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Prevalence of Rotavirus Infections in Small Ruminants

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Abstract: In the present study, fecal samples from different localities at Giza governorate, Egypt were collected from 228 sheep lambs and 79 goat Kids 1-8 week old suffered from acute diarrhea and gastroenteritis during winter season. They were examined for detection of rotavirus antigen using latex agglutination test (LAT) and ELISA technique. Results revealed that out of 228 fecal samples of lambs 28 (12.3 %) and out of the 79 goat kids 6 (7.9%) were positive to Rotavirus antigen by serial testing using ELISA and LAT, meanwhile by parallel testing 40(17.5%) lambs, 10(13.2%) goat kids were positive to Rotavirus antigen with 95.2% and 93.7% observed proportion agreement between both tests respectively in lambs and goat kids. Using RT- PCR technique for confirmation of rotavirus detection, all positive samples were confirmed positive for detection of rotavirus genome except one ELISA positive goat kid. The results also indicated that goat kids are less frequently infected with Rotavirus infection than lambs, also Rota virus antigen was not detected in diarrheic lambs of less than three week old and in diarrheic goat kids less than 2 week old and 90% of the reported cases occur between 3-7 weeks old in both species. It was concluded that the LAT can be used to quickly identify the Rotavirus in cases of neonatal young small ruminant diarrhea and it would facilitate the rapid implementation of effective control measures at herd level, but at individual level to minimize the false negative reactors and to estimate the prevalence of infection parallel testing using both LAT and ELISAs. PCR is recommended to increase the overall sensitivity.

Key words: LAT · ELISA · RT- PCR · Diarrhea

INTRODUCTION

Scour is considered as an important disease in lambs and goat kids. It is a complex multi factorial condition involving animal, environmental, nutritional and infectious agents. Neonatal diarrhea induced by Rotavirus causes significant economical losses due to high morbidity, mortality, treatment cost and reduced growth rate of infected animals [1]. Ovine Rotavirus has been identified as causing neonatal lamb diarrhea in United Kingdom, Japan and United States [2-5]. In Egypt, Rotavirus was detected in fecal samples of lambs and goat kids in Giza [6] and the virus was isolated from diarrheic lambs and goat kids bred under intensive system which results in some economic losses [7]. Therefore, the current study was conducted to throw light on the association between Rotavirus and diarrhea among new born lambs and goat kids in relation to age susceptibility and to evaluate the currently used diagnostic tests.

MATERIALS AND METHODS

A total 304 fecal samples was collected from newly born lambs (N= 228) and goat kids (N = 76) from 1 day and up to 8 week old from different localities at Giza governorate, Egypt. These animals were suffering from diarrhea and gastro intestinal manifestation during winter lambing season whereas the highest prevalence of ovine rotavirus infection was expected according to Samad and Ahmed [8] and Habashi [7].

Latex Agglutination Test (LAT): The test was done using commercial "Slidex Rota-Kits". The kit was supplied by Bio-Merrieux, France and includes latex Rotavirus-monoclonal antibody, negative and positive control suspension to Rota virus antigen.

Enzyme Linked Immuno Sorbent Assay (ELISA): Detection of Rotavirus antigen was carried out on the fecal samples suspension by using a commercial double

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antibody sandwich ELISA kit (*Rota Ag detection Kit, C.E.R Division immunologie, Belgium*) according to the instruction manual.

Reverse Transcription Polymerase Chain Reaction (RT- PCR): A pair of primers amplifying a 294 bp fragment of VP7 of type A Rotavirus have been designed according to *Chinsangaram et al.* [9].

The sequence of the upstream primer was 5'-ACCACCAAATAGACACCAGC-3', the sequence of downstream primer was 5'-CATGCTTCTAATGGAAGC-3'.

RNA was extracted from the fecal samples by QIA amp viral RNA mini kit (Qiagen) following manufacturer's instructions. RT- PCR was performed using Superscript one step RT- PCR system (Life Technologies, Roch ville, Mod). 5µl of extracted RNA was mixed with primers (0.4µM) and RNase-free water was added to a total volume of 24µl. the mixture was heated at 95°C for 4' and then quickly cooled to 4°C. The superscript 2X- reaction mix (25µl) and RT-Taq mix (1µl) were then the reaction was carried out at 50°C for 30min and denaturation at 94°C followed by 40 cycles of PCR product amplification (94°C for 30 S, 52°C for 30 S, 72°C for 1min and final extension at 72°C for 7'. Amplification products were visualized in ethidium bromide stained gels.

RESULTS

Detection of Rotavirus Antigen: It is clear from Table 1 that by using LAT out of 228 lambs fecal samples, 31 (13.6%) were positive and among 76 goat kids, 7 (9.2%) were positive. Meanwhile, by using ELISA it was noticed that Among 228 lambs fecal samples, 36 (15.8%) were positive and out of 76 goat kids, 10(13.2%) were positive.

