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SEROPREVALENCE OF SOME COMMON RESPIRATORY VIRAL INFECTIONS OF SHEEP

MERVAT, I.I. ABD-EL-MONIEM
Virology Dept., Animal Health Research Institute, Dokki, Cairo.

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SUMMARY

Respiratory tract diseases represent a major causes of economic loss of sheep. This study was planned to know some light on respiratory viral infection in sheep.

A total of 300 sheep serum samples were collected from 2 Egyptian Governorate (Kafer El-Sheikh and Alexandria). The collected sera represented age varied from (3-12 months). These samples were tested serologically by Enzyme Linked Immunosorbent Assay "ELISA" Technique and Serum Neutralization Test "SNT" for detecting specific antibodies of parainfluenza virus type 3 (PI-3), infectious bovine rhinotracheitis virus (IBR) and adenoviruses. Serological examination revealed the presence of specific antibodies against respiratory viruses used. Out of 300 sheep serum samples (7.8%, 16.2% and 17.2%) for IBR, PI-3 and adenovirus respectively ere detected by ELISA. While, they were 3.9%, 11.0% and 9.3% respectively by SNT.

INTRODUCTION

Sheep represent one of the most important domestic livestock in Egypt, it represented about 2 480 000. Respiratory diseases represent one of the serious problems for this species causing economic loss in sheep. Several viruses are incriminated in respiratory infection of sheep as Infectious Bovine Rhinotracheitis (IBRV), Parainfluenza virus type 3 (PI-3) and Ovine adenovirus (OAV).

BHV-1/IBR causes various disease conditions in sheep including respiratory tract disorders, fetal pneumonia. This species considered potential host for BHV-1/IBR and involved in the interspecies transmission among domestic animals (Whetstone and Evermann, 1988).

Parainfluenza -3 virus is associated with respiratory infection of sheep (pneumoentritis) with high mortality rate.

Adenovirus isolated from nasal secretion of sheep (Adair et al., 1984) with respiratory manifestation.

The present study investigates the seroepidemiological manner for the prevalence of PI-3, IBR and adenovirus specific antibodies in sheep in2 localities in Egypt using two serological tests (Enzyme Linked Immunosorbent Asay (ELISA) and Serum Neutralization Test (SNT).

MATERIAL AND METHODS

1. Animals:

A total of 300 sheep serum were used in this study obtained from 2 localities, Kafr El-Sheikh and Alexandria (150 each). These serum were used for the sero-survey for PI-3, IBR and adenovirus. These animals were suffering from respiratory manifestation include increased respiratory rate, elevated temperature, sero-nasal and lacrimal discharge, coughing, depression and anorexia.

2. Viruses:

All viral strains were obtained from animal health Research Institute, Dokki, Giza.

3. Viral antigens.

Positive and negative viral antigen were locally prepared from infected and non infected MDBK cell culture with reference virus according to Edward et al. (1983). The viral antigens were used in ELISA technique.

4. Conjugates:

Antisheep horse reddish peroxidase conjugate was supplied by Animal Health Research Institute, Dokki, Giza Egypt.

5. Positive Immunesera:

Standard Positive hyperimmune serum of IBR, PI-3 and adenovirus were supplied from serum and Vaccines Production and Research Institute, Abbassia, Cairo, Egypt. Control negative was new borne calf serum.

6. Serum Neutralization Test (SNT):

Beta procedure (Constant virus-variable serum) was used to quantitative virus neutralizing antibodies in serum samples according to Carbery and Lee (1966).

7. Virus Titration on MDBK cells:

A microtiter tissue culture plate, 96 wells was used for titration of the virus according to Frey and Liess (1971), the virus suspension was diluted in sterile vial ten-fold manner ranged from 101 till 108 using MEM from each vial dilution 100 µl, then transferred into individual wells (8 well per dilution) 100 µl suspension containing 50 000 cells were added in each well containing the virus. The plates were incubated at 37°C in CO_2 in-

cubator with daily microscopic examination to detect CPE. Once the cytopathic effect has been detected, the viral titer was estimated by Reed and Muench (1938).

