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Serological Study for Detection of Rota Virus in Sheep in Baquba City

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ABSTRACT

Rotavirus (RV) is one of more important viruses of small ruminant are one of the major causes of productivity losses. The study was done to Determine the prevalence of RV in sheep at different regions in Baquba city. This study was designed in order to detect the infection with Rotavirus.

The study was carried out in Diyala province. The objectives of this study included serological detection of rotavirus antigen in feces of sheep clinically presented with diarrhea. This study was extended from October / 2021 to February / 2022. . Serological detection of rotavirus antibodies for sheep is done by using ELISA technique. The age of animals was range from one month up to 6 months. They were collected from different farms and veterinary clinics in Baquba city.

All forty five samples that collected were tested in the laboratory of microbiology in the college of veterinary medicine – university of Diyala. The results were showed that were 16 samples out of the 45 were infected with Rotavirus, while 29 samples out of the 45 samples were negative, this indicates that the infection appeared in the animals of Baquba city due to a lack of vaccination.

Key words: Rotavirus, sheep, ELISA, Baquba

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INTRODUCTION

The main pathogen for viral gastroenteritis that affects both people and animals is rotavirus A (RVA) [1]. It is endemic throughout the world and causes an estimated 258,173,300 cases of diarrhea and 128,500 deaths of children under the age of five per year

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[2]. The most dangerous species discovered in ruminant diarrhea episodes is RVA [3, 4]. Usually, neonatal ruminant animals between one and two weeks old are affected. This is due to the fact that milk feeding can support RVA ability to survive in a variety of gastrointestinal pH levels and promote viral infection of the intestine's epithelial cells. Thus, milk feeding may explain why non-weaned neonatal animals have a higher risk of developing diarrhea [5].

The rotavirus genome is made up of 11 double-stranded RNA segments and is encased in a recognizable three-layered icosahedral protein capsid. The protein VP2 forms the first layer, with a copy of the proteins VP1 and VP3 at each vertex. [4] The protein VP6 helps to build the second layer. Spike protein VP4 and structural glycoprotein VP7 make up the topmost protein layer. The buoyant density of viral particles in CsCl is 1.36 g/ml and they have a diameter of up to 100 nm. [7] Rotaviruses typically attack the epithelial cells of the villus' tip, which line the gastrointestinal tract. They are remarkably resistant to both the digesting enzymes (lipases and proteases) in the gastrointestinal tract as well as the typically restrictive pH of the stomach because to their triple protein coatings. [12]

Usually, newborn ruminant animals between one and two weeks old are affected. This is due to the fact that milk nutrition can support RVA's ability to survive in a variety and encourage viral invasion of the epithelial cells in the intestines. Thus, milk feeding may explain why non-weaned neonate animals have a greater risk of developing diarrhea [5].

Goat neonatal diarrhea outbreaks carry roughly the same disease load as those described for sheep. Infectious agents such as along with parasites, germs, viruses, and management techniques can induce diarrhea in goats. [3].

For a better grasp of ongoing RVA illness surveillance is crucial to understanding the epidemiology and evolution of RVA strains in domestic animals. This investigation attempted to ascertain the frequency of RVA infections and how they affected sheep in Iraq who experienced diarrhea. RVA strains have traditionally been distinguished using the genes for the outer capsid proteins VP7 (glycoprotein, G) and VP4 (protease-sensitive protein, P). As of right now, RVA has 36 different G genotypes and 51 unique P genotypes, numbered P[1]-P[36] and P[1]-P[36], respectively [6]. The predominant genotypes of bovine RVA found during field studies were G6, G10, and less commonly G8. They commonly connect to [5], P[11], and less frequently P[1] [7, 8]. The most frequently documented genotype combinations in sheep are G6P[5], G6P[1,] and G10P[11] [9]. While G6P[11] has been recognized as the predominant ovine strain in India [12], genotypes G8P[1] and G8P[14] have been discovered in calves from Spain. Most ovine G-types, including G10, G3, G6, and G9, migrate together with [4].

