA, Iraq.

Introduction

Camels are considered to place an important influence of economy in Iraq and different Arabic regions. These animals are well-known for their capacities to tolerate severe environmental arid typed conditions. Iraq is considered having the share of the world camels as 0.2% as stated by the (Sikkema *et al.*, 2019); however Iraq in 1970, had higher populations than that in 2017, 266,000 and 78,196 camels, respectively. One of the detrimental intruders to good productions in Camel calf is mortalities in calves as diarrhea in those animals leads to high mortality rates (Hamzah *et al.*, 2019). It has been considered that 30% and 100% rates of morbidity and mortality, respectively were noticed for the diarrhea related disease in calves of camels. Microorganisms such as bacteria and viruses are considered as the major etiological agents inducing diarrhea in calves of camels such as *Escherichia coli*, *Salmonella* spp. and *Closteridium perferingens* for bacterial causes and *Rotavirus* (RV), *Coronavirus* and *Parvovirus* for viral causes (Ali, 2003).

RV virus is responsible for a clinical sign, diarrhea, of the bowels which is the great reason behind death of youngsters in not well-established health system countries plus inflicting vital economical malady in newborn animals of the many livestock (Trojnar *et al.*, 2009; Kindler *et*

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al., 2013). In newborn animals, a non-viremic infection can be seen, with noticeably low time windows manifesting great diarrheal sign with subsequent intense loss of bodily fluids. Disease severity can be augmented via synchronic types of infection induced by secondary infectious agents (Yilmaz *et al.*, 2017; Hamzah and Hasso, 2019). Destruction to the enterocytes can be mediated by the presence of this viral diarrhea, enteric nervous system-related activation, or the enterotoxin of the RV (Dirwal *et al.*, 2019).

Materials and Methods

Animals and sample collection

In the beginning, 470 camel calves (CCs) were general-health-based investigated. The current work included 175 diarrheic and non-diarrheic CCs (DCCs) and (NDCCs), respectively, located in middle and south Iraq. Data collected for those animals were animal temperature, morbidities and mortalities, seasonal related information, age and gender plus health information regarding any clinical signs observed or told by the owner using a questionnaire paper included animal related and owner sections according to (Hamzah and Hasso, 2019). The animal ages ranged between 1 to 60 days.

One hundred seventy-five fecal samples from DCCs and NDCCs were collected during a period of 12 months (February, 2018 to January, 2019) from different animal farms and sales locations of some provinces of Babylon (21 samples), AL-Najaf (19 samples), Karbalaa (22 samples) and Al-Qadisiyah (21 samples), Wasit (19 samples) Thi-Qar (19 samples) Mothana (22 samples) Mesan (14 samples) Basra (15 samples) using aseptic condition to prevent contamination of the samples, placed in sterile plastic bags and ice-based transported the lab. Fecal samples were stored at -20°C until the required tests performed.

One-step *Rotavirus* rapid test

One-step *Rotavirus* rapid test (OSRRT) was performed using One-Step *Rotavirus* Rapid Test Device kit (Aconcompany, Germany).

Table 1: The numbers of samples used in the One Step Rotavirus Rapid Test plus the positive results in counts and percentages categorized according to the gender of the camel calves.

Positive rate (%)	No. of positive sample	No. of samples	Type of tested calves		Gender
35	14	40		DCCs	Male
9.6	5	52		NDCCs	
37.1	13	35		DCCs	Female
8.3	4	48		NDCCs	
20.6	36	175			Total

The results can be interpreted as following:

Enzyme-linked immunosorbent assay

An ELISA reader was used to measure the optical densities at 450nm absorbance. Resulted values at ≥0.150 were considered as positive findings and values at <0.150 were evaluated as negative findings (Schwartz, 1992).

Results

One Step Rotavirus Rapid Test

• Classification of the results according to gender: The results of the OSRRT showed the presence of the RV-antigenic positive reactivity (RAPR) in 36 (20.6%) out of 175 collected fecal samples. The results indicated higher positive rates of the DCCs, 35% and 37.1%, in both males and females, respectively, than those in NDCCs, 9.6% and 8.3%, in both males and females, respectively. Although the samples numbers in the DCCs were somehow fewer than those from NDCCs, the positive findings were higher in the DCCs than those from the NDCCs. Moreover, with the fewest number of samples regarding the female DCCs, they showed the highest positive rates, 37.1%, for the detection of the RV antigen in the fecal samples, table 1.

