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Molecular Epidemiology and Associated Risk Factors for Bovine Rotavirus Infection in Cattle and Buffalo Calves in Different Governorates in Egypt

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Abstract

BOVINE ROTAVIRUS (BRV) remains the most recognized worldwide pathogen causing acute diarrhea in calves under one month of age. The current study was designated to estimate the prevalence of Bovine Rotavirus infections in cattle and buffalo calves in different localities in Egypt under different management systems and study the role of some risk factors associated with BRV infections as well as the role of previously infected animals as reservoirs of infection. Fecal samples of 222 diarrheic calves, 5 recently calved cows and 17 apparently healthy calves previously infected with BRV were screened for the presence of BRV antigen by the Rapid Rotavirus immunochromatographic assay (ICA). The overall prevalence of BRV infection using the ICA was 25 (11.26%). The prevalence of BRV infection was investigated in relation to different epidemiological variables including, the animal species, sex, age, breed, dam parity, areas of study, rearing system, method of colostrum feeding and pre-calving vaccination of dams. Different risk factors were proved to influence the prevalence of BRV infections in the current study. The enzootic situation of BRV infection has been supported and proved in this study in different localities in Egypt. BRV previously infected recovered calves have been proved to remain as carriers and reservoirs leading to maintenance of BRV infection in the herd. Rotavirus double-stranded RNA was extracted from the 25 fecal samples and subjected for one-step RT-PCR targeting the NSP5 gene, and two-step RT-PCR targeting the partial length VP4 gene. The presence of the viral genome was confirmed by amplification of NSP5 gene and VP4 gene with 155 bp and 856 bp expected product size, respectively. Partial nucleotide sequencing of VP4 gene and P-typing indicated that VP4 gene of BRV belongs to the P[11] genotypes.

Keywords: Bovine Rotavirus, Carriers, Egypt, Immunochromatographic assay, VP4 gene.

Introduction

Bovine Rotavirus (BRV) is a major cause of calf diarrhea worldwide [1,2], with a prevalence ranging from 7% to 94% worldwide, as reported previously[3]. It is the most recognized pathogen causing acute diarrhea in calves under one month of age worldwide [4].

BRV belongs to the genus Rotavirus within the family Reoviridae. Rotaviruses are icosahedral and nonenveloped, with 32 capsomers and 11 segments

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of ds RNA (16–21 kbp), and are well protected by an inner and outer capsid layer, giving a wheel-like appearance to the virion [5–7]. The genome encodes six structural proteins (VP1–VP4, VP6, and VP7) and six nonstructural proteins (NSP1–NSP5/6)[8]. The segmented nature of this virus favors genetic reassortment and the generation of new strains that may differ in pathogenicity, virulence, and interspecies transmission [9].

Diarrhea induced by BRV is usually mild to severe [10]. The clinical signs include depression, anorexia, mild fever, and diarrhea. Diarrheic feces vary from liquid to pasty, often yellowish mucoid and occasionally bloody [11,12]. The infectious cycle of BRV infection clarifies that the virus targets enterocytes, causing specific lesions in the intestines and mesenteric lymph nodes and resulting in morbidity and mortality in calves younger than three weeks of age [13].

Continuous monitoring of emerging and re-emerging BRV strains is essential for a better understanding of the viral ecology within a region and to improve the vaccination programs implemented by updating vaccine strains.

The present study generally addresses calf diarrhea, with the principal aim of determining the role of diarrhea caused by BRV infection through estimation of the prevalence of BRV infection in cattle and buffalo calves in different localities in Egypt under different management systems, studying the role of some risk factors associated with BRV infection and the role of BRV previously infected carriers as an important component of the infection chain of the disease, genotyping of BRV circulating serotypes detected in this study and their correlation with the serotypes included in the employed vaccines.

Material and Methods

Study area and animals

A total of 222 calves at different localities in Beni Suef, El-Behira, and Alexandria governorates belonging to 6 herds as well as individually reared calves were clinically and epidemiologically examined for BRV infection from October (2021) to March (2023). In addition, 22 apparently healthy animals, including five apparently healthy recently calved cows and 17 previously BRV-diseased calves, were targeted for studying the carrier state of BRV infection (Table 1).

Inclusion criteria employed in the selection of animals

a. Diarrheic calves (n=222) aged between birth and 45 days of age that were clinically affected by diarrhea and exhibited systemic disturbances were included in this study.

b. A total of 22 apparently healthy animals, including five apparently healthy recently calved cows and 17 previously BRV-infected calves, 2 months after their clinical recovery were the target for investigation of the carrier state of BRV infection.

Calves were examined for their suckling reflex, septicemia, degree of dehydration and body temperature. The consistency of the feces, odor, color, and presence or absence of mucus and blood in the feces were checked according to methods described previously [14].

Rapid detection kit used for detection of BRV infection.

Fecal samples from 222 diarrheic calves as well as 5 recently calved cows and 17 apparently healthy calves previously infected with BRV were screened for the presence of BRV antigen by immunochromatographic assay (ICA) (Haicang, Xiamen, China) following the manufacturer's instructions.

Molecular detection and characterization of BRV infection in calves via polymerase chain reaction (PCR)

Fecal samples were diluted with 10% sterile phosphate-buffered saline (PBS, pH 7.2) and used for the extraction of viral RNA. Total RNA was extracted via a GeneJET Viral RNA Purification Kit (Thermo Scientific, Lithuania catalogue no. k0821).

One-step RT–PCR targeting the NSP5 gene was performed via a Verso 1-step RT–PCR kit (Thermo Fisher, Lithuania) to confirm the presence of BRV. The primers used for amplifying the NSP5 gene BRV A were F-5'-GAT ATT GGA CCA TCT GAT TCT GCT TCA AA -3' and R-5'-GAA ATC CAC TTG ATC GCA CCC AA-3'[15].

Two-step RT–PCR targeting the partial VP4 gene was performed. cDNA synthesis was performed with a high-capacity cDNA reverse transcription kit (Thermo Fisher Lithuania catalogue no. 4374966). P typing was performed through the amplification of the partial-length VP4 gene (856 bp) via the following primers: F-5'-TTCATTATTGGGACGATTCACA and R-5'-CAACCGCAGCGGATATATCATC-3[4] and PCR Master Mix (Applied Biotechnology, Egypt). The cycling conditions for one- and two-step RT–PCR are shown in Table (2).

