



## Original Research Article

### Detection of border disease in ovine using ELISA in Iraq

Khawlah M.I. Al-Rubayie\* and Saleem A. Hasso

Department of Veterinary Internal and Preventive Medicine, College of Veterinary  
Medicine - Baghdad University, Iraq

\*Corresponding author

#### A B S T R A C T

##### Keywords

Border  
disease  
virus;  
sheep;  
ELISA  
Iraq

A study was conducted to detect the border disease (BD) in Iraqi sheep. Specific antibodies were detected against the causative agent of (BD) virus in ovine sera by testing (552) sera samples in six governorates (Baghdad, Babel, Anbar, Salah Alden, Najaf and Karbala). All sera samples were examined by ELISA kits specific to border disease, the positive total samples percentage was 30.35%, whereas the proportions according to the governorates were 36.9% (Baghdad), 27.1% (Babel), 32.6% (Anbar), 21.7% (Salah Alden), 30.4% (Najaf) and 32.6% (Karbala). Odds ratio (OR) estimations for disease for each of Baghdad, Babel, Anbar, Najaf and Karbala compared to Salah Alden were 2.11 (P=0.02), 1.34 (P=0.39), 1.74 (P=0.18), 1.80 (P=0.03), 1.74(P=0.18) respectively. The presence of antibodies against (BDV) indicates the presence of the causative agent in Iraq.

## Introduction

Border disease (BD) is a congenital virus disease of sheep and goats first reported in 1959 from the border region of England and wales. (Hughes et al., 1959). BD virus (BDV) is a pestivirus genus within the family flaviviridae and is closely related to bovine virus diarrhea virus (BVDV) and classical swine fever virus(CSF) (Becher, 1994 ; Paton et al., 1995); (Fauquet et al., 2005). Synonyms are "hairy shaker disease" and "fuzzy-lamb syndrome", as some BD lambs show characteristic signs of body tremors and/or hairiness; This syndrome in sheep is a congenital, teratogenic infection (Terpstra, 1981).

Border disease virus (BDV) is an important sheep pathogen causing significant losses in sheep farming (Nettleton et al., 1998). Distribution of the BDV is worldwide (Czopowicz, et al., 2011). Prevalence rates vary in sheep from 5 to 50% between countries and from region to region ( Nettleton et al., 1998). Clinical signs in sheep include barren ewes ,abortion , stillbirth and the birth of small weak lamb syndrome (Nettleton, 2004). The BD cases a new-born kid presenting central nervous system (CNS) signs (Loken et al., 1982)

Infection of fetuses can result in the birth of persistently infected (PI) lambs (Keyvanfar and Karimi, 1997). The virus spreads from sheep to sheep with (PI) animals being the most potent source of infection (Laude and Gelfi, 1979). Vertical transmission plays an important role in the epidemiology of the disease. Pestiviruses may also spread by ear-tagging, castration and oral infusion, and even through rectal examination using gloves contaminated with feces from a PI animal (Lang-Ree et al., 1994). Sheep may also be infected following close contact with cattle excreting the closely related BVDV (Wensvoort et al., 1989). Both BVDV and BDV infect cattle, sheep, goats, and many other wild ruminants and pigs but in clinical conditions, BVDV is mostly found in cattle, whereas both BDV and BVDV can be isolated from sheep (Strong et al., 2010). BDV infections in pregnant goats result in abortion and malformation in fetuses and neonates (Depner et al., 1990; Nettleton et al., 1998). The BDV has been reported in sheep in Iran (Keyvanfar and Karimi, 1997).

Czopowicz et al., (2011) detected antibodies against border disease in goat in Poland; it was also detected in sheep in Spain (Mainar-Jaime and Vazquez-Boland, 1999) and in sheep in Turkey (Yazici et al., 2012) as well as in Austria (Schiefer et al., 2006). A new BDV subgroup was reported in Turkey (Oguzoglut et al., 2001). No survey has been done in small ruminants (sheep and goats) neither on the antigen nor the antibody of BDV in Iraq.

## Materials and Methods

Five hundred fifty two ovine blood samples were collected without anticoagulant, sera were separated by

centrifugation and stored at -20 °C until serological testing.

SVANOVIR BDV- Abs is an indirect ELISA (Enzyme immune sorbent assay) for the detection of border disease virus in small ruminants, it was used according to the company's instructions to detect the antibody to BDV in sheep sera.

Control positive and negative reference sera were included in every test. These should give results within predetermined limits for the test to be considered valid.

Ovine sera samples were tested to determine the rate of infection of BDV in Iraq.