• Classification of the results according to age of the camel calves: Although the samples numbers in the DCCs of age at >30 days were relatively the lowest than those from NDCCs at the same age category and the rest of the DCCs and NDCCs of age at ≤30 days, the positive findings, infection rates by RV, were the highest in the DCCs of age at >30 days than those from NDCCs at the same age category and the rest of the DCCs and NDCCs of age at ≤30 days. Although the sample tested from NDCCs of both age categories were the highest, 50 sample/each, the infection rates were the lowest, 14% and 4%, respectively, table 2.

• Classification of the results according to the months of the sample collection: For the highest infection rates, the results in March revealed 90% and 44.44% for both DCCs and NDCCs, respectively. However, April

Table 2: The numbers of samples used in the One Step Rotavirus Rapid Test plus the positive results in counts and percentages categorized according to the age of the camel calves.

Ages (days)	No. of tested sample		Positive sample	Infection rate (%)
>30	DCCs	39	22	56.4
	NDCCs	50	7	14
≤30	DCCs	36	5	13.8
	NDCCs	50	2	4
Total	175		36	20.6

Table 3: The numbers of samples used in the One Step Rotavirus Rapid Test plus the positive results in counts and percentages (infection rates) categorized according to the months of the year when the samples were collected in.

Months	No. of tested sample		Positive sample	Infection rate (%)
January	DCCs	11	2	18.18
	NDCCs	9	0	0
February	DCCs	11	4	36.36
	NDCCs	8	2	25
March	DCCs	10	9	90
	NDCCs	9	4	44.44
April	DCCs	8	6	75
	NDCCs	8	2	25
May	DCCs	10	6	60
	NDCCs	9	1	11.11
June	DCCs	4	0	0
	NDCCs	8	0	0
July	DCCs	6	0	0
	NDCCs	9	0	0
August	DCCs	4	0	0
	NDCCs	8	0	0
September	DCCs	3	0	0
	NDCCs	8	0	0
October	DCCs	4	0	0
	NDCCs	8	0	0
November	DCCs	1	0	0
	NDCCs	8	0	0
December	DCCs	3	0	0
	NDCCs	8	0	0
Total	175		36	20.6

demonstrated the 2nd highest infection rates, 75% and 25%, for both DCCs and NDCCs, respectively. Furthermore, in May, the results revealed the 3rd highest infection rates, 60% and 11.11%, for both DCCs and NDCCs, respectively.

• Classification of the results according to the provinces of the sample collection: For the highest infection rates, the results Mothana revealed 66.67% and 25% for both DCCs and NDCCs, respectively. Furthermore, in Najaf, the results revealed the 2nd highest infection rates, 40% and 11.11%, for both DCCs and NDCCs, respectively.

Enzyme-linked immunosorbent assay

• Classification of the results according to the gender of the camel calves: The results indicated higher positive rates, 31.42% and 4.17%, of DCCs and NDCCs, respectively, in females than those from male calves, 30% and 3.85%, respectively. Although the samples numbers in the female calves were fewer than those from male calves, the infection rates were higher in the female calves than those in male calves. Furthermore, with the least number of samples related to the female DCCs, they revealed the highest infection rate, 31.42%, for the detection of the RV in the fecal samples, table 5.

Table 4: The numbers of samples used in the One Step Rotavirus Rapid Test plus the positive results in counts and percentages (infection rates) categorized according to the regions that the samples were collected from.

The region	No. of tested sample		Positive sample	Infection rate (%)
Babylon	DCCs	10	4	40
	NDCCs	11	1	9.09
Najaf	DCCs	10	4	40
	NDCCs	9	1	11.11
Karbala	DCCs	9	3	33.33
	NDCCs	13	0	0
Al-Diwaniyah	DCCs	10	1	10
	NDCCs	11	0	0
Wasit	DCCs	8	2	25
	NDCCs	11	1	9.09
Thi-Qar	DCCs	9	2	22.22
	NDCCs	10	1	10
Mothana	DCCs	12	8	66.67
	NDCCs	12	3	25
Mesan	DCCs	3	1	33.33
	NDCCs	11	0	0
Basra	DCCs	4	2	50
	NDCCs	11	1	9.09
Total	175		36	20.6

• Classification of the results according to the age of the camel calves: Although the samples numbers in the DCCs of age at >30 days were relatively the lowest than those from NDCCs at the same age category and the rest of the DCCs and NDCCs of age at ≤30 days, the infection rate of RV, was the highest, 51.28%, in the DCCs of age at >30 days than those from NDCCs at the same age category and the rest of the DCCs and NDCCs of age at ≤30 days. Although the samples tested from NDCCs of both age categories were the highest, 50

Table 5: The numbers of samples used in the ELISA plus the positive results in counts and percentages categorized according to the gender of the camel calves.