Gene sequencing and sequence analysis

The PCR products were purified via a PCR Purification Kit® (Thermo) following the manufacturer's instructions. Sequencing was performed by Wuhan Hecegene Technology Co., Ltd., Wuhan, China, in both the forward and reverse directions via the same primer sets that were used for amplification of each gene. A BLAST search

(http://www.ncbi.nlm.nih.gov/BLAST) was conducted for sequences. Phylogenetic analyses and sequence alignments were conducted via MEGA X software [16]. A tree was created via the maximum likelihood method with 1,000 bootstrapped data sets. The Kimura 2-parameter was used as a model, and the tree was obtained initially via the neighbor-joining and BioNJ algorithms [17]. The maximum composite likelihood (MCL) was estimated as a matrix of pairwise distances. Nucleotide and amino acid identities were determined via Geneious® 7.1.3, Build 2014-03-17, Java Version 1.7, Copyright © 2005-2014 Biomatters Ltd.

Statistical analysis

The data were presented as the mean ± standard deviation (SD) and analyzed using GraphPad Prism software. The F test was performed to determine whether there were significant differences among the groups. P-values less than 0.05 were considered statistically significant.

Results

In the present study, a total of 222 diarrheic cattle and buffalo calves aged between birth to 45 days of age at different localities in Beni Suef, El-Behira, Alexandria and Al-Faioum governorates and under different epizootiological situations were examined for BRV infection. Prevalence of BRV infection was estimated by the ICA in relation to different epidemiological variables including, the animal species, sex, age, breed, dam parity, the study area, rearing system, method of feeding of colostrum and pre-calving vaccination of dams against enteric pathogens. The overall prevalence of BRV infection revealed 25 (11.26%), (Table 3).

Clinical abnormalities observed in the 25 diarrheic positive ICA calves are shown in (Fig.1, Table 4).

The presence of the viral genome was confirmed by amplification of NSP5 gene with 155 bp expected product size, (Fig. 2a). The amplification of cDNA was performed using a primer sequence that flanked a region of 856 bp within the VP4 gene confirming the presence of BRV (Fig. 2b). Prevalence of BRV - PCR positive diarrheic calves in relation to different epidemiological variables are shown in Table (3)

Detection of the carrier state of BRV infection in cows and calves using the ICA and PCR are shown in Table (5)

Partial nucleotide sequencing of VP4 gene was carried out. The generated sequence was submitted to GenBank (BRV A/ Egy-BSU/2023) (OR992003). Blast analysis of sequenced PCR products (partial length VP4 gene segment) proved that PCR bands visualized in expected locations of agar gel electrophoresis belonged to BRVA and P-typing indicated that VP4 gene of BRV belongs to the P[11] genotypes (Fig 3, 4).

Discussion

BRV diagnosis, as one of the important causes of acute undifferentiated diarrhea, requires laboratory testing to confirm the clinical suspicion. In the present study, a total of 222 cattle and buffalo diarrheic calves aged between birth and 45 days of age at different localities in Beni Suef, El-Behira, and Alexandria governorates belonging to 6 herds as well as individually reared calves were tested via rotavirus ICA. The overall prevalence of Rotavirus via the ICA was 25 (11.26%).

Concerning species susceptibility, the obtained results revealed that BRV infection was diagnosed in both cattle (12.22%) and buffalo calves (7.14%) with no significant difference at p- value 0.355, although a mathematically higher percentage of BRV infection was detected in cattle calves than buffalo calves. Similar results were reported in Assiut governorate [18]. Concerning sex as a risk factor for BRV infection, the obtained results revealed that BRV infection was diagnosed in both males 16 (13.22%) and females 9 (8.91%) with no significant difference at p-value 0.314, although a mathematically higher percentage of BRV infection was detected in males than females. Reviewing the literature, there was no significant difference in the rate of BRV infection between male and female calves statistically indicating a non-sex-linked disease as reported [19].

Concerning the age of newborn calf as a risk factor for susceptibility to infection by BRV the results obtained in this study revealed that BRV infection was diagnosed in 18 (14.63%) calves from birth up to 15 days, 7 (9.33%) in calves of 16-30 days and 0 (0.00 %) in calves of 31-45 days. It is obviously clear that the majority of cases of BRV infection were diagnosed in the first two weeks followed by the 3rd and 4th weeks with non-significant differences. The results obtained in the current study are supported by those of Vega et al., (2011) who reported that BRV diarrhea affects calves between 7-21 days of age, but the chances of acquiring infection decrease with increase of age [20]. Higher prevalence of BRV was recorded at 8-15 days of age with a rate of (12.31%). Such high susceptibility in the early stage of life may be attributed to the immature immune system of newborn calves which require 30 days to perform as adults as explained by Hosein [21]. In addition, such susceptibility may be explained on the bases that milk uptake by newborn calves provide a good environment for BRV survival under a wide range of gastrointestinal pH levels

Regarding the susceptibility of calves in different breeds as a risk factor, significant difference at p-value 0.03 was recorded in the percentage of BRV infection between native 12 (8%) and foreign breeds

13 (18.06%) with mathematically foreign breeds had higher rate than Native breed. This finding disagreed with that previously concluded by Youssef and Zaitoun [12] who found a non-significant difference in the prevalence of BRV infection between different breeds of calves. The breed of the animal is an important host determinant that influences the immune response and disease severity as reported and explained [22]. Generally, calf diseases are reported to be significantly higher in crossbred than native animals [23]. The higher percentages of cross breed to viral infection may be due to differences in digestive efficiency of absorption of antibodies in colostrum between different breeds besides differences in the amounts of digestive enzymes [18].

Prevalence of BRV infection in relation to dam parity in this study revealed the highest non-significant prevalence rate 16 (16.33%) of BRV infection of dams of the first calving. This was in comparison with 5 (13.89%), 2 (6.67%), 1 (4.55%) and 1 (2.78%) of the 1st, 2nd, 3rd, 4th and 5th calving, respectively. This indicates the higher susceptibility of calves of the first calved dams. Morrill et al., [24] found that Parity numbers influence the colostrum composition and increasing parity number increases the IgG concentration. In addition, Gulliksen et al., [25] reported that older cows are exposed to pathogens for a longer time during their life than younger cows, producing colostrum with higher antibody levels.

Distribution and prevalence of BRV-infected calves in different localities investigated in this study as estimated by Rotavirus ICA revealed 14 (8.81%), 11(19.3%) and 0 (0.00%) in Beni Suef, El-Behira and Alexandria governorates respectively. BRV infection in Assiut governorate was estimated as 14.60 [12] and 29.8 % [26]. Variations in the prevalence of BRV infection in calves in different studies may be due to geographical variation, difference in hygienic measures, environmental conditions, management system involving the pregnant dams and newborn calves and difference in age groups subjected for investigation and the tests employed for detection of infection on the levels of both sensitivity and specificity. Prevalence of BRV infection in relation to rearing system revealed 11(17.46%), 4 (28.57%) and 10 (6.9%) for calves reared in individual boxes, calf rearing units and calves mixed with other animals respectively. Calf rearing units showed the highest prevalence of BRV infection compared with calves mixed with other animals that was significant at p-value 0.001. Such unexpected finding may be attributed to enzootic nature and the presence of carriers as well as environmental contamination in the investigated localities under the different systems of management.

