10 years - **ANNIVERSARY EDITION *TRAKIA JOURNAL OF SCIENCES***
Trakia Journal of Sciences, Vol. 10, No 1, pp 76-78, 2012

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

Original Contribution

COMPARATIVE SEROLOGICAL STUDIES OF BOVINE VIRAL DIARRHEA DISEASE IN CATTLE HERD POPULATIONS IN REPUBLICS OF IRAQ AND BULGARIA BY USING SEROLOGICAL TEST

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ABSTRACT
Bovine viral diarrhea (BVD) is endemic in cattle all over the world. It caused highly economic losses worldwide. This study demonstrated presence of antibodies of bovine viral diarrhoea virus in blood sera samples collected from cattle herds in Republic of Iraq and Bulgaria by using ELISA method in 2010. Blood sera samples collected from cows in different ages, all animals were not vaccinated.
The number of tested cows in Republic of Bulgaria were 309, and in Republic of Iraq - 188. The highest seropositive results were found in animals from Iraqi farms (33 %), while the lowest seropositive samples-in Bulgarian farms (22.3 %).

Key words: bovine viral dearrhea, ELISA, Republic of Iraq, Republic of Bulgaria

INTRODUCTION

Bovine Virus Diarrhea Virus (BVDV) as an important member of the Pestivirus genus is a very common agent affecting livestock production throughout the world. Serological studies have shown that the presence of antibodies to BVDV in cattle is 12 - 90% (1-5). Economic losses are directly related to multiple clinical forms of the infection that vary from the subtle enteric infection to the fatal mucosal disease caused by a combination of cytopathic (cp) and non-cytopathic (ncp) biotypes of the virus (6 - 8). The other consequence with epidemiological importance is the birth of persistently infected (PI) calves. PI calves are born into herds as a result of intrauterine infection with NCP biotype at early gestation. PI animals serve as a virus source by shedding the virus life along with various body excretions and are therefore responsible for the presence of the virus within herds (9).

For epidemiological purposes, it is important to detect the viremic status of animals in addition to their serological status. As a general approach for distinguishing PI animals from those infected transiently (acute), the most common way is to repeatedly isolate the virus in, at least. Sensitive diagnostic methods for rapid identification and elimination of persistent carriers in the herds are therefore of significant importance (10). Common detection of the virus in clinical samples is carried out using standard techniques of isolation in cell cultures or identification of viral antigens by immunoperoxidase and immunofluorescence tests or by demonstration of BVDV antigens in serum by ELISA. Some of these methods are laborious and less sensitive, other are expensive due to high-cost, examinations of individual animals. Therefore, molecular analytical methods have been commonly used for the detection of BVD virus in different clinical samples using RT-PCR followed by visualization on agarose gels. These methods are more sensitive than common ELISA assays (11).

In Bulgaria the disease establish in 1966 and isolation of Bulgarian strain in 1975 (12). More recently study identificated the presence of BVDV antibodies in blood serum samples and demonstrated reproductive problems in

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affected cattle herds, which revealed that highest percentage in calves (13).

In Republic of Iraq, very little studies about BVDV, but some research revealed that first demonstrated of disease in year 1977 (14). BVDV antibodies in cattle were tested in different areas in Iraq. In year 2004 demonstrated cytopathogenic strain in cultured cells in vitro (15). Another study detected the virus in buffalos and cow herds, also demonstrated persistent infection (16).

MATERIAL AND METHODS

Animals

Three herds from 3 different regions in Bulgaria included Gabrovo, Haskovo and Lovech these herds not vaccinated and the animal were in different ages.

In Iraq, samples were collected from two region in South of Iraq - Basrah city and Thi-qar city. Animals selected randomly because there were no big herds their, only 3-8 cows owned by every farmer in these two cities.

Serological tests

Samples of blood were collected in 10 ml tubes without anticoagulant and centrifuged at 2500 rpm for 20 minutes in order to obtain sera samples. These bovine sera were refrigerated for one day then tested for detected of antibodies to BVDV by commercial kits (BVDV antibodies test kit: IDEXX Herd Laboratories).

The test were carried out depend on the manufacture instructions. Briefly, the antibodies detected kits required the sera incubated with antigens well coated in microplate of kits then washing to remove bound materials after that a specific peroxidase-conjugated monoclonal antibody was added for being detected with substrate/chromagen solution.

The color absorbance generated by the substrate-chromagen reaction was measured by using spectrophotometer (ELISA reader) at single wave 450 nm.

RESULTS AND DISCUSSION

The results of study revealed presence of disease in all herds in the two countries in different values which summarized in **Table 1**. The serum samples percentage of the above values were showed that the highest percentage of seropositive samples found in Basrah city (46.8 %), that indicate to highly distributed disease in this area when we compared results taken from Thi-Qar city (19.1 %), the reason may be related to high humid weather in Basrah city because it is lower area in Iraq and it located near Arabian gulf that may help virus to survive a long time compared to dried and highly heated weather in Thi-Qar city. Thi-Qar conclusion building upon the physiological features of virus which is not survive more than 30 minutes in highly temperature area about 56 C^0 (14).

Table 1. *The results of tested animals in the two countries*

Herd name (Region)	No. of tested animals	Positive result	Negative result
Velkovtci (Gabrovo)	199	56	143
Krepost (Haskovo)	55	13	42
Chavdarci (Lovech)	55	0	55
Thi-Qar (Thi-Qar, Iraq)	94	18	76
Basrah (Basrah, Iraq))	94	44	50

The mean problem of cattle populations in most Iraqi cities is absence of regular data about animal's sources, because farmers not keeping same cows may sell or slaughter them.

In Republic of Bulgaria the highest percentage occur in Velkovtci herd in Gabrovo city about (28.1 %), while in Chavdartci in Lovech city were 0 %. That may indicate two different ideas, first one that the herd is clean from infection other thing the herd has PI animals which carry the virus life-span for ensuring the second opinion by test the animal with ELISA Ag kit, or by other virus detecting methods such as reverse transcriptase - polymerase chain reaction (RT-PCR).

Conclusion from above results that the disease is demonstrated in two countries and must be build solutions to control and cleaning herds from this complicated virus due to high economic losses when it distribution between cattle herds or other animals susceptible to infection.

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