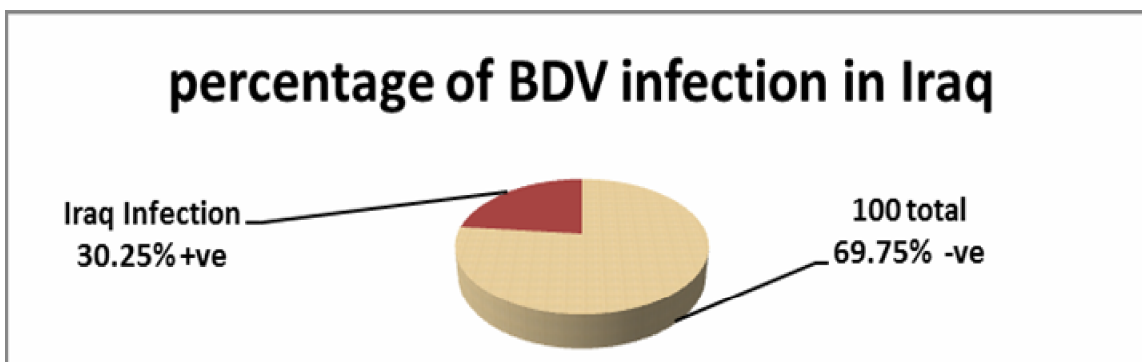
Data were analyzed by using SAS Program. Proportions of incidence were estimated. Also OR were estimated for each governorate compared to Salah Alden which considered as base.

## Results and Discussion

Out of 552 ovine sera samples collected randomly from six governorates examined by ELISA kits (SVANOVIR® BDV- Ab) specific to border disease, the positive total samples were 167 (30.25%) (fig-1) and according to the governorates were in Baghdad 34 +ve sera samples with 36.9%, Babel 25 +ve in 27.1%, Anbar 30 +ve in 32.6%, Salah Alden 20 +ve in 21.7%, Najaf 28 +ve in 30.4% and Karbala 30 +ve in 32.6% (Table -1-). The presence of antibodies against (BDV) indicates the presence of the disease in Iraq.

Odds ratio estimations for BDV disease for each of Baghdad, Babel, Najaf and Karbala compared to Salah Alden were 2.11 (P=0.02), 1.34 (P=0.39), 1.74 (P=0.18), 1.80 (P=0.03), 1.74 (P=0.18)

**Fig.1** BDV Antibody percentage of in ovine in Iraq



**Table.1** The percentage of BDV antibodies in the capital (Baghdad) and some governorate of Iraq

Governorate	Samples	Negative	Positive	Percentage Positive
Baghdad	92	58	34	36.9 %
Babble	92	67	25	27.1 %
Anbar	92	62	30	32.6 %
Salah Alden	92	72	20	21.7 %
Najaf	92	64	28	30.4 %
Karbala	92	62	30	32.6 %
Total	552	385	167	30.25 %

**Table.2** The odds ratio in capital and some governorate compared with Salah Alden

Odds ratio of incidence in governorate compared with Salah Alden				
Governorates	Odds ratio	95 % CI	Z statistic	P
Salah Alden	1			
Baghdad	2.11	1.09 to 4.04	2.246	0.02
Karbala	1.74	0.90 to 3.36	1.338	0.18
Najaf	1.80	1.03 to 3.13	2.076	0.03
Babel	1.34	0.68 to 2.63	1.648	0.39
Anbar	1.74	0.90 to 3.36	1.338	0.18

respectively (Table 2). Out of 552 ovine sera samples examined by ELISA 167 +ve in (30.35%) this result is agrees with the results which indicated that the overall seroprevalences of pestivirus antibody in sheep and goat were 46.62% and 32.871% (Prevalence of pestivirus in Iran) respectively. The results indicate that the rate of infection with BDV was relatively high in Baghdad, about 36.9 % of total tested samples (fig 2) this coordinate with BVD infection rate in the same governorate (Baghdad) in cattle (khawlah M.I.Al-Rubayie and Saleem A.Hasso., 2012). Using ELISA in 552 tested sheep sera, the overall detected seropositive of pestivirus was 36.9% highest seroprevalence was detected in sera collected from the capital Baghdad because it is near to Iran from one side and the present of many small ruminant needed for religious condition by the visitors to Al- Kademia, while the lowest seroprevalence (21.7%) was detected in Salah Alden because the visitors were lesser to this governorate . In Al-Anbar it was (32.6%) mostly due to it being a border governorate to Syria and Jordan but still less than Baghdad as in (fig.2). BDV antibodies percentage in Karbala and Najaf were 32.6%, 30.4% respectively, the percentages were higher because of the many visitors from whole world especially Iranian to the holey shrines. Babel is a governorate with lesser amount of visitor than the above (Table-1-). The highest seroprevalence records were recorded in Iran and Sudan.

