Journal of Animal and Veterinary Advances 10 (10): 1283-1286, 2011
ISSN: 1680-5593

Serological Evaluation of Sheep Border Disease in Sarab City, Iran

Mohammad Ali Dadkhah, Mahdi Yeganehzad, Farid Sarkarati and Behnaz Nadery
Department of Veterinary Medicine, Islamic Azad University,
Sarab Branch, Sarab, Iran

Abstract: Border disease has always been considered as a sheep viral disease with relatively high prevalence all over the world, involving in abortion syndromes and creating of weak and abnormal lambs in countries where sheep husbandry industry is of high importance. Border Disease Virus (BDV) belongs to the genus *Pestivirus* within the family Flaviviridae and leads to abortion or birth of abnormal lambs in pregnant ewes. Such lambs often perish and constantly excrete virus, if continue their life time. Persistently infected ewes keep the virus for time and excrete it through saliva, faeces and urine. Considering that Sarab is regarded as an important bestial pole in northwest of Iran, it is necessary to study diseases damaging industry of animal husbandry in order to offer solutions required to control and prevent the diseases through identifying its causes. In present study, blood samples were collected from 100 ewes of different farms found in the region, sera were separated and stored at -30°C until conducting the experiments. Infection rate of the samples and antibody titer were distinicted through use of Serum Neutralization (SN) test on sera in laboratory. Finally it was found that 16% of the understudy samples were infected by the virus and had a distinictive antibodytiter.

Key words: Serological, border disease, flaviviride, pestivirus, ewe, SN test, antibody

INTRODUCTION

Border is a congenital viral disease first reported in a sheep in border region of Wales and England in 1959 (Chalmers *et al*., 1990). The agent belongs to the genus *Pestivirus* within the family Flaviviridae. Disease 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 (Nettleton and Entrican, 1995). Almost all isolates of Border viruses are non-cytopathic in cell culture. Distribution of the virus is worldwide and prevalence rates varies from 5-50% between countries and from region to region within countries. In Iran, the viral infection varies in different provinces (Keyvanfar and Karimi, 1997).

Prior to border, a contagious and apparently new disease in bovines at one of the regions of New York characterized by fever, mouth and digestive system erosion and ulcers and sever diarrhea (Olafson *et al*., 1946). Considering serological features and different serum reactions, the virus causes Border may not be distinguished from viral diarrhea and swine pest agents such that agent of these three diseases are known to be of the same genus or undistinguishable variety of one genus. Historically, studies conducted on swine pest virus in America at the end of the past century were initial studies considering this genus of viruses (Loken *et al*., 1990). Swine pest agent has been named as swine pest bacillus in the left unknown reports. In 1904, Shouintz and Doursea found that body liquids of infected pigs can transmit the disease touninfected ones (Loken, 1995). Viral nature of the agent was considered after transmissibility of the agent from micropore filters was confirmed (Charke and Osburn, 1975).

The disease is usually transmitted in two horizontal (contact) and vertical (congenital) ways. Among horizontal transmission which often is hidden, respiratory transmit is of high importance (Derbyshire and Barlow, 1976). In the second way, i.e., vertical one, the disease in transferred by pregnant sheep to their generation and lead to Hairy shaker disease. Of course, pregnancy stage or fetus age in uterus is one of the important factors involved in appearing of viral pathogenesis in fetus (Chalmers *et al*., 1990). If fetus was infected to the virus before completing of its immune system, it will be unable to respond the antigen, the virus will be distributed in all parts of its body and it will die. The infection will permanent in embryo's body if it can continue its life (Barlow *et al*., 1975).

Corresponding Author: Mohammad Ali Dadkhah, Frhanghian Alley, Islamic Azad University, Sarab Branch, Sarab, East Azarbaijan, Iran

In mature sheep, the infection is always subclinical. But infection in pregnant ewes leads to born of abnormal lambs or stillbirth. Abnormal lambs do not grow well and are light weighted at birth (Sansom *et al*., 1979). The conformation occurs in different ways including unusual fleece, muscles trembling, hypomyelinogenesis in nerve fibers and nervous system and deformity of bones (dome-shaped). In experimental and natural conditions, it seems that some 20 week aged lambs are recovered and no symptoms of tremor and unusual fleece are observed in them. These lambs grow slowly are about 20% light weighted from their coevals and their mutton is of low quality (Keyvanfar and Karimi, 1997).

In necropsy findings, apparent lesions are seen as microcephaly. In histology, reduction of myelin growth is important. There are deficiency and increase in lipid materials constituting myelin and esterified cholesterols of nerve tissue, respectively (Oguzoglu *et al*., 2009).

Nerve lesions disappear in the living 20 weeks aged lambs, microcephaly becomes normal considering weight and chemical compounds and their myelin normalized. Disease agent may be isolated by tissue culture. In addition, other manifestations such as Arthrogryposis, Hydrocephaly, Spongiosis in brain cells and cerebellum defects (Shimi, 1996). The disease is diagnosed considering clinical symptoms, epidemiological status and microscopic study of the brain tissue. It is possible to identify virus antigen through immunofluorescence reaction. Isolating the virus from tissue culture and serum tests including immunodiffusion, complement fixation test, Virus neutralization assay, immunofluorescence reaction are regarded as different ways for laboratory diagnosis of the disease (Akkina and Raisch, 1990). It should be noted that no disease is similar to this one and clinical symptoms and microscopic study of lesions is generally sufficient for diagnosing of the disease nature (Buxton and Rase, 1977). To prevent the disease, suspected or infected ewes should be kept away from others for 3 weeks after mating (Chalmers *et al*., 1990). All infected lambs should be slaughtered. Bovin infected with MD should be isolated from sheep because it is thought that these bovin can infect the sheep if they are raised together (Barlow *et al*., 1975).