As shown in Table 2 with reference to the previously done by Pai *et. al.*, [10], the LAT test has not 100% sensitivity: 81.6% and has not 100% specificity: 98.1%, the Estimated true prevalence is 16.1% rotavirus infected lambs (95% CI 12: 22%) and 10.7% rotavirus infected goat kids (95% CI 5–20%) and the estimated true prevalence for the LAT in both lamb and goat kids is closer to apparent prevalence ELISA results (=15.8%, 13.2% in lambs and goat kids respectively.

The present results also indicated that the prevalence of Rotavirus infection was higher in lambs than goat kids and as shown in Table 3 and Fig. 1 focused the relationship between Rotavirus infection and age, whereas it was found that the viral antigen was not detected in diarrheic lambs less than 3 weeks age and in diarrheic goat kids less than 2 weeks age and the highest prevalence was at 3-7 week age which constitute 90% of

Table 1: Rotavirus detection in fecal samples of lambs and goat kids by LAT and ELISA

		LAT	ELISA		
Animal species	No. collected samples	No. Positive samples (%)	No. Positive samples (%)	No. Positive Serial testing ELISA and LAT (%)	No. Positive Parallel testing ELISA and LAT (%)
Lambs	228	31(13.6)	36(15.8)	28(12.3)	40 (17.5)
Goat kids	76	7(9.2)	10(13.2)	6(7.9)	10 (13.2)

Table 2: Evaluation of LAT and ELISA correlation with reference to LAT

		LAT				ELISA
Animal species	No. collected samples	%AP[a]	%TP[b]	%PPV[c]	%NPV[d]	%AP[a]
Lambs	228	13.6	16.1	96.93	96.57	15.8
Goat kids	76	9.2	10.7	95.14	97.84	13.2

a= Apparent prevalence, *b*= True prevalence, *c*= positive predictive value *d*= negative predictive value

Table 3: Relationship between age of lambs and goat kids and prevalence of Rotavirus infection

	No. Positive (%)	
Age in weeks	Lambs	Goat Kids
1	0(0.0)	0(0.0)
2	0(0.0)	1(10.0)
3	5(12.5)	2(20.0)
4	10(25.0)	2(20.0)
5	9(22.5)	1(10.0)
6	6(15.0)	2(20.0)
7	6(15.0)	2(20.0)
8	4(10.0)	0(0.0)
Total	40(100)	10(100)

90% of cases occurred between 3-7 weeks old

Table 4: Results of RT- PCR for detection of Rotavirus nucleic acid in positive fecal samples by LAT and/or ELISA

Positive samples by LAT and/or ELISA			No. Positive by RT- PCR
Positive by LAT and ELISA	Lambs	28	28
	Goat Kids	6	6
Positive by LAT only	Lambs	3	3
	Goat Kids	1	1
Positive by ELISA only	Lambs	8	8
	Goat Kids	4	3
Total (%)		50	49 (98%)

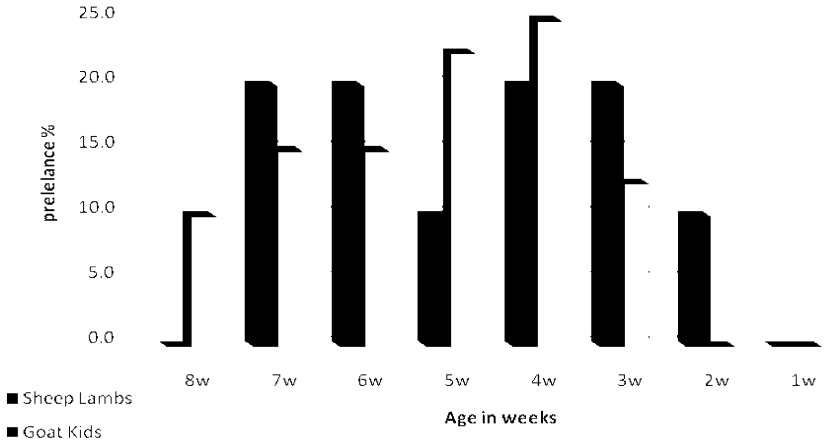

Fig. 1: Prevalence of Rotavirus infection Relationship to age of diarrheic lambs and goat kids

Photo 1: RT- PCR-based amplification of group A rotavirus RNA. Lane 1, 100-bp DNA ladder; Lane 2, negative control (distilled H2O used as a template); Lanes 3 to 8 results presenting positive samples

the reported positive diarrheic cases with Rotavirus infection in both sheep lambs and goats kids.

