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Molecular investigation of abortions induced by Bunya virus / or Flavivirus infections in Mazandaran province, northern Iran

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The aim of this study was to identify border disease (BD) in aborted embryos or lambs with neurological disorders in different area of Mazandaran province, northern Iran. In this study, 75 aborted lambs and embryos were evaluated by Real time PCR using a commercial kit consisting of reverse transcript and polymerase enzyme that performs both reverse process and DNA polymerase activity. Brain and spinal cord samples were collected from each fetus by necropsy. In addition, in some cases, blood samples were collected randomly from sheep and aborted fetuses. After coagulation, the serum was isolated and stored at minus 20 ° C until serological testing. We recorded only one case of infection with the border disease virus (BDV) in an embryo as well as PCR showed negative for brucella, listeria, campylobacter, and mycoplasma, and other viral agents, parasitic and fungal infectious agents. Therefore, this study suggests that aborted embryos and lambs with neurological problems need to be examined by molecular methods for pathogens such as Border virus.

Keywords: Border disease, Abortion, Real time PCR, Neurological, Mazandaran

INTRODUCTION

Border disease (BD) is a worldwide viral disease of sheep and goat caused by border disease virus (BDV) that occasionally infects cattle and wild ruminants. BDV belongs to the family Flaviviridae (genus Pestivirus). BDV is capable of crossing the placenta and infecting the fetus with various consequences.

Infection of pregnant ewes with BDV before foetal immunocompetence at 85th day of gestation can be associated with the birth of persistently infected animals (PIs), resulting in the continuous shedding of the virus throughout their lives and maintaining pestiviruses in a flock (Nettleton et al. 1998 and 1990).

This disease causes economic losses to the sheep breeding industry due to barrenness, abortion, still birth, weak lambs, etc. (Fernández-Sirera et al. 2012; Mishra et al. 2012; Oguzoglu et al. 2009; Cabezon et al. 2010). Postnatal infection in sheep has been described to be mild and characterized by mild pyrexia and transient lymphopenia, and seroconversion (Nettleton et al. 1998).

BDV epidemics have been reported in certain areas of the world (Giangaspero et al. 2011; McFadden et al. 2012; Marco et al. 2008), but is remains underreported in Iran where clinical signs may be unnoticed because of other endemic diseases, e.g., brucellosis, bluetongue, peste des petits ruminants (PPR). Here we confirm the identification of BDV isolated from aborted lambs (sheep) in Iran.

MATERIALS AND METHODS

Sample collection

This study was performed on 75 aborted and dead lambs suspected of having neurological disorders at different area of Mazandaran province, northern Iran. Brain and spinal cord samples were collected from each fetus by necropsy.

RNA Extraction

Samples taken from the brain and spinal cord of aborted fetuses were used for Real time PCR. RNA was extracted from samples using Qiaamp viral RNA extraction kit (Qiagen company; Cat No 5206) following the manufacturer instructions.

Real time PCR methods

The reaction was performed using a commercial kit consisting of reverse transcript and polymerase enzyme that performs both reverse process and DNA polymerase activity. Furthermore, setting of the threshold and the quantification cycle (C_q) values were established based on the default settings. The genes and primers used are listed in Table 1.

Table1: The genes and primers used in PCR

BDV87F	CCG TGT TAA CCA TAC ACG TAG TAG GA
BDV237R	GCC CTC GTC CAC GTA GCA
BDV136T	VIC-CTCAGGGATCTCACCACGA-NFQ-MGB

Amplification and qPCR measurements were performed using the Roche Light Cycler 480 in 25 µl total volume consisting of 22.5 µl of BVD Master Mix, and 2.5 µl of RNA. The cycling conditions were: 42 ° C for 5 min, 95 ° C for 10 s, 40 cycles of 95°C for, and 60 ° C for 34 s. The assays were also assessed using clinical samples from positive control subjects.

RESULTS AND DISCUSSION

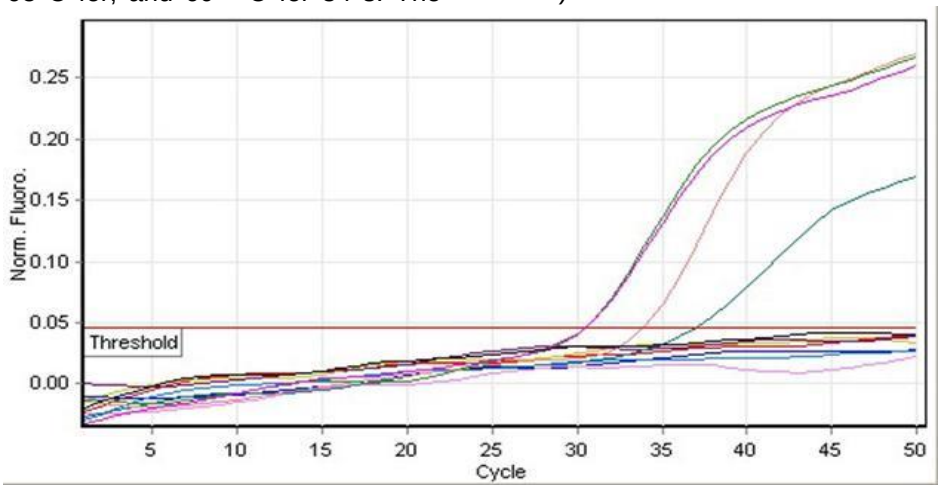
In this study, samples taken from the brain and spinal cord of aborted fetuses were evaluated using Real time PCR. The results of this study showed that only a sample of 75 available samples was positive for BDV (Figures 1 and 2).

