A Serological Survey on Prevalence of Sheep Border Disease in Iran

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Summary

This survey was carried out via serum neutralization and ELISA tests with NADL strain of BVD virus. The sheep sera were collected from 8 different provinces of Iran. By SN test the rate of infection in all provinces was 13.5%. Chaharmahal-Bakhtiary and Khorasan showed highest (21.2%) and lowest rate (4.1%) rate of infection. By ELISA the total rate of infectivity came out to be 11.9% while by SN test using border disease virus (isolated from Iran) this rate was 12.8%. Statistical analysis revealed no significance differences in these results. According to the age, the lambs between 4-8 months showed lowest (8.1%) and those over 18 months had the highest (18.3%) infection rate.

Keywords: border disease, Pestivirus, serum neutralization, ELISA, Iran

Introduction

Members of the genus Pestivirus in the family Flaviviridae infected several species of domestic and wild ruminants and also pigs. Diseases caused by Pestiviruses encompass the bovine viral diarrhea/mucosal disease (BVD/MD) in cattle, Border disease (BD) in sheep and goats and classic swine fever (CSF) in pigs (Becher 1994, Paton 1995,Wensvort *et al* 1989).

Serological investigations in some countries have indicated widespread infection with pestiviral infections in different species of ruminant (Carlson & Blak 1994, Fener *et al* 1993 Piter 1995).

In those countries where sheep raising industry and wool production are an important sources of national income the border disease because of its vast distribution, as a cause of abortion, wool deformation, congenital defect and/or causing weak lamb syndrome is one of the problems that must be pay much attention to. The border disease virus (BDV) is a causative agent of abortion, congenital abnormalities and weak lamb syndrome. Thus more attention must be paid to

transmission of the virus from sheep to cow. Other important aspects of BDV are its helpfulness in understanding the problems in immunology, embryology, enzymology and biochemistry (Hechert & Dubuc 1994, Vantsis *et al* 1980).

Sedighinejad (1996) data showed that the infection rate of BVD in cattle in Iran varied from 22% to 90% by ELISA. Because of much concern about the BVD in Iran, this study was carried out to determine the prevalence of BD in some sheep raising in different areas. It has been found out that small antigenic differences between BDV strains cause differences in serological tests results (Hechert & Dubuc 1994, Huck *et al* 1975). The main object of using BD virus isolated in Iran in SN test was to compare the results from SN and ELISA tests and to access a standard method for detecting the disease in the country.

Materials and Methods

Samples. With respect to climate and geographical similarities in Iran from seven provinces; Markazi, Isfahan, Chaharmahal-Bakhtiary, West Azarbyjan, Khuzestan, Khorasan and Mazandaran were chosen. By cluster random sampling method, sheep were collected and grouped according to and age such as first group 1- 4, second group, 4-18, and third group 18 month and over. In each related herd in the provinces, where number of raising sheep were more than 250, 8 blood samples were taken. Sera were separated and stored at -30C.

Cell line and culture media. R.BK (Razi Bovine Kidney) cell line, MEM and Hanks media were used. FCS was purchased from Sebak GmbH Co (Germany) and required buffer was made in microbiology laboratory of veterinary faculty Tehran University according to Bottcher 1993.

Antigen. NADL strain and a BVD strain isolated from Iran (Kargar-Moakhar; personal communication) were used as antigen. ELISA kits were providing by Sovanovir Company (Sweden). According to the manufacturer's instruction they were able to detect antibodies in sera and milk against BDV.

200 TCID50 of BDV and NADL strain of BD virus as antigens and R.BK cell line were used in SN test. All sera samples were diluted two-fold from 1/2 to 1/64. The sera that had titer over 1/2 were kept for other examinations. By using 200 TCID50 of virus, the sera with titer 1/8 and more also titer 1/10 for 100 TCID50 of virus were recorded as positive.

In SN test using NADL strain all sera with titer below 1/8, and about 10% of negative samples without any titer were chosen randomly. While 479 sera samples were tested by using BDV-Iran. 244 sera samples were selected randomly from 8

different herds in each province, each of 4.

Statistical analysis. Mac Nemar analysis was applied for comparison of SN and ELISA results.

Results

The results of SN test using NADL strain are showed in table and figure 1. Among

Table 1. *A comparative results of SN (NADL strain) and ELISA tests according to different provinces of Iran*

Province	Samples	Positive by SN	Infection rate by SN	Tested by ELISA	Positive by ELISA	Infection rate by ELISA
Chaharmahal	392	83	21.2	32	6	18.7
Isfahan	342	38	11.1	31	3	9.7
Markazi	341	30	12.4	30	4	13.3
Mazandaran	436	74	15.9	32	5	15.6
Khorasan	224	9	4.1	31	1	3.2
Khuzestan	218	32	14.5	32	4	12.5
W.Azarbayjan	224	19	8.5	31	3	9.7
Total	2104	258	13.5	219	26	11.9