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Only one RVA study in Iraq looked into dromedary camel infection. Using commercially available, Al-Mutairi [14] discovered one RVA positive sample out of 408 (0.2%) fecal samples, while using RT-PCR, eight out of 109 samples (7.3%) were RVA positive. Of these eight findings, five samples came from animals with diarrhea that were four months old, and three samples came from asymptomatic animals who were three years old. The name of the variant of camel rotavirus found in Iraq is Rotavirus Camel-2010/G10P. The Iraqi camel strain and the ovine and bovine strains later shared similarities, as revealed by phylogenetic research [15]. According to an early research from the US Sheep Experiment Station, 46% of lamb deaths were caused by diarrhea [16]. Lambs are susceptible to a range of infectious agents, environmental factors, dietary status, and complicated, multifactorial diseases that can cause diarrhea. Salmonella spp., RV, Escherichia coli, Cryptosporidium sp. are the four main infectious agents that induce sheep experiencing diarrhea their month one of existence [17]. Ovine RVs can be classified as either RVA or RVB, but little is known about the etiology of lamb RVs. Numerous investigations revealed a significant morbidity rate (75–100%) and striking mortality when neonatal rotavirus outbreaks [18]. The stool samples from lambs with and without diarrhea that were examined in these studies contained ROV in 16–100% of the cases [19]. Ovine RVAs have been reported in a number of countries across the world, and depending on the epidemiologic setting, the diagnostic assay employed, etc., their detection rates may approach 60%, frequently with a mortality rate of 10-15% [20]. India has published several reports. [21].

When an epidemic occurred in India's Kashmir, 25% incidence, [22] found an association between RVA and lamb diarrhea. A subsequent investigation discovered RVA in about 25% of the 96 samples of diarrheal stools [23].

Only a limited amount of information is available globally regarding the DNA of ovine RV strains. The largest data set was published in India, where 500 samples that were collected over a three-year span were tested for RVs using a combination of antigen detection and molecular methods, and 52 strains were subsequently genotyped for the VP7 and VP4 genes. The bulk of the 52 isolates carried the P[24] VP4 gene [25], with the genotype G6 being the most prevalent (48%) and G10 being the second-most frequent (36%). At least three ovine RVA strains were reported in China, and G10P was the only antigenic combination that could be determined[15]. (Lp14, Lamb-NT, CC0812). Two of these genotypes, Lamb-NT and CC0812, have had their entire genomes [26], showing conserved arrangements of the backbone genes.

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In sheep sera from Europe, a sizable serosurvey carried out identified neutralizing antibodies to seven G serotypes in Spain, with the highest titers and frequency against serotypes G10, G3, and G6 [27]. One Spanish strain characterization study genotyped a single ovine RVA from an epidemic in lambs between 50 and 60 days old as G8P [28] A second Spanish RVA variant was identified as G8P[29] (OVR762] [30]. Four lamb RVA variants were identified in the UK, each with distinct characteristics, including LRV1, G3P[31]; LRV2a, G6P[11]; LRV2c, G9P[32]; and K923, G10P[33].

However, there aren't many reports accessible in Iraq, especially, as a way to ascertain the frequency of the Rotavirus in its invasive form in local sheep in Baquba City, for the current research was conducted.

MATERIALS AND METHODS

Study design and sample collection

Between October 2021 and February 2022, a cross-sectional survey was carried out. Independent of the development of clinical symptoms, fecal samples from various age groups was collected from the farms involved in the study during a single visit. Either single-species or mixed-species farms existed. For each property that was inspected, the sample size was calculated using the Chi square. The quantity of animals in the farm under investigation served as the basis for sampling. To choose the animals for this study, a systematic random sampling method was used.