In some cases, blood samples were collected randomly from sheep and aborted fetuses. After coagulation, the serum was isolated and stored at minus 20 ° C until serological testing, but the results were negative for Brucella, Listeria, Campylobacter, and Mycoplasma, as well as for other viral, parasitic and fungal infectious agents.

Quantitation data for Cycling A. Green was shown in figure 1 and 2, revealing BDV positive BDV samples of brain and spinal cord

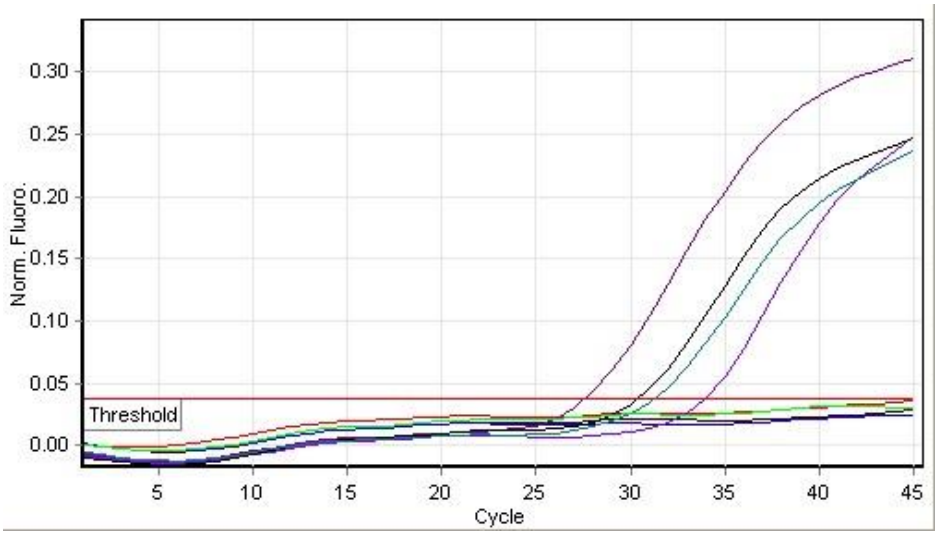
This is the report confirming the presence of BDV isolated from aborted lambs (sheep) in Iran by real time RT-PCR but negative for Brucella, Listeria, Campylobacter, and Mycoplasma, as well as for other viral, parasitic and fungal infectious agents. The most commonly used tests for detection of BDV PI sheep are virus isolation, BVDV Ag-ELISA or BDV specific real-time RTPCR (OIE, 2017).

BDV has been described to be associated with prenatal and postnatal infections in animals, leading to reproductive disorders, and birth of unviable lambs, as well as mortality during epidemics and consequent economic losses and outbreaks in some countries, leading to huge economic losses (McFadden et al. 2012; Dubois et al. 2008; Giangaspero et al. 2011; Marco et al. 2015).



No.	Colour	Name	Type	Ct	Given Conc (Copies)	Calc Conc (Copies)	% Var
1		Ghaffari b	Unknown				
2		Ghaffari b	Unknown				
3		Joybar b	Unknown				
4		Joybar	Unknown				
5		Nazaryan b	Unknown				
6		Nazaryan	Unknown				
7		Gasemi	Unknown	36.91			
8		Pos Type1	Unknown	33.87			
9		Pos Type2	Unknown	30.47			
10		Pos BDV	Unknown	30.45			

Figure 1: Real time PCR reaction of BDV positive control and positive expression of BDV in fetal spinal cord sample



No.	Colour	Name	Type	Ct	Given Conc (Copies)	Calc Conc (Copies)	% Var
1		Arab	Unknown				
2		Sepehr	Unknown				
3		Asghari	Unknown				
4		Ghasemi pos	Unknown	27.58			
5		Type1 pos	Unknown	33.96			
6		Type2 Pos	Unknown	30.35			
7		Border Pos	Unknown	31.29			

Figure 2: Real time PCR reaction of BDV positive control and positive expression BDV in fetal brain sample

Our findings revealed that BDV was present in the sheep from Mazandaran, Northern Iran, and indicating necessity of monitoring and comprehensive investigation (i.e., Molecular epidemiological and genetic studies) on BDV in Iran. It is worth remarking that this can be considered as target site of BDV investigations to provide ample evidence for the detection of origin, and control of BDV in this region.

CONCLUSION

This is study is a report on the detection of BDV isolated from aborted lambs (sheep) in Iran, suggesting that aborted embryos and lambs with neurological problems need to be examined by BDV real time RT-PCR assay development.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHOR CONTRIBUTIONS

AT, MNSH and JJ drafted, designed and performed the experiments and also wrote the manuscript. All authors read and approved the final version.

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