Infection rate (%)	Positive sample	No. of tested samples	Type of examined calves		Gender of the calves
30	12	40		DCCs	**Male**
3.85	2	52		NDCCs	
31.42	11	35		DCCs	**Female**
4.17	2	48		NDCCs	
15.43	27	172			**Total**

Table 6: The numbers of samples used in the ELISA plus the positive results in counts and percentages categorized according to the age of the camel calves.

Ages (days)	Type of tested calves	No. of tested sample	Positive sample	Infection rate (%)
>30	DCCs	39	20	51.28
	NDCCs	50	4	8
≤30	DCCs	36	3	8.83
	NDCCs	50	0	0
Total	175		27	15.43

samples/each, the infection rates were the lowest, 8% and 0%, respectively, table 6.

• Classification of the results according to the months of the sample collection: For the highest infection rates, the results in March uncovered 80% and 33.33% for both DCCs and NDCCs, respectively. However, April revealed the 2nd highest infection rates, 75% and 12.5%, for both DCCs and NDCCs, respectively. Furthermore, in May, the results showed the 3rd highest infection rate, 40%, for DCCs.

Table 7: The numbers of samples used in the ELISA plus the positive results in counts and percentages (infection rates) categorized according to the months of the year when the samples were collected in.

Months	No. of tested sample		Positive sample	Infection rate (%)
January	DCCs	11	2	18.18
	NDCCs	9	0	0
February	DCCs	11	3	27.27
	NDCCs	8	0	0
March	DCCs	10	8	80
	NDCCs	9	3	33.33
April	DCCs	8	6	75
	NDCCs	8	1	12.5
May	DCCs	10	4	40
	NDCCs	9	0	0
June	DCCs	4	0	0
	NDCCs	8	0	0
July	DCCs	6	0	0
	NDCCs	9	0	0
August	DCCs	4	0	0
	NDCCs	8	0	0
September	DCCs	3	0	0
	NDCCs	8	0	0
October	DCCs	4	0	0
	NDCCs	8	0	0
November	DCCs	1	0	0
	NDCCs	8	0	0
December	DCCs	3	0	0
	NDCCs	8	0	0
Total	175		36	20.57

Table 8: The numbers of samples used in the ELISA plus the positive results in counts and percentages (infection rates) categorized according to the regions that the samples were collected from.

Region	Type of tested Calves	No. of tested sample	Positive sample	Infection rate (%)
Babylon	DCCs	10	3	30
	NDCCs	11	1	9.09
Najaf	DCCs	10	3	30
	NDCCs	9	1	11.11
Karbala	DCCs	9	1	11.11
	NDCCs	13	0	0
Al-Diwaniyah	DCCs	10	1	10
	NDCCs	11	0	0
Wasit	DCCs	8	1	12.5
	NDCCs	11	0	0
Thi-Qar	DCCs	9	2	22.22
	NDCCs	10	0	0
Mothana	DCCs	12	6	50
	NDCCs	12	2	16.67
Mesan	DCCs	3	1	33.33
	NDCCs	11	0	0
Basra	DCCs	4	1	25
	NDCCs	11	0	0
Total		175	23	13.14

• Classification of the results according to the provinces of the sample collection: For the highest infection rates, the results Mothana revealed, 50% and 16.67%, for both DCCs and NDCCs, respectively. Furthermore, in Najaf, the results revealed the 2nd highest infection rates, 30% and 11.11%, for both DCCs and NDCCs, respectively. However, Babylon showed almost the same results as the infection rates obtained from Al-Najaf in which, 30% and 9.09%, for both DCCs and NDCCs, respectively. Furthermore, the infection rate, 33.33%, from the province of Mesan can be the 2nd highest for the DCCs.