The rectal fecal samples contained 5–10 g of feces that were taken directly from the rectum or soon after defecation. The gathered fecal samples were placed in clean containers and labeled with details about the sampled animal, such as its species, age, and state of health, as well as the date of sampling, the owner's name, and the location of the farm. Collection containers were placed in an ice box and brought to the lab as soon as was practical. Collected Samples stored at –20^{o}C for further ELISA analysis.

ELISA test:

To identify RVA antigen, a direct, sandwich, double-well, ELISA kit (Bio-X Diagnostics, Rochefort, Belgium) was used to examine all fecal samples. A microplate reader was used to measure the optical density at 450 nm. (BioTek Instrument, The Rotavirus Antigen ELISA Kit is a qualitative procedure for the detection of Rotavirus nucleoprotein antigen in complex sample matrices derived from both human and veterinary sources. Inc. Winooski, USA). The manufacturer's instructions were followed for the validation, calculation, and interpretation of the According to manufacturer instructions, specific sandwich, double-well, ELISA kit (Bio-X Diagnostics, Rochefort, Belgium) was applied to detect RVA antigen in fecal samples of study population.

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Statistical analysis:

Statistical analysis program SPSS was used to analysis the data, by using chi-square test and frequency. P value at the 95% confidence level was computed for significant result.

Results and discussion:

Understanding RVA epidemiology and the disease's geographic spread requires conducting field studies of RVA infections in sheep and characterizing the disease's disseminated genotypes. Using the data from these surveys, disease control strategies can be improved [5, 9]. The RVA infection in ruminants (sheep) in Iraq has not previously been documented until this study. To look into the prevalence of RVA and how it affects livestock in Iraq who are at risk for developing diarrhea.

Previous study on RVA infections using an ELISA test showed that RVA is one of the main causes of diarrhea in sheep, with a prevalence of 11.6 and 28.8% in diarrheic neonatal and preweaned calves, respectively [18, 19]. In the current study, both diarrheal and non-diarrheic animals had RVA detected with a lower proportion (ELISA test: 35% detection). The proportion positive was higher (55%) if only animals with diarrhea were considered, in contrast to the prevalence of both diarrheal and non-diarrheic sheep overall.

Table 1: Gender and distribution of rotavirus infection

			Data		Total
			Infected	Non infected	
groups	male	Count	7	15	22
		% within groups	31.8%	68.2%	100.0%
		% within data	43.8%	51.7%	48.9%
	female	Count	9	14	23
		% within groups	39.1%	60.9%	100.0%
		% within data	56.3%	48.3%	51.1%
Total		Count	16	29	45
		% within groups	35.6%	64.4%	100.0%
		% within data	100.0%	100.0%	100.0%

