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Invited Review

A Review of Border Disease Virus Infection in Ruminants: Molecular Characterization, Pathogenesis, Diagnosis and Control

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ABSTRACT

This review includes the recent studies of virological diagnosis, agent characterization, pathogenesis, and control of Border Disease (BD) infection caused by viruses in the genus Pestivirus. BD virus (BDV), closely related to Bovine Viral Diarrhea Virus and Classical Swine Fever Virus, is classified in the genus Pestivirus of the family Flaviviridae. Based on phylogenetic analysis, ovine pestiviruses are segregated at least into six clusters, using the 5'-NTR and N^{pro} gene region sequences. Phylogenetic analysis of BDV isolated from Turkey isolates reveal that the virus form a distinct cluster as BDV-7 and BDV-3, which isolates are being more closely related to CSFVs than to the known BDV-3 subgenotype that contains pig-derived Pestivirus obtained from sheep. BD infection is characterized by abortions, congenital abnormalities, and stillbirths, the birth of small weak lambs and persistent infections of the offspring in small ruminant flocks. On postmortem examination, porencephaly, hydranencephaly, hydrocephalus, and cerebellar hypoplasia are most marked lesions in aborted fetuses and neonatal animals. The gross and histologic lesions are generally consistent with bluetongue, akabane, and enzootic ataxia of small ruminants. Therefore, labeling the Pestivirus viral antigen and genome is essential to clarify the differential diagnosis. BD is reported worldwide and leads to significant losses in the ruminant population. In addition, the infected small ruminants play a key role in the spread of Pestivirus infections among ruminant population by interspecies transmission. The great importance of these reservoirs should not be forgotten in Pestivirus control programs.

Keywords: Border disease, Pestivirus, Molecular characterization, Control

Ruminantlarda Border Hastalığı Üzerine Bir Derleme: Moleküler Tiplendirme, Patogenezis, Tanı ve Kontrol

ÖZET

Bu derleme; küçük ruminant sürülerinde abortlar, kongenital anomalili yavrular ve yaşama gücü zayıf yavruların doğumu ile karakterize olan ve pestivirusların neden olduğu Border Hastalığı (BD) hakkında son yıllarda gerçekleştirilen virolojik tanı, etken karakterizasyonu, patogenezis ve enfeksiyonun kontrolüne yönelik çalışmaları içermektedir. Sığır virus diare virus ve klasik domuz vebası virusu ile çok yakınlığı olan BD virus (BDV), Flaviviridae ailesinin Pestivirus genusunda yer alır. 5'-NTR ve N^{pro} gen bölgesini hedef alan filogenetik analizler koyun pestiviruslarını en az 6 alt grupta sınıflandırmaktadır. Türkiye küçük ruminantlardan elde edilen izolatların filogenetik analizlerinin sonuçları; BDV'ların, bilinen BDV-3 subgenomundan farklı olarak, BDV-3 ve BDV-7 olduğunu ortaya koymuştur. Her iki izolat genetik olarak aynı genustan klasik domuz vebası ile çok yakın ilişkili bulunmuştur. BD atıklar, konjenital anomaliler, yaşama gücü zayıf ve persiste enfekte zayıf yavru doğumları ile karakterizedir. Nekropsi muayenesinde, atık ve neonatal küçük ruminantlarda porensefali