Prevalence of BRV diarrhea in relation to the method of colostrum feeding, revealed 10 (6.9%) and 15 (19.48%) in calves fed colostrum naturally and calves fed artificially respectively, Natural colostrum suckling revealed significant lower percentage of infection at p-value 0.007. This signifies the importance of natural suckling. First-milking colostrum is an important source of nutrients and passively maternal immunoglobulins, essential to safeguard the newborn calf against infection in the first few days of life and their after. The calf is born agammaglobulinemic, including those directed and specific against enteropathogens causing diarrhea. The calf will acquire these antibodies only from colostrum [27].

In this study, the prevalence of BRV infected diarrheic calves derived from vaccinated dams was 15 (19.48%) while of those derived from non-vaccinated calves was 10 (6.9%). This could be attributed to the use of BRV strains that are strange from the virus circulating serotypes in the area of investigation. The inactivated vaccines used against BRV infection must cover the prevailing strains in the area. The calf immunity is directed against the specific vaccine's strain. Unfortunately infection may happen by different circulating strains as reported by [28]. Several researches have verified vaccine failure due to inadequate managing conditions of calves and pregnant dams or may be due to antigenic differences between vaccine and circulating BRV strains, even if the employed vaccine and the circulating strains shared partially their surface antigen specificities. Both BRV infection and immunization elicits nonspecific and acquired humoral and cellular immune responses [29]. This clarifies the importance of immunization of pregnant dams shortly before delivery, to enhance protective levels of antibody in colostrum.

Interestingly, some researchers reported little or no increase in protection against BRV infection among calves suckling BRV-immunized cows [30]. Therefore, continuous checking of emerging bovine BRV strains is an essential operation that should be carried out continuously for a better understanding of the viral ecology within a locality and to update the vaccine strains.

Clinical abnormalities observed in Rotavirus ICA positive diarrheic calves in this study, revealed that the body temperature did not exceed 40°c which is identical to most cases of uncomplicated viral infections. Usually BRV infection is a non-febrile disease, unless complicated by secondary infections [31]. Out of the 25 infected calves, 17 (68%) showed negative suckling reflex that revealed significant highest prevalence of BRV infection at p-value at 0.007. Negative suckling reflex is an indication of high susceptibility to enteric infection as these calves obtained no sufficient protective colostrum. Therefore, the crucial plan established for prevention and control of BRV infections includes enhancing the immunity of newborn calves through dam immunization and reducing the exposure of calves to infection through strict hygienic measures and adoption of good management system [26]. Severe

dehydration was observed in 11 (44%), this refers to loss of body fluids through diarrhea. Diarrhea in calves occurs due to interference of absorptive surface function of small intestine as reported by [20,32]. Affected calves require immediate fluid therapy and electrolytes to combat electrolyte imbalance, electrolyte imbalance and metabolic acidosis.

Clinical examination of calves revealed different forms of diarrhea, including watery, yellow mucoid and bloody diarrhea, at rates of 9 (36%), 14 (56%) and 2 (8%), respectively. Diarrhea is associated with different degrees of dehydration, which may be attributed to the loss of body fluids. Watery to pasty yellowish diarrhea followed by dehydration were the prominent clinical findings reported previously by several authors [11,12,13]. These findings may be attributed to the different etiological agents and coinfections involved in each case and secondary infections. Diarrhea and dehydration can be explained on the basis of the pathophysiological changes in the intestinal tracts of BRV-infected calves, in which BRV can escape unaffected by the acidic pH of the stomach and digestive enzymes in the gut as a result of the presence of the triple protein coat possessed by the virus. BRV invades surface epithelial cells of the villi of the small intestine, inducing stunts and exfoliation of the villi of the small intestine, resulting in a reduction in absorption capacity and in the secretion of digestive enzymes, resulting in profuse viscous fluid containing undigested and unabsorbed nutrients in the intestinal lumen [12].

In this study, amplification of BRV RNA from 25 positive rapid rotavirus test (ICA) fecal samples from diarrheic calves was carried out. Rotavirus double-stranded RNA (dsRNA) was extracted from 25 fecal samples and subjected to one-step RT–PCR targeting the NSP5 gene and two-step RT–PCR targeting the partial-length VP4 gene. The amplification of cDNA was performed via a primer sequence that flanked a region of 856 bp within the VP4 gene, confirming the presence of BRV.

Prevalence of BRV -PCR positive diarrheic calves revealed 14 (56%), including 11 (50%) in cattle calves and 3 (100%) in buffalo calves. These also included 9 (50%) in calves of 1 to 15 days, 5 (71.43%) in calves of 16 to 30 days and 0 (0%) in calves of 31 to 45 days. Nine males (56%) in and 5 females (55.56%) were detected. Among PCR positive cases, 7 (58.33%) native cross breed and 7 (53.85%) native breeds were detected. Nine (60%) calves derived from vaccinated dams and 5 (50%) calves derived from non-vaccinated dams. Eight (72.73%) calves reared in individual boxes, one (25%) calf reared in calf rearing unit and 5 (50%) calves reared mixed with other animals were PCR positive. Interestingly, the association between the prevalence estimated by the ICA and those estimated by the ICA and the different risk factors followed the same pattern.

The PCR technique is considered a sensitive and rapid genomic detection tool that can be used to detect the genomic RNA extracted from fecal samples by obtaining cDNA after reverse transcription with reverse transcriptase, followed by PCR amplification via specific gene primers. The sensitivity of these molecular methods is approximately two- to threefold greater than that of other diagnostic assays [33].

In the current study a total of 22 apparently healthy animals from different localities including 5 apparently healthy recently calved cows and 17 apparently healthy calves, 2 months post their recovery from severe diarrhea due to previous BRV infection in previously infected herds were selected. Fecal samples were collected from these animals and subjected to the Rotavirus ICA for detection of BRV infection. Fecal samples of the 17 calves derived from vaccinated dams were processed in 6 pooled samples revealed 2 (33.33%) and 6 (100 %) positive result for Rotavirus ICA and PCR respectively. Fecal samples of the 5 recently calved cows were processed in one pool that revealed 0 (0 %) and 1 (100%) positive result for Rotavirus ICA and PCR respectively.