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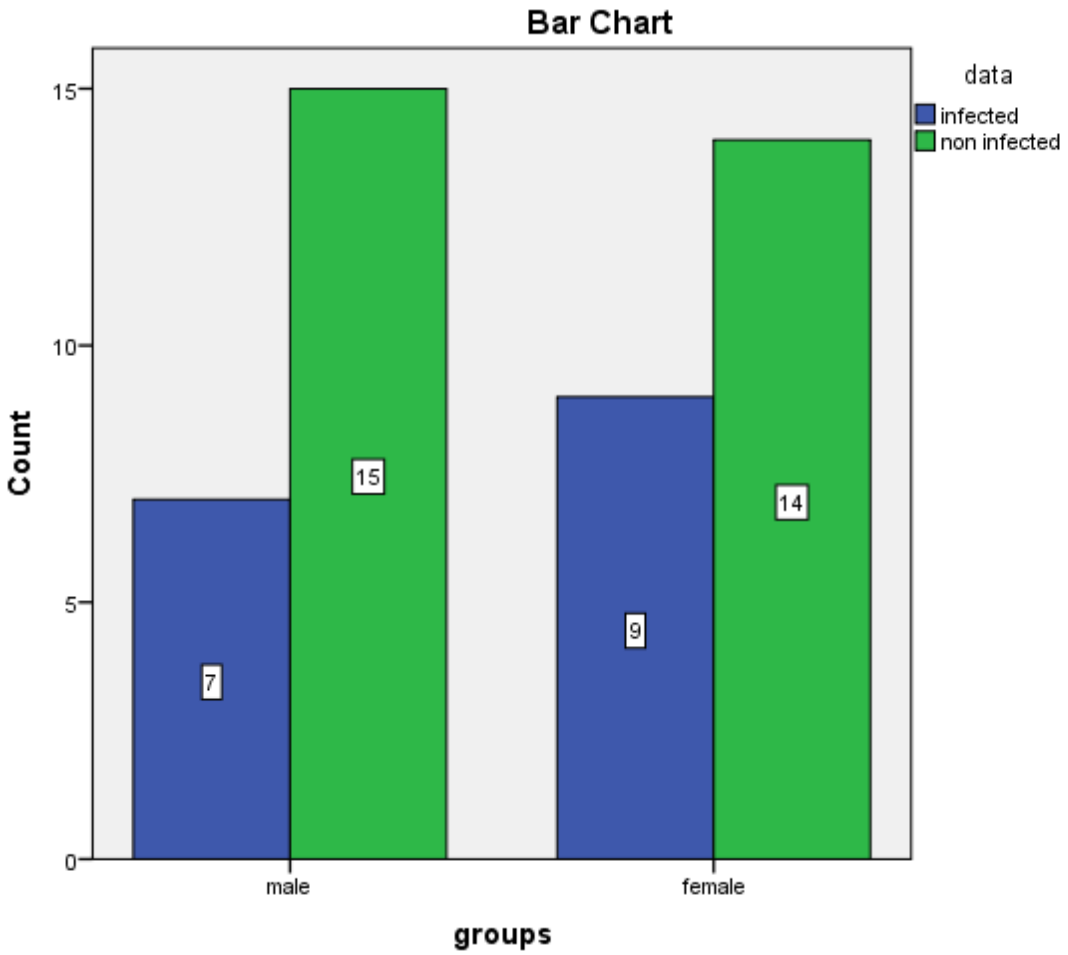

Figure 1: Effect of Gender in distribution of rotavirus infection

If samples were only taken from young, diarrheal animals, the prevalence might have been greater based on these findings. Depending on the various diagnostic techniques, hygienic precautions, and management techniques used in the farms, there can be variance in the rates of RVA detection from animal specimens. Adult animals that can act as non-diarrheic carriers for RVA are less likely to become infected when newborn lambs are isolated from their mothers and other adult animals [20, 21].research as the age group that is most prone to developing RVA infection [5, 22, 23].

Discussion

The present study was carried out because of the importance of rotavirus infection as a cause of acute gastroenteritis among sheeps beside the lack of such previous study in Diyala province. Study samples were collected from Serological detection of this experiment was carried out in the Virology Laboratory, College of Veterinary Medicine, Diyala

University, Diyala, Iraq, while the practical experiment was carried out in Canaan Baladruz, Shahrban, Bahris, Diyala Governorate.

Note that this study lacked the scarcity of samples that are sent abroad and conducting tests on them, and this is due to the personal financial aspect as the laboratory materials are very expensive and this was considered one of the obstacles in the study In examining the PCR of these samples collected in the study.

Understanding RVA epidemiology and the geographic distribution of the various genotypes requires field studies of RVA infections with the characterisation of disseminated genotypes in ruminants. These surveys' findings can be used to enhance disease prevention efforts [5, 9]. This study is the first to pinpoint RVA genotypes in ruminants (sheep) in Iraq. It is also the first study look into the prevalence of RVA and its role as a risk factor for diarrhea in sheep in Iraq.

in the present study, RVA was detected in 1.2 and 0.9% of fecal specimens from sheep by ELISA, respectively, The incidence of ovine and caprine RVAs have been reported from different countries around the world. Based on epidemiological conditions and diagnostic tests used, their prevalence rates varied. The investigations show that viruses seen in sheep originated in either the human or the cattle species. [19]. Because cattle and sheep are frequently mated together, rotavirus infections in sheep pose a possible risk to cattle. Since there is no vaccine available for sheep, vaccines for cattle may be utilized in sheep. [24].