The significant of OR was shown in two governorates (Baghdad and Karbala). These result could be attribute to existing of religious locations in those two governorates which could make them more exposed to BDV disease as a result of large number of visitors (carriers) coming from Iran

The presence of antibody in the serum indicate the presence of BDV, this is the first recording of this specific disease in Iraq. It is imperative to conduct further studies, by PCR genotyping and virus isolation to confirm our results.

### **Acknowledgement**

We thank our GOD faithfulness and helpfulness to do this study.

### **References**

- Becher, P.1994. Molecular characterization of Border disease virus, a pestivirus of sheep. *Virology* 198:542
- Czopowicz M, Jarosław K., Schirrmeier H, Bagnicka E, Szalus-Jordanow O, Nowicki M, Witkowski L., Frymus T 2011.
- Depner, K.R., Hubschle, O.J., Liess, B., 1990. Transplacental BVD virus transmission after experimental inoculation of goats in different pregnancy stages. *Dtsch. tierärztl. Wochenschr.* 97, 421–423.
- Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA 2005: *Virus Taxonomy, Classification and Nomenclature of Viruses*. Eighth Report of the International Committee on Taxonomy of Viruses. Academic Press, London. 1162 pp.
- Hughes L.E., Kershaw G.F. 1959: "B" or Border disease, an undescribed disease of sheep. *Veterinary Record*, 71, 313–317
- Keyvanfar, H. and N.karimi, 1997. *Veterinary Virology* .3rd Edn.,Tehran University Publication .Tehran
- Khawlah M.I.Al-Rubayie; Saleem A.Hasso 2012.detection of bovine viral diarrhea

- mucosal disease BVD-Din buffaloes and cows using ELISA, the Iraqi J.Vet.Med.361:45-50;2012
- Krametter-Froetscher R., Kohler H., Benetka V., Moestl K., Golja F., Vilcek S., Baumgartner W. 2007b: Influence of communal Alpine pasturing on the spread of Pestiviruses among sheep and goats in Austria: first identification of Border Disease Virus in Austria. *Zoonoses and Public Health*, 54, 209–213
- Lang-Ree JR, Vatn T, Kommissrud E, et al. Transmission of bovine viral diarrhoea virus by rectal examination. *Vet Rec* 1994; 135:412-413
- Laude H, Gelfi J. Properties of Border disease virus as studied in a sheep cell line. *Arch Virol* 1979;.346-62:341 .PubMed –
- Löken T, Bjerkås I, Hyllseth B. Border disease in goats in Norway. *Res Vet Sci* 1982; 33:130-131.
- Thabti F., Fronzaroli L., Dliissi E., Guibert J.M., Hammami S., Pepin M. & Russo P. 2002. Experimental model of border disease virus infection in lambs: comparative pathogenicity of pestiviruses isolated in France and Tunisia. *Vet. Res.*, 33, 35–45.
- Nettleton, P.F. 2004. Border disease. In: J.A.W. Coetzer and R.C. Tustin, Eds. *Infectious Diseases of Livestock* 2nd edn.. Pp: 970-974. Oxford University Press
- Nettleton P.F., Gilray J.A., Russo P., Dliissi E.1998: Border disease of sheep and goats. *Veterinary Research*, 29, 327–340.
- Oguzoglu T.C., Floegel-Niesmann G., Frey H.R. & Moennig V. 2001. Differential diagnosis of classical swine fever and border disease: seroepidemiological investigation of a pestivirus infection on a mixed sheep and swine farm. *Dtsch Tierarztl. Wochenschr.*, 108, 210–213.
- Paton D.J., Sands J.J., Lowings J.P., Smith J.E., Iyata G. & Edwards S. 1995. A proposed division of the pestivirus genus into subgroups using monoclonal antibodies, supported by cross-neutralization assays and genetic sequencing. *Vet. Res.*, 26, 92–109.
- Schiefer P, Krametter-Frötscher R, Schleiner A, Loitsch A, Golja F, Möstl K, Baumgartner W 2006. Seroprevalence of antibodies to ruminant pestiviruses in sheep and goats in Tyrol Austria. *Dtsch Tierarztl Wochenschr.* 1132:55-58.
- Strong R, La Rocca SA, Iyata G, Sandvik T: Antigenic and genetic characterisation of border disease viruses isolated from UK cattle. *Vet Microbiol* 2010, 141:208–215.
- Terpstra C. Border disease. Virus persistence, antibody response and transmission studies. *Res Vet Sci.*1981; 30:185-191. – PubMed
- Wensvoort G, Terpstra C, De Kluyver EP. Characterization of porcine and some ruminant pestiviruses by cross-neutralization. *Vet. Microbiol.* 1989; 20:291-306. PubMed –
- Yazici Z, Serdar MS, Gumusova SO, Albayrak H 2012. Molecular diagnosis and seroepidemiology of pestiviruses in sheep. *Veterinarski Arhiv* 821 35-45