To explain the results of the carrier state obtained in this study, adult cattle have been considered BRV reservoirs by other researchers [34]. Transmission from the environment to susceptible calves seems more likely. Adults and previously infected recovered calves remain important sources of infection, leading to maintenance of infection in the herd by the same circulating viruses. In addition, a researcher reported that serological examinations revealed that 10.27% of calves 9–12 weeks of age harboured BRV in their feces [12]. From an epidemiological point of view, the results of this study highlight the maintenance mechanisms of BRV infection in newborn calves in the investigated areas. This can be explained by the ability of the virus to persist on cattle farms in the form of its survival in the environment as well as the presence of previously infected calves as carriers for Rotaviruses. This signifies that the characters of the infection chain of BRV infection is influenced by both the reservoiring process in previously infected calves as well as the prolonged survival of the virus in the environment.

Nucleotide sequencing, comparative sequence and phylogenetic analysis were carried out in the current study for P typing of BRV strains to investigate the potential antigenic disparities between the circulating virus strain serotypes in the areas of the study and those present in the employed vaccines. Partial nucleotide sequencing of the VP4 gene was carried out. The generated sequence was submitted to GenBank (Bovine Rotavirus A/Egy-BSU/2023) (OR992003). Blast analysis revealed that the PCR

bands visualized at the expected locations via agar gel electrophoresis belonged to BRVA, and P typing indicated that the VP4 gene of rotavirus belongs to the P[11] genotype.

Interestingly, the P genotype profiles of BRV field strains circulating in the area under investigation indicated that P[11] was the dominant P type, which completely differed from the employed vaccines (Rota-vec ® corona (G6P5), KOLI-BIN ® and ROTAGAL® (G6P1)). These results explain the significantly greater increase in the prevalence of infection, with a p-value of 0.007, observed in calves derived from vaccinated dams and clarify the failure of the commercial vaccines employed in this study to protect calves through passive immunity after colostrum intake.

Sequencing analyses of the VP4 gene revealed that the sequence obtained in the present study (OR992003) is closely related to the Egyptian BRV A sequence available in the database and shares 98.7% nucleotide and 100% amino acid identities with MW751823.1Egypt 2021 and MW751826.1/Egypt 2021. However, the nucleotide and amino acid identities were 98.5% and 99.2%, respectively, with those of MK961092.1/Egypt_2017 due to the presence of unique residues at positions aa 40-V and 235-G. Compared with those of the other bovine rotavirus A strains isolated from different geographical areas, the nucleotide identities ranged from 94.1 to 95.7%, and the amino acid (AA) identities ranged from 96.6% to 98.9%.

To represent the evolutionary relationships among sequence obtained in the current study (OR992003) and available BRV sequence in the database, a VP4-based phylogenetic tree was generated using the neighbor-joining method on nucleic acid sequences. The tree showed two tight genetic clusters. Sequence obtained in the current study (OR992003) was clustered in one with other Egyptian strains available in the database (MW751823.1Egypt/2021, MW751826.1/ Egypt/ 2021, and MK961092.1/Egypt/2017). The other Bovine Rotavirus_A isolated from different geographical area (LC133550.1 USA 1983, MZ913004.1 Turkey 2008, LC591088.1 Japan 2020, MW771108.1 South Africa 2012, OP169135.1 China 2021, JX470491.1 Canada 2009, EU311200.1 India 2007, OL988983.1 Ireland 2013, MW771163.1 Mozambique 2016 MK376895.1 Bangladesh 2016) showed less relationship with our strain and placed in a separate cluster.

Conclusion

The enzootic situation of BRV infection has been supported and proved in this study in different localities in Egypt. Continuous checking of emerging BRV strains is an essential operation for a better understanding of the viral ecology all over the country and to update the vaccine strains. Different important risk factors were found to influence the prevalence of BRV infections. BRV previously infected recovered calves have been proved to remain as carriers and reservoirs leading to maintenance of BRV infection in the herd. P genotype profiles of BRV field strains circulating in the area under investigation indicated that P [11] was the dominant P type which is completely differ from the serotypes present in the employed vaccines. The sequence obtained in the current study (OR992003) is closely related to the Egyptian Bovine Rotavirus A available in database.

Author contributions

All authors contributed in creating this article and approved the final manuscript.

Conflict of interest statement

Authors declare no conflict of interest

Funding statement

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Ethical of approval

The study protocol was approved by the Animal Research Ethics Committee of the Faculty of Veterinary Medicine, Beni-Suef University, with a letter for the approval and credibility of work No. 022--403, in accordance with the international guidelines for animal research.

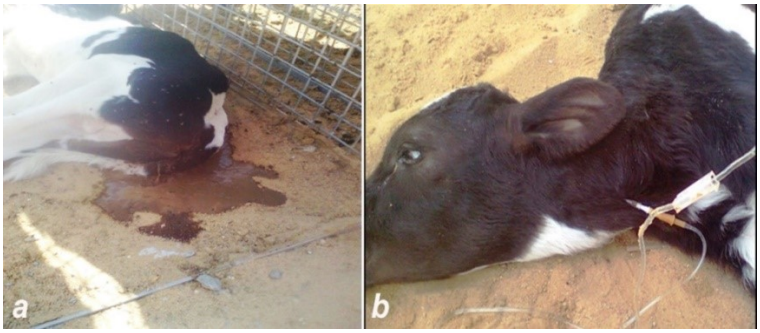

Fig.1. Clinical abnormalities observed in diarrheic calves

a. A calf showing bloody diarrhea
b. A calf suffering from severe dehydration with sunken eyes

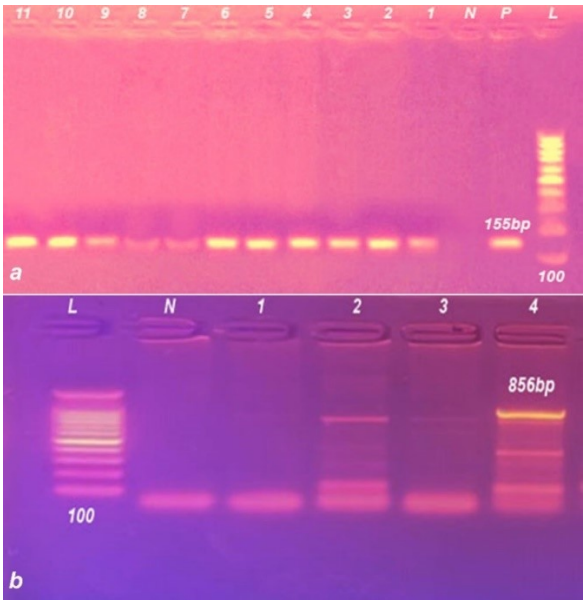

Fig. 2. Results of Rotavirus RT-PCR

a. Amplification of target gene NSP5 flanked the 155 bp. by one-step RT-PCR; Lane L: 100bpDNA ladder, Lane P: positive control, Lane N: negative control, Lanes 1: 11: Fecal samples.

b. Amplification of target gene VP4 gene flanked the 856 bp. by Two-step RT-PCR; Lane L: 100bpDNA ladder, Lane N: negative control, Lanes 1- 4 fecal samples.