MATERIALS AND METHODS

In this study, blood samples were collected from 100 ewes of different flocks, sera were separated and stored in freezer at -30°C until conducting the experiments. Then, sera were sent to laboratory in order to conduct required tests and infection rate of the samples was distinicted through use of Serum Neutralization (SN) test (Shimi, 1996). In SN test, the initial step is choosing the cell which is appropriate for cell culture required for the experiment. In this research, BK-KH cell line was used for SN test considering that ruminant pestiviruses are well propagated in cell cultures with bovine and sheep origin and their cytopathic strains create distinguishable CPE in cell cultures after some days. Iran's BD virus and NADL strain of BVD create appropriate CPE in this cell line after 4 days. MEM (Minimum Essential Medium) media is the appropriate for this cell line. Of course, Hanks media can also be used for cell propagation after habituating the cell in the mentioned media. To conduct the study, different virus dilutions and different standard positive and negative dilutions of sera were prepared from Savanovir Institute of Sweden (Fig. 1). Then sera samples were taken out of freezing conditions and 1/2, 1/4, 1/8, 1/16, 1/32 dilutions of each serum sample were prepared at MEM cell culture media. About 0.5 mL of each dilution was mixed with 0.5 mL of each diluted virus and the glass tubes were put in 37°C incubator for 1 h to neutralization the samples. For each series of study, some culture tubes are served as the controls for virus, serum and cell. Cell control tubes should have complete cellular layers and no effects of CPE should be seen there. Then, virus control tubes are read. The first tube had 0.2 mL of virus and therefore created distinguishable CPE. The 2nd, 3rd, 4th and 5th tubes had 2.5, 1.75, 0.875, 0.437 $TCID_{50}$ of virus, respectively. As a result, CPE was created in the Ist, 2nd and 3rd tubes but not in the 4th and 5th ones. These results indicate correctness of initial titer of virus used in SN test. After reading the control tubes resulted from the samples will be read. Tubes include samples are read from 1/2 and those tubes no CPE has been created in them will be noted as serum titer. All samples qualified the titer over 1/2 are specified for the next experiments. In this study, the sera with titer over 1/8 of virus were recorded as positive.

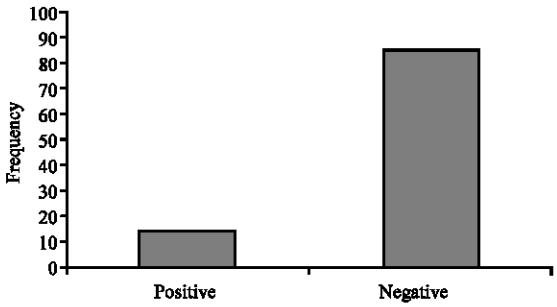

Fig. 1: Infection rate of sheep border disease in collected sera

RESULTS AND DISCUSSION

Considering that Sarab is regarded as an important bestial pole in northwest of Iran and Moghan tribes immigrate to the region every year, this research which has been conducted as a field research demonstrated that 16% of different samples-collected from cattle found throughout the region were infected by the virus of this disease and had distinictive antibody titer. This indicates the importance of the subject and necessity of exercising practical approaches to prevent and control the disease.

Most researchers attribute manifestation of clinical symptoms of Border disease in sheep to several factors including kind of virus, maternal immunity, animal's race and other animal's individual factors such that just very few percent of pregnant ewes give birth to abnormal lambs if the virus enters a sensitive population (Shimi, 1996). Lambs which are somehow infected by the virus during embryonic period, live substantially for a shorter period. Comparing with other lambs at the same group, they have short lifetime, less growth rate and more sensitivity (Al-Muarrawi, 1986).

Appearing of congenital deformities in a similar population depends on the embryo's age. If the virus has a contact with fetus which is younger than one month i.e., before formation of its immune system, immune tolerance state is created in the fetus and the lamb keeps the virus in its body and constantly excrete it without having a serum with distinguishable cell (Fener *et al*., 1993).

Persistently Infected (PI) lambs is one of the most important points which should be considered. Although, these animals are infected by the virus, they can not be identified through serological tests. Concurrent application of tests, antigen-antibody detection in an infected population is one of the best plans for identifying patients (Derbyshire and Barlow, 1976). Considering that virus culture/isolation are almost practically impossible in all infected populations due to sensitivity of virus, difficulty of isolation and diagnosis plan, use of other methods such as ELISA and gel diffusion have been recommended for detecting virus antigens (Barlow *et al*., 1975).

CONCLUSION

Regarding cycling of ruminant pestiviruses among bovine and sheep populations and also observing clinical cases of Border disease in some points of the country, it seems that rate of infection to virus of this disease at different parts of the country reflexes BVD status. Conducting several studies, researchers suggested uses of inactivated or mild vaccine of BVD to prevent Border. High titer of antibody reflects widespread of the disease among sheep population of the country. This requires more attention of health sector authorities to prevent losses imposed by this disease to the industry of animal husbandry in the country (Keyvanfar and Karimi, 1997).

ACKNOWLEDGEMENT

The study was supported financially by Islamic Azad University of Sarab Branch.

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