For this reason, vaccination for cattle with region-type specific vaccine may be advised for endemic locations. Similar to how one vaccine given to infants Rotashield contains monkey strains and another RotaTeq cow strains. Finding dominant genotypes in the area of concern is a crucial consideration when picking a vaccination strain. [12].

The present study revealed the rate of females infection with rotavirus in have a significant higher rate of rotavirus infection than Males , similar results have been reported in the previous studies done in iraq. [33]. in Iran found that the percentage of males was higher than that of females. Some studies found a relation between rotavirus diarrhea and male gender as a risk fact . [17] This study did not agree with international studies in india where it showed a variation in the rates of rotavirus infection in sheep. [21]

The presence of RVA in small ruminants' feces was not associated with the development of diarrhea in the current investigation. Additionally, there was no statistically significant correlation between the occurrence of RVA and various age groups. [22] This may be because there were only nine

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samples from sheep in this investigation that were used to identify RVA. There aren't many research on small ruminants conducted worldwide where RVA has been recognized as a cause of diarrhea in young lambs and children. [11] in Turkey. Compared to other studies conducted in different countries in the world, it was close to The results [20] Earlier study on RVA infections in Kuwait using an ELISA test revealed that RVA is among the most common causes of diarrhea, with prevalence rates of 11.6 and 28.8% from diarrheic neonates. [9].

Diarrhea was the cause of 46% of Sheep deaths, according to a previous study done at the US Sheep Experiment Station. In addition to animal vulnerability, nutritional state, environmental factors, and a number of infectious pathogens, diarrhea in Sheep is a complicated, multifactorial disease. In the first month of life, Escherichia coli, RV, Cryptosporidium sp., and Salmonella sp. are the four main infectious causes of diarrhea in Sheep. [16]

Based on these findings, it is conceivable that the prevalence may have been higher if the samples had only been taken from young sheep that were experiencing diarrhea. [13]. The various diagnostic techniques, hygienic precautions, and management techniques used in the farms may affect the variation in RVA detection rates from sheep specimens. The frequency of RVA infection in adults, which can act as non-diarrheic carriers, is reduced when newborn sheep are isolated from their dams and older animals. [17]

Previous study has established the significance of RVA as the main viral cause of dysentery, has classified RVA as a newborn animals viral scours [5, 22]. In general, thought that malabsorption was the primary cause of RVA gastroenteritis [23]. However, there is also proof that it is brought on by NSP4, an enterotoxin produced by the RVA [23, 24]. According to this, the first two weeks of life are when most cases of RVA diarrhea in sheep and livestock occur. Numerous studies have indicated that this age group is the most vulnerable to developing an RVA illness [5, 22, 23].

In the current study, there was no correlation between small ruminants experiencing diarrhea and having RVA found in their feces. Furthermore, there was insignificant numerically correlation between age groups and the prevalence of RVA. This might be as a result of the study's limited sample size for sheep-derived RVA. Only a few studies on small ruminants have been conducted worldwide where RVA has been found as a cause of diarrhea in newborn lambs and children [11, 16, 25, 26, 27].

Conclusion:

The results of the present study suggest that RVA might be a risk factor for diarrhea in ovine sheep of various ages; further study is needed to investigate this idea while taking other risk factors into consideration. This research provides evidence that RVA is present in sheep populations in Iraq. But shows that in these animals, it does not pose a danger of gastroenteritis. Iraqi humans have not yet been exposed to the RVA strains discovered in ruminant animals in this study.

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