8. Test proper:

All sera were inactivated in water bath at 56°C for 30 minutes, 50 µ of maintenance media were added to all wells of the microplates. 50 µ of tested sera were added to the individual well of the first column to give starting dilution of 1:2, then 2 fold serial dilution were carried out up to 128, every plate contained cell control and virus control. 50 µl of virus containing 100 $TCID_{50}$ were added to all wells. The plate was agitated to mix the virus-serum mixture and then incubated for 1/2 hour at incubator at 37°C monolayer culture (MDBK) was dispersed with trypsin and resuspended with maintenance 2% foetal calf serum, each well received 100 µl suspension containing 50 000 cells. The plate was then incubated at 37 °C in CO_2 incubator with daily microscopic examination to detect CPE in virus control. End point was expressed as the highest dilution of serum inhibited complete viral CPE. The neutralization titer was considered positive at 16, 32 and 8-16 for IBR, PI-3 AND adenovirus respectively.

9. Solid phase enzyme linked immunosorbent assay (ELISA) Technique:

It was used for detection of specific antibodies against PI-3, IBR and adenovirus in serum samples according to the method described by Voller et al. (1976).

RESULTS

As showed in table (1) and Fig. (1), the results of SNT technique revealed that the positive reactors for PI-3 specific antibodies among sheep were 19%, while the positive reactors for IBR specific antibodies among sheep were 4% and 21% for adenovirus, respectively.

While, the results of ELISA technique, as showed in table (1), the positive reactors for PI-3, IBR and adenovirus specific antibodies among sheep were 25%, 9% and 26% respectively.

As showed in table (1 & 2) and Fig. (1), the prevalence of antibodies against PI-3, IBR and adenovirus by ELISA were 25%, 9% and 28% respectively. While by SNT, the prevalence of antibodies were 19%, 4% and 21% for PI-3, IBR and adenovirus, respectively. Comparison between ELISA and SNT for detection of specific antibodies against PI-3, IBR and adenovirus in sheep in table (3) and Fig. (1). It was noticed that ELISA was significantly sensitive than SNT for detection of specific antibodies against all the viruses used in this study.

Table (1): Prevalence of PI-3, IBR and adenovirus specific antibodies among sheep using SNT.

Type of virus	Governorates	Total numbers	Numbers of+ve cases	% of +ve cases	Average
PI=3	Kafr El-Sheikh	150	39	26%	19%
	Alexandria	150	18	12%	
IBR	Kafr El-Sheikh	150	-	-	4%
	Alexandria	150	12	8%	
Adenovirus	Kafr El-Sheikh	150	27	19%	21%
	Alexandria	150	36	24%	

Table (2): Prevalence of PI-3, IBR and adenovirus specific antibodies among sheep using ELISA.

Type of virus	Governorates	Total numbers	Numbers of+ve cases	% of +ve cases	Average
PI=3	Kafr El-Sheikh	150	48	23%	25%
	Alexandria	150	27	18%	
IBR	Kafr El-Sheikh	150	9	6%	9%
	Alexandria	150	18	12%	
Adenovirus	Kafr El-Sheikh	150	39	2%	26%
	Alexandria	150	38	26%	

+ve control of PI-3-1.104 O.D.
-ve control of IBR- 1.468 O.D.
+ve control of adenovirus 0.516 O.D.
Samples above O.D. of each virus considered +ve.

Table (3): Comparison between SNT and ELISA technique.