Discussion

The results of the OSRRT showed the presence of the RV-antigenic positive reactivity (RAPR) in 36 (20.6%) out of 175 collected fecal samples. These results identified that the RV is actually present and making a camel calf diarrhea in the tested animals and this agrees with Abbas *et al.*, (1992); (Abbas *et al.*, 1993) who reported the same as our observed results regarding the morbidity rate induced by the RV in the camel calves. Infectious agents that affect camel calves leading to diarrhea as a primary health symptom or condition are arranged in the group of viruses especially RVs and coronaviruses. As a primary infectious condition, diarrhea in camel calves may

introduce severe changes to the intestines of these animals (Al-Ruwaili *et al.*, 2012). It has been recognized that camel calf diarrhea were caused mainly by bacterial agents such as *E. coli* and *Proteus* spp (Abubaker *et al.*, 2006; Fouda and Al-Mujalii, 2007). The present work results came a bit higher regarding the incidence rate than that reported by Al-Ruwaili *et al.*, (2012) who detected about 18% of the incidence rate caused by RV in the camel calves tested in Saudi Arabia. Interestingly, serological tests showed that most infections came from RVs that led to the occurrence of diarrhea in those animals in about 31.4% (Ali *et al.*, 2005). The detailed classification according to the gender indicated a mild higher level of detection in males (56.5%) than that revealed in females (43.5%) (Ali, 2005). Our results disagree with Ali *et al.*, (2005) who detected the highest rates in females than that in males from clinically healthy camel calves. However, the current data from sick calves provided higher level of seropositivity of the RV detection in males than that seen females. This completely agrees with Ali, (2003) who revealed the same as our results related to this section.

Some studies revealed that camel calves at ages of older than 30 days of old had higher incidence rates of RV Ali *et al.*, (2005) who reported similar to our findings in which the incidence rates of the camel calf diarrhea caused by RVs were mostly higher in calves aged between 18 to 36 months (Ali *et al.*, 2005). However, Al-Ruwaili *et al.*, (2012) have detected that camel calf diarrhea was mostly observed in ages at day 1 to 3 months of old. The main reason for such differences is that calves under 3 months of old have a less developed immune system than that in older calves making them prone to the disease. For older calves than 3 months of old, recovered calves or claves in contact with infected animals become asymptomatic and many cases could be detected at these ages (Steyer *et al.*, 2008; Hwang and Kim, 2018). According to that immunity of the calves, but not the route of infection or the viral dose, may increase that chances of developing the infection by RV or not (Al-Ruwaili *et al.*, 2012).

These results agree with the fact that the most cases of camel calf diarrhea can be occurred during the end of winter and the early spring as the most cases, in the current study, were recognized in March, April and May but with sequent-descending levels. Those data agree with Muktar *et al.*, (2015) who concluded that camel calf diarrhea can be seen in any time of a year, but it can commonly be noticed during the winter season with emphasis on late winter and the beginning of the spring season in some regions of the world such as North America and this completely matches up our high incidence rates of this health condition during the end of winter and the early of spring, March and April, respectively. During winter, little or no growing of grass in Iraq is documented which indicates why low incidence rates can be seen during this time of the year as these rates may go up higher during the end of winter and early spring due to increases in the grass growing are observed leading to aggregation of animals from different provinces of Iraq especially those with low presence of green pastures into one or two major provinces that provide with these necessities (Elford, 2013; Streicher *et al.*, 2017).

Using ELISA, the results revealed that the RV is actually found and inducing a camel calf diarrhea in the tested animals and this matches up with Schwartz, (1992); (Abbas *et al.*, 1993) who documented the same as our results related to the morbidity rate caused by the RV in the camel calves. Infectious causative agents that influence camel calves encouraging diarrhea as a primary health symptom or condition belong to viral groups especially RVs and coronaviruses. As a primary condition, diarrhea in camel calves may cause extreme alterations in the animal intestines (Al-Ruwaili *et al.*, 2012). It has been observed that camel calf diarrhea were resulted mainly due to bacterial causative agents such as *E. coli* and *Proteus* spp (Abubaker *et al.*, 2006; Fouda and Al-Mujalii, 2007). The present work findings brought higher levels of the incidence rates than those read by Al-Ruwaili *et al.*, (2012) who revealed an incidence rate at ~18% induced by RV in the tested Saudi Arabia camel calves. The study improves the presence of the *rotavirus* in the camel calves in the tested cities from Iraq.

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