Nucleic Acid Detection (RT- PCR): As shown in Table 4 and, Photo 1, 49 fecal samples out of all the positive samples of both lambs and goat kids by LAT and/or ELISA were found positive by RT- PCR for detection of the Rotavirus group A nucleic acid except only one goat kids sample was previously detected positive by ELISA which confirm 98% of the positive result of parallel testing using both LAT and ELISA for specific antigen detection.

DISCUSSION

Rotavirus is ubiquitous intestinal pathogen that have been isolated and suspected of infecting virtually every avian and mammalian species, including human being [11- 13]. Ovine rotavirus was isolated from intestinal content of lambs suffering from diarrhea [14]. Although rotavirus gastroenteritis is primarily a problem in neonates and very young animals, symptomatic and asymptomatic infection among adults can occur [15]. The Current study was conducted aiming to study the prevalence of rotavirus infection in lamb and goat kids1-8 weeks old

from different localities in Giza governorate during winter season though testing field samples by LAT in comparative with ELISA and finally all the positive samples by were confirmed by nucleic acid detection method "RT- PCR".

Diagnosis of rotavirus infection should be based on various clinical manifestations field test and specific laboratory methods. LAT, ELISA and PCR have become more standard methods for the diagnosis of Bovine and Ovine Rotavirus [1].

LAT is a rapid, simple, inexpensive field test with moderate sensitivity, but high specificity in comparative with ELISA which can be used for diagnosis of rotavirus infections [10]. ELISA was widely used for detection of rotavirus antigen since it was highly sensitive in comparison with other techniques [16, 17]. Although ELISA is now commonly used in rotavirus antigen detection, it cannot be detected in a drawback is that virus antibody complexes formed after the onset of the intestinal immune response [1].

Our results revealed that by LAT 13.6%, 9.2% of diarrheic lambs and goat kids were positive for Rotavirus infection. Meanwhile, 15.8%, 13.2% were positive by ELISA in lambs and goat kids respectively. There were 3 samples from lambs and 1 samples from goat kids found positive only by LAT, 8 samples from lambs and 4 samples from goat kids were found positive only by ELISA with 95.2% and 93.7% observed proportion of agreement between both the LAT and ELISA in lambs and goat kids respectively. According to Pai *et al.* [10] the reference LAT sensitivity is 81.7% and specificity is 99.5%, the estimated true prevalence of infection rotavirus is 16.1% in sheep lambs and 10.7% in goat kids. This estimated true prevalence using LAT become closer to apparent prevalence ELISA results (=15.8%, 13.2% in lambs and goat kids respectively) which confirm the high sensitivity of the ELISA in relation to LAT (Table 2).

RT-PCR has been described for detection and/or typing of different rotavirus isolates the results obtained by RT- PCR and ELISA in detection limit of 6×10^2 particles of fecal samples as coincides with Chinsangaram *et al.* [9]. PCR is being used more commonly as a tool for the diagnosis of rotavirus. Its advantages are very high sensitivity and its ability to detect viral RNA as effectively in samples containing virus-antibody complexes as in those composed mainly of fully infectious virions. The disadvantages of RT- PCR are cost and the need for specialized equipment and technically proficient staff [1].

Inspection of the obtained results, it was clear that samples considered negative by LAT should be analyzed by more sensitive second assay like (ELISA or RT- PCR) to minimize the false negative reactors in order to assure an appropriate diagnosis as suggested by Thais *et al.* [18]. For reason we accounted the overall results in our study to the parallel testing results of both the LAT and the ELISA for the final interpretation in order to increase the overall sensitivity and it was found that 17.5% of diarrheic lamb and 13.2% goat kids were positive to rotavirus infection and the prevalence of rotavirus infection was higher in lamb as shown in Table 1 which indicated that goat kids are more resistant to rotavirus infection than lambs as previously reported by Kaminjolo and Adeisyum [19].

Based on these results, the incidence of rotavirus infection in relation to the age was also studied and the virus was not detected in lambs less than three weeks old or in goat kids less than two weeks old and 90% of rotavirus reported diarrheic cases in both species occurred during age between 3-7 weeks old. These results agreed also with that of Kaminjolo and Adeisyum [19], but not agreed with Susan [5] who found that rotavirus cause diarrhea in lambs and goat kids 2-14 days of age. This condition may be attributed to the presence of rotavirus as an endemic disease in our breeds, whereas no vaccination programs were used so that the disease appears after the maternal antibodies have been ceased.

In conclusion, LAT as is rapid, simple diagnostic tests that can be used to quickly identify the Rotavirus neonatal Lamb diarrhea and would facilitate the rapid implementation of effective control measures at herd level. For accurate estimation of the prevalence of infection, parallel testing using both LAT and ELISAs or PCR is recommended to increase the overall sensitivity. It necessary to improve young animals hygienic measures and implement vaccination.

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