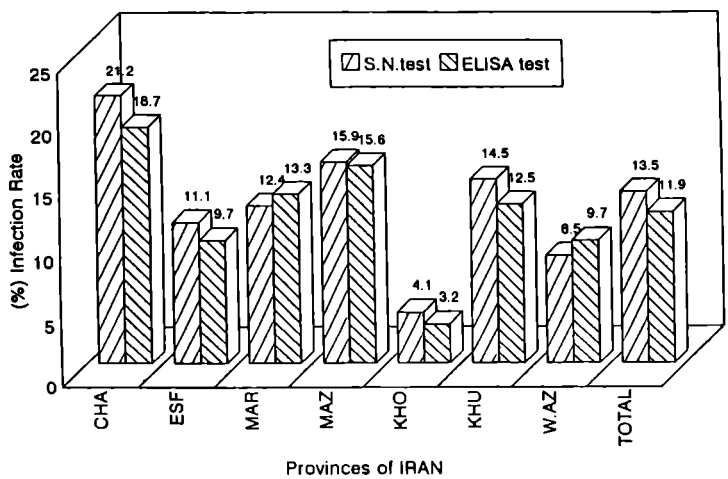

Figure 1. *A comparative results of SN (NADL strain) and ELISA tests according to different provinces of Iran*

2104 tested sera samples 285 (13.5%) were positive. The Chaharmahal Bakhtiary (21.2) and Khorasan (4.1%) had the highest and lowest rate, respectively.

Using BD-Iran strain by SN and ELISA tests the infection rate were 12.8% and 11.9% respectively. According to the ages, the youngest sheep group (4-8 mon) showed the lowest (1.8%) and sheep over 18 mon had the highest (14.3%) rate of infection (Table and figure 2). All of the 180 negative sera tested by SN using NADL strain and also examined by BD-Iran strain were negative, too. Therefore, it can conclude that all negative sera and false negative cases of them are equal in two different tests.

From 224 tested samples by ELISA, 5 samples could not be read due to some technical problems, however these samples were examined by SN test and were found to be negative. Out of 224 sera samples examined by ELISA 26 samples showed positive (11.9%) results.

Table 2. *A comparative results of SN (NADL strain) and ELISA tests according to age groups*

Age groups	Tested by SN (NADL)	Positive by SN (NADL)	Infection rate by SN (NADL)	Tested by ELISA	Positive by ELISA	Infection rate by ELISA
1-4 mon	187	24	12.8	19	2	10.5
4-18 mon	872	77	8.8	74	6	8.1
>18 mon	1045	184	17.6	126	18	14.3
Total	2104	285	13.5	219	26	11.9

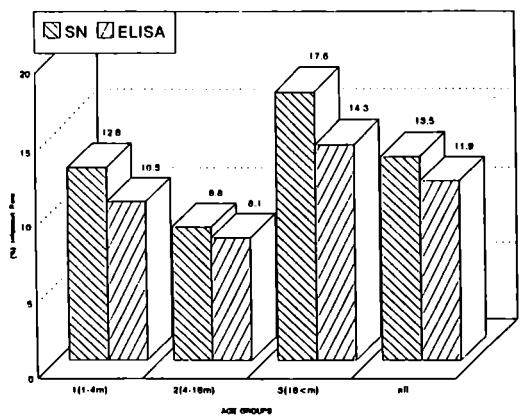

Figure 2. *A comparative results of SN (NADL strain) and ELISA tests according to age groups*

Discussion

The results indicate that the incidence of infection with border disease virus was relatively high, about 13.5% of total tested samples. This is coordinated with BVD infection rate in the same provinces in cattle (Sedigghinejad 1996).

It is suggested that the source of infection for cattle and sheep might be common. The occurrence of lowest rate of infection at the age group between 4-18 mon may be due to the diminishing of maternal antibodies, and the incidence of 10.5% infection rate at 1-4 mon old may be due to the presence of maternal antibody or intrauterine infection after immune system maturation. In this particular time of age if could differentiate maternal antibodies from nonmaternal antibodies, then the infection rate would be much lesser than it is present. The highest rate of infection in sheep over 18 mon may be due to direct contact with infected sources.

The statistical analysis (Mac Nemar) indicated that about 95.8% coordination there is between SN and ELISA. There is no significant differences (Z=1) in their results. Each of these two tests could be used for serological diagnosis. However because ELISA test indicates recently infected sheep and also give more specific results (98.7-99%) this test could be a comparable base for other serologic test. The sensitivity and specificity of SN test according to homologues and heterologus strains has been determined about 90-92% and 85-89%, respectively (Fenton *et al* 1991,1990).

With respects to the results obtained in this survey, it is suggested that for investigating the prevalence of the disease or screening, the SN test may be more useful, but for an accurate diagnosis the ELISA test is recommended. Another important point that may be noted is the presence of PI sheep in infected herd. These animals cannot be detected by usual serological methods thus the other techniques such as immunocapture ELISA, PCR or immunoblotting to detect the antigen must be used.

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