Technique used		PI-3	IBR	Adenovirus
ELISA	No.	300	300	300
	%	25%	9%	26%
SNT	No.	300	300	300
	%	19%	4%	21%

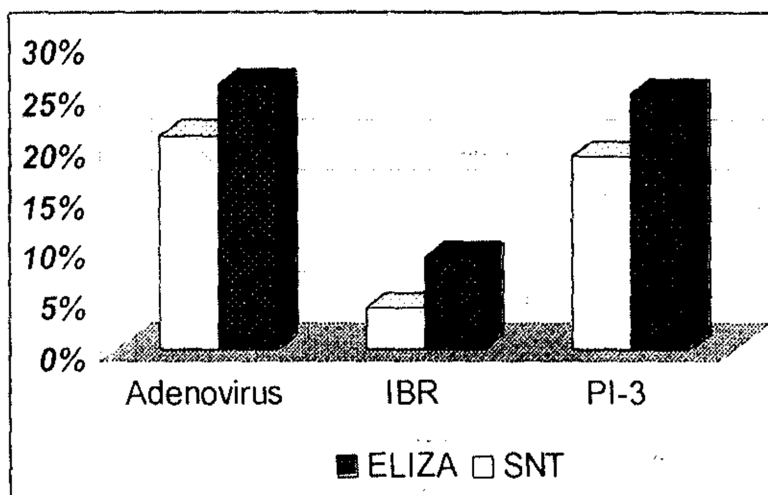

Fig (1): Comparison between SNT and ELISA for detection of specific antibodies against used viruses in sheep

Table (4): Single and mixed infection for the three viral pathogens determined by ELISA .

No. of infection	Type of infection	Number of cases
Two infection	PI-3 + Adenovirus	90
	IBR + Adenovirus	30

DISCUSSION

Respiratory tract diseases represent one of the serious problems among sheep causing economic loss and considered the cause of decreases in number of sheep. Several viruses incriminated in respiratory infection of sheep as parainfluenza virus type 3, infectious bovine rhinotracheitis virus and adenovirus.

The present work was planned to illustrate screening of sheep sera for presence of PI-3, IBR and adenovirus specific antibodies using two serological tests, ELISA technique and serum neutralization.

As shown in table (1 & Fig. 1), the results of screening using ELISA and SNT, the comparison revealed that ELISA more sensitive than SNT to detect specific antibodies against PI-3, IBR and adenoviruses. Out of 150 sheep sera using ELISA, the percentage of the examined viruses were 25%, 9% and 28% respectively, were recorded, while by SNT, the prevalence of antibodies were 19%, 4% and 21% respectively.

So ELISA technique was recommended as a choice test for detection of specific respiratory viral antibodies prevalence among the tested sheep. This results agree with Herring et al. (1980); Edwards et al. (1984); Cerny and Schuller (1988); Obi and Ibu (1990); Ratan et al. (1992) and Boyle

et al. (1994); Saber et al. (1996); Genedy (1999); Schaller et al. (2000); Amal et al. (2001); Hanaa

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Also, these results agree with Robinson (1983); Adair et al. (1884) who recorded PI-3 antibody prevalence ranged between 4-30% among sheep. While in case of IBR, these results agree with Herring et al. (1980); Wafula and Mushi (1985); Arab et al (1989); Salem et al. (2000); Amal (2001) and Said (2002) who recorded IBR antibody prevalence ranged between 5-14% among sheep.

Finally, the result of adenovirus antibody prevalence ranged between 8-26% among sheep, this

findings agree with Goyal et al. (1988); Momtaz (1993) and Ganady (1999).

The sensitivity of ELISA as detect all antibodies of virus while SNT detect protective antibody alone. This agree with Herring et al. (1980); Saber et al. (1996) and Said (2002) who described ELISA for detection of respiratory viral antibodies. They stated that all positive sera in SNT were positive by ELISA and negative SNT sera were positive by ELISA.

Mixed infection by more than one virus may indicate high susceptibility of animal to infection by more than one virus due to low immunity and other stress factors as poor nutrition, bad weather, bad hygiene, lack of vaccination. The nature of respiratory tract as an open system for inhaled viruses, open the way for other microorganism.

From the present study, it is concluded that this ELISA test as a reproducible and practical clinical tool for resolving the immune status of a population to multiple etiologic agents, so that early management decisions regarding vaccination can be made at the same time reproducible rapid results can be obtained. According to our results, we advice use a combined virus vaccine.

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