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LC133550.1USA_1983                NMVTVRSLNA ELNQVRCEGG HYSFALPVGS WPVMQGGSVI LTFDGVTLST QFTDIYVSLN5 LRFRFRCAVS EPSFRVTGTR ISNLIGLPAA  [ 90]
MZ913004.1Turkey_2008             .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [ 90]
OR992003 EGY/BSU/2023             .......... .......... .......... .........V .......... .......... .......... .......... .......... ..........  [ 90]
MW751823.1Egypt_2021              .......... .......... .......... .........V .......... .......... .......... .......... .......... ..........  [ 90]
MW751826.1Egypt_2021              .......... .......... .......... .........V .......... .......... .......... .......... .......... ..........  [ 90]
MK961092.1Egypt_2017              ----------.......... .......... .......... .......... .......... .......... .......... .......... ..........  [ 90]
LC591088.1Japan_2020              .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [ 90]
MW771108.1South_Africa_2012       .......... .......... .......... .......... .......... .......... .......... .......... ......A... ..........  [ 90]
OP169135.1China_2021              .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [ 90]
JX470491.1Canada_2009             .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  [ 90]
EU311200.1India_2007              .......... .......... .......... .......... .....I.... .......... .......... .......... .......... ..........  [ 90]
OL988983.1Ireland_2013            .......... .......... .......... .......... .........N VS..T.T... .......... .......... .......... ..........  [ 90]
MW771163.1Mozambique_2016         .......... .......... .......... .......... .....I.... .......... .......... .......... .......S.. ..........  [ 90]
MK376895.1Bangladesh_2016         .......... .......... .......... .......... .....I.... .......... .......... .......... .......... ..........  [ 90]

LC133550.1USA_1983                NEMGDQQYYE AAGRFSLILL VPSNDDYQTP IANSVTVRQD LERQLDEMRR EFNELSANIA LSQLIDLALL FLDMFSMFSG IQSTVEAAKS  [180]
MZ913004.1Turkey_2008             .......... ........S. .......... .......... .......... .......... .......... .......... .......... ..........  [180]
OR992003 EGY/BSU/2023             .......... ........S. .......... .......... .........K .......... .......... .......... .......... ..........  [180]
MW751823.1Egypt_2021              .......... .--------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------  [180]
MW751826.1Egypt_2021              .......... .--------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------  [180]
MK961092.1Egypt_2017              .......... ........S. .......... .......... .........K .......... .......... .......... .......... ..........  [180]
LC591088.1Japan_2020              .......... ........S. .......... .......... .......... .......... .......... .......... .......... ..........  [180]
MW771108.1South_Africa_2012       .......... ........S. .......... .......... .......... .......... .......... .......... .......... ..........  [180]
OP169135.1China_2021              .......... ........S. .......... .......... .........K .......... .......... .......... .......... ..........  [180]
JX470491.1Canada_2009             .......... ........S. .......... .......... .......... .......... .......... .......... .......... ..........  [180]
EU311200.1India_2007              .......... ........S. .......... .......... .......... .......... .......... .......... .......... ..........  [180]
OL988983.1_Ireland_2013           .......... ........S. .......... .......... .......... .......... .......... .......... .......... ..........  [180]
MW771163.1Mozambique_2016         .......... ........S. .......... .......... .......... .......... .......... .......... .......... ..........  [180]
MK376895.1Bangladesh_2016         .......... ........S. .......... .......... .......... .......... .......... .......... .......... ..........  [180]

LC133550.1USA_1983                FATSVMKKFR KSDLAKSVNS LTDAITDAAG SISRSSTLRS VNSAASVWTD ISDIVDSTDN VVAATATAAA KKFKVKEFTT EFNGVSFD [268]
MZ913004.1Turkey_2008             .......... .......... .......... .......... .......... .......... .......... .......... .......... ........  [268]
OR992003 EGY/BSU/2023             .......... .......... .......... .......... .......... ...VG..... .......... .......... .......... ........  [268]
MW751823.1Egypt_2021              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------  [268]
MW751826.1Egypt_2021              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------  [268]
MK961092.1Egypt_2017              .......... .......... .......... .......... .......... ...V...... .......... ....------ -------- --------  [268]
LC591088.1Japan_2020              .......... ..N....... .......... .......... .......... .......... .......... .......... .......... ........  [268]
MW771108.1South_Africa_2012       .......... .......... .......... .......... .......... .......... .......... .......... R......... ........  [268]
OP169135.1China_2021              .......... .......... .......... .......... .......... ...V..T... .......... .......... .......... ........  [268]
JX470491.1Canada_2009             .......... .......... .......... .......... ...T...... .......... ..S....... .......... .......... ........  [268]
EU311200.1India_2007              .......... .......... .......... .......... .......... .......... .......... .......... .......... ........  [268]
OL988983.1Ireland_2013            .......... .......... .......... .......... .......... .......... .......... .......... .......... ........  [268]
MW771163.1Mozambique_2016         .......... .......... .......... .......... .......... .......... .......... .......... .......... ........  [268]
MK376895.1Bangladesh_2016         .......... .......... .......... .......... .......... .......... .......... .......... .......... ........  [268]
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Fig. 3. Deduced amino acid sequence of outer capsid protein VP4 gene of Bovine Rotavirus_A. Dots indicate identical amino acid

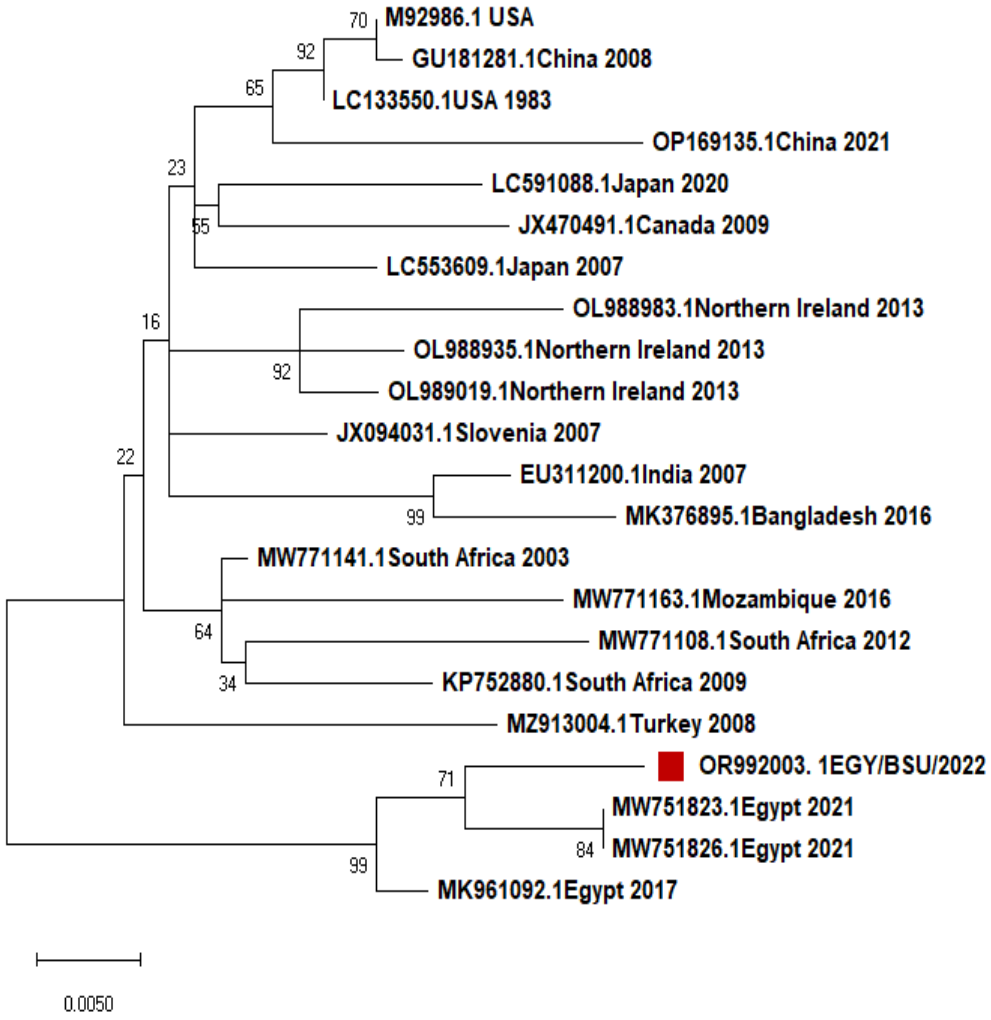

Fig. 4. Phylogenetic analysis of outer capsid protein VP4 gene of Bovine Rotavirus A; The tree was generated using MEGA X program by the neighbor-joining analysis. Bootstrap confidence values were calculated on 1000 replicates according to the maximum likelihood approach. Sequences obtained in this study are labeled red.

TABLE 1. Investigated herds in different localities.

Animal population	Locality	No. of diarrheic sampled calves
Herd 1	El-Nobaria (El-Behira)	24 Cattle Calves
Herd 2	El-Nobaria (El-Behira)	5 Cattle Calves
Herd 3	Abo-El-matamer (El-Behira)	6 Buffalo Calves 22 Cattle Calves
Herd 4	Al-Ameria (Alexandria)	6 Cattle Calves
Herd 5	Beni Suef governorate	35 Buffalo Calves 5 Cattle Calves
Herd 6	Beni Suef governorate	14 Cattle Calves
Individual calves	Beni Suef governorate	1 Buffalo Calves 104 Cattle Calves
Total		180 cattle calves 42 buffalo calves **222**

TABLE 2. Cycling condition for RT-PCR

Cycling condition for One Step RT-PCR							
Gene	**Reverse transcription**	**Primary Denaturation**	**Secondary Denaturation**	**Annealing**	**Extension**	**Final Extension**	**No. of cycles**
NSP5	**50˚C 30 min.**	**95˚C 5 min.**	**94˚C 30 sec.**	**60˚C 30 sec.**	**72˚C 30 sec.**	**72˚C 7 min.**	**35**
Cycling condition for VP4							
VP4 gene		**95˚C 5 min.** **95˚C 5 min.**	**95˚C 45 sec.** **95˚C 45 sec.**	**46˚C 45 sec.** **46˚C 45 sec.**	**72˚C 60 sec.** **72˚C 60 sec.**	**72˚C 7 min.** **72˚C 7 min.**	**35**

TABLE 3. Prevalence of Rotavirus infection using the Rotavirus ICA and PCR in relation to different epidemiological variables

Parameter	**Animals (222)**	**Positive ICA (n=25) (11.26%)**	**PCR positive samples (n=14) (56%)**
Species	Cattle calves (180)	22 (12.22%)	11 (50%)
	Buffalo calves (42)	3 (7.14%)	3 (100%)
Age	Birth to 15 days (123)	18 (14.63%)	9 (50%)
	16 to30 days (75)	7 (9.33%)	5 (71.43%)
	31to 45 days (24)	0 (0)	0 (0%)
Sex	Male (121)	16 (13.22%)	9 (56%)
	Females (101)	9 (8.91%)	5 (55.56%)
Breed*	Native cross breed (150)	12 (8%)	7 (58.33%)
	Foreign breed (72%)	13 (18.06%)	7 (53.85%)
vaccination status of dams*	Vaccinated (77)	15 (19.48%)	9 (60%)
	Non vaccinated (145)	10 (6.9%)	5 (50%)
Rearing system*	Individual boxes (63)	11 (17.46%)	8 (72.73%)
	Calf rearing unit (14)	4 (28.57%)	1 (25%)
	Mixed with other animals (145)	10 (6.9%)	5 (50%)
Area of study	Beni Suef (159)	14(8.81%)	6 (42.86%)
	El-Behira (57)	11 (19.3%)	8 (72.73%)
	Alexandria (6)	0 (0)	0 (0)
Method of colostrum feeding*	Natural (145)	10 (6.9%)	4 (40%)
	Artificial (77)	15 (19.48%)	10 (66.67%)
Dam party	1st (98)	16 (16.33%)	10 (62.25%)
	2nd (36)	5 (13.89%)	2 (40%)
	3rd (30)	2 (6.67%)	1 (50%)
	4th (22)	1 (4.55%)	0 (0)
	5th (36)	1 (2.78%)	1 (100%)

*Statistically significant

TABLE 4.Clinical abnormalities observed in Rotavirus ICA positive diarrheic calves.

Signs	Finding	Number of positive calves (n=25)	PCR (n=14)
Body Temperature °c	≤ 37 (10)	0 (0%)	0 (0)
	37.1 to 38 (18)	2 (8%)	2 (100%)
	38.1 to 39 (78)	12 (48%)	7 (58%)
	39.1 to 40 (104)	11 (44%)	5 (45.45%)
	> 40 (12)	0 (0%)	0
Suckling ability*	Positive (96)	4 (16%)	4 (100%)
	Relative (41)	4 (16%)	4 (100%)
	Negative (85)	17 (68%)*	6 (35.29%)
Dehydration	Normal (73)	1 (4%)	1 (100%)
	Mild (34)	4 (16%)	2 (50%)
	Moderate (41)	9 (36%)	3 (33.33%)
	Severe (74)	11 (44%)	8 (72.73%)
Attitude	Standing (143)	12 (48%)	6 (50%)
	Recumbent (79)	13 (52%)	8 (61.54%)
Condition of feces	Watery (90)	9 (36 %)	2 (33.33%)
	Mucoid (123)	14(56%)	11 (78.57%)
	Bloody (9)	2 (8%)	1 (50%)
Total		25	14

*Statistically significant

TABLE 5. Results of detection of the carrier status of Bovine Rotavirus.

Parameter	N. of examined animals	Positive BRV(ICA)	Positive PCR
Calves born to vaccinated dams	17 6 pooled samples	2 (33.33%)	6 (100%)
Calves born to non-vaccinated dams	0	0(0%)	(0%)
Recently calved cows	5 1 pooled sample	0(0%)	1 (100%)

References

1. Greenberg, H.B. and Estes, M.K. Rotaviruses: from pathogenesis to vaccination. *Gastroenterology*, **136**(6), 1939-1951 (2009). doi:10.1053/j.gastro.2009.02.076
2. Rocha, T.G., Silva, F.D., Gregori, F., Alfieri, A.A., Buzinaro, M. and Fagliari, J.J. Longitudinal study of bovine rotavirus group A in newborn calves from vaccinated and unvaccinated dairy herds. *Trop. Anim. Health Prod.*, **49**(4), 783-790(2017). doi:10.1007/s11250-017-1263-2
3. Alfieri, A., Parazzi, M.E., Takiuchi, E., Medici, K. and Alfieri, A.F. "Frequency of group a Rotavirus in diarrhoeic calves in Brazilian cattle herds, 1998–2002," *Trop. Anim. Health Prod*., **38**, 521(2006).
4. Falcone, E., Tarantino, M., Di Trani, L., Cordioli, P., Lavazza, A. and Tollis, M. Determination of bovine rotavirus G and P serotypes in Italy by PCR. *J. Clin. Microbiol.,* **37**(12), 3879-3882(1999). doi:10.1128/JCM.37.12.3879-3882.1999
5. Estes, M.K., and Kapikian, A.Z. Rotaviruses.In Fields Virology. Edited by Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE. Philadelphia: Lippincott, Williams & Wilkins. *5th ed,* 1917–1974(2007).
6. Desselberger, U. Rotaviruses. *Virus Res*. **190**,75-96 (2014). doi:10.1016/j.virusres.2014.06.016
7. Ghonaim, A.H., Hopo, M.G., Ghonaim, N.H., Jiang, Y., He, Q. and Li, W. The Epidemiology of Circulating Rotavirus Associated with Diarrhea in Egyptian Kids and Calves: A Review. *Zoonoses.* **3**(1),0004 (2023). doi:10.15212/zoonoses-2023-0004
8. Steele, D., Geyer, A. and Gerdes, G.H. "BRV infections," in Infectious Diseases of Livestock, *Oxford University Press, Oxford, UK,* 1256–1264(2004).

9. Crawford, S.E., Ramani, S., Tate, J.E., Parashar, U.D., Svensson, L., Hagbom, M., Franco, M.A., Greenberg, H., O'Ryan, M. and Kang, G. Rotavirus infection. *Nat Rev Dis Prim.,* **3**, 17083 (2017). doi:10.1038/nrdp.2017.83

10. Kassem, I.K., Magouz, A., Desouky, A.Y. and Hagag, M.F. Isolation and Identification of Rotavirus Infection in Diarrheic Calves at El Gharbia Governorate. In: (2017). *https://api.semanticscholar.org/CorpusID:251839904*

11. Abdel-Rady, A., Ma, A. and Youssef, Z. Clinical Study with Rapid Serological Detection of Rotavirus Infection in Diarrheic Neonatal Calves. *Acta Sci. Vet. Sci.,* **4**, 2582-3183(2022). doi:10.31080/ASVS.2022.04.0304

12. Youssef, Z. and Zaitoun, A. Epidemiological Study of Rotavirus Infection in the Diarrheic Neonatal Calves. *Acta Sci. Vet. Sci.,* **4**(3), 34-39 (2022). doi:10.31080/asvs.2022.04.0327

13. Barua, S.R., Rakib, T.M. and Rahman, M.M. Disease burden and associated factors of rotavirus infection in calves in south-eastern part of Bangladesh. *Asian J. Med. Biol. Res.* 201987, Published online (2019). https://api.semanticscholar.org/CorpusID:201987140

14. Constable, P., Hinchcliff, K., Done, S. and Grünberg, W. Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs and goats: *Elsevier Health Sciences*, 1116(2017).

15. Schroeder, M.E., Bounpheng, M.A., Rodgers, S., Baker, R.J., Black, W., Naikare, H., Velayudhan, B., Sneed, L., Szonyi, B. and Clavijo, A. Development and performance evaluation of calf diarrhea pathogen nucleic acid purification and detection workflow. *J. Vet. diagnostic Investig.,* **24**(5), 945-953(2012). doi:10.1177/1040638712456976

16. Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.,* **35**(6), 1547-1549(2018). doi:10.1093/molbev/msy096

17. Kimura, M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.,* **16**(2), 111-120(1980); doi:10.1007/BF01731581

18. Zaitoun, A., Abdel-Hakim, O. and Youssef, Z.M.A. Enteric Rota and Corona viruses' infection in neonatal calves. *Assiut Veterinary Medical Journal,* **64** (156), 8-17(2018).

19. Yahaya, K. and Veronique, A. Prevalence of rotavirus infection in diarrheic newborn calves in Abidjan region, Ivory Coast. *GSC Biol. Pharm. Sci.,* **5**(2), 082-087(2018). doi:10.30574/gscbps.2018.5.2.0116

20. Vega, C., Bok, M., Chacana, P., Saif, L., Fernandez, F. and Parreño, V. Egg yolk IgY: protection against rotavirus induced diarrhea and modulatory effect on the systemic and mucosal antibody responses in newborn calves. *Vet. Immunol. Immunopathol*., **142**(3-4), 156-169 (2011). doi:10.1016/j.vetimm.2011.05.003

21. Hosein, H.I. Infectious diseases of domestic animals. Dar El- Salam Publ Cairo, Egypt. (2024);*5th. Ed.*

22. Islam, S., Barua, S.R., Zonaed, Siddiki, A.M., Masuduzzaman, M., Hossain, M.A. and Chowdhury, S. Comparison of diagnostic tests for detection of bovine rotavirus a in calf feces. *Maced. Vet. Rev.,* **44**(1), 37-45(2021). doi:10.2478/macvetrev-2020-0033.

23. Ali, M. A study on the occurrence of calf diseases in some selected dairy farms of Bangladesh. *Asian Journal of Medical and Biological Research*, **1** (1), 39-46(2015).

24. Morrill, K.M., Conrad, E., Lago, A., Campbell, J., Quigley, J. and Tyler, H. Nationwide evaluation of quality and composition of colostrum on dairy farms in the United States. *J. Dairy Sci.,* **95**(7), 3997-4005(2012). doi:10.3168/jds.2011-5174

25. Gulliksen, S.M., Lie, K.I., Sølverød, L. and Østerås, O. Risk factors associated with colostrum quality in Norwegian dairy cows. *J. Dairy Sci.,* **91**(2),704-712(2008). doi:10.3168/jds.2007-0450

26. Abou El-Ella, G.H., Mohamed, A. and Aamer, A. Prevalence of enteropathogens associated with neonatal calf scour in cattle and buffalo calves using (FASTEST® STRIPS) rapid Field test. *Assiut Veterinary Medicine Journal*, **59** (138), 19-22(2013). doi:10.21608/avmj.2013.171602.

27. Edwards, S.A., Broom, D.M. and Collis, S.G. Factors Affecting Levels of Passive Immunity in Dairy Calves. *Br Vet J.,* **138**(3), 233-240(1982). doi:https://doi.org/10.1016/S0007-1935(17)31087-4

28. Silva, L.C., Sanches, A.A., Gregori, F., Brandão, P.E., Alfieri, A.A., Headley, S.A. and Jerez, J.A. Research in Veterinary Science First description of group A rotavirus from fecal samples of ostriches (Struthio camelus) q. *Res. Vet. Sci.,* **93** (2),1066-1069(2012). doi:10.1016/j.rvsc.2011.12.007

29. Offit, P.A., Hoffenberg, E.J., Santos, N. and Gouvea, V. Rotavirus-specific humoral and cellular immune response after primary, symptomatic infection. *J. Infect. Dis.,* **167**(6), 1436-1440(1993) . doi:10.1093/infdis/167.6.1436.

30. Snodgrass, D.R., Fahey, K.J., Wells, P.W., Campbell, I. and Whitelaw, A. Passive immunity in calf rotavirus infections: maternal vaccination increases and prolongs immunoglobulin G1 antibody secretion in milk. *Infect. Immun.* **28**(2), 344-349(1980). doi:10.1128/iai.28.2.344-349.1980

31. Chauhan, R.S. and Singh, N.P. Epidemiology of rotavirus infection in calves in India. International *Journal of Animal Sciences.* **11**, 221–223(1996).

32. Nataraju, S.M., Chattopadhyay, U.K. and Krishnan, T. A study on the possibility of zoonotic infection in rotaviral diarrhoea among calves and buffalo calves in and around Kolkata, India. *Eur. Rev. Med. Pharmacol. Sci.,* **13**(1), 7-11 (2009).

33. Soltan, M.A., Tsai, Y.L. and Lee, P.Y.A. Comparison of electron microscopy, ELISA, real time RT-PCR and insulated isothermal RT-PCR for the detection of Rotavirus group A (RVA) in feces of different animal species. *J. Virol. Methods*, **235**, 99-104(2016). doi:10.1016/j.jviromet.2016.05.006

34. Crouch, C.F. and Acres, S.D. Prevalence of rotavirus and coronavirus antigens in the feces of normal cows. *Can J. Comp. Med. Rev.,* **48**(3), 340-342(1984).

دراسة الوبائية الجزيئية وعوامل الخطر المرتبطة بعدوى فيروس الروتا (BRV) في الأبقار والجاموس في محافظات مختلفة في مصر

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الملخص

يعد فيروس الروتا البقري (BRV) هو المسبب الرئيسي في جميع أنحاء العالم لحالات الإسهال الحاد في العجول التي يقل عمرها عن شهر واحد . تهدف الدراسة الحالية إلى تقدير مدى انتشار عدوى فيروس الروتا البقري في الأبقار وعجول الجاموس في مناطق مختلفة في مصر تحت أنظمة رعايه مختلفة ودراسة دور بعض عوامل الخطر المرتبطة بعدوى فيروس الروتا البقري وكذلك دور الحيوانات المصابة سابقًا في العدوى. تم فحص عينات براز مجمعة من 222 عجول مصابة بالإسهال، و5 أبقار حديثة الولادة، و17 عجل سليم ظاهرياً ومصاب سابقاً بفيروس BRV بواسطة الاختبارات المناعية السريعه لفيروس الروتا (ICA) كان اجمالي معدل الانتشار لعدوى BRV باستخدامICA 25(تمت دراسة مدى انتشار الإصابة بفيروس BRV فيما يتعلق بالمتغيرات الوبائية المختلفة بما في ذلك، نوع الحيوان، الجنس، العمر، السلالة، نظام التربية، طريقة التغذية والتطعيم قبل الولادة. ثبت أن عوامل الخطر المختلفة تؤثر على انتشار عدوى فيروس BRV في الدراسة الحالية. لقد تم دعم وإثبات الوضع المتوطن لعدوى فيروس BRV في هذه الدراسة في مناطق مختلفة في مصر. ثبت أن العجول المصابة بفيروس BRV سابقًا تظل بمثابة ناقلات وخزانات تؤدي إلى الحفاظ على عدوى BRV في القطيع. تم استخراج الحمض النووي لفيروس الروتا من 25 عينة برازية وتم إخضاعها لتقنية RT-PCR في خطوة واحدة تستهدف جينNSP5 ، و RT-PCRفي خطوتين تستهدف جين .VP4 تم تأكيد وجود الجينوم الفيروسي عن طريق تضخيم جين NSP5 وجين VP4 بحجم منتج متوقع يبلغ 155 و856 ، على التوالي. أشار تسلسل النوكليوتيدات الجزئي لجين VP4 ونمط P إلى أن جين VP4 لـ BRV ينتمي إلى الأنماط الجينية P11

الكلمات الداله: فيروس الروتا البقري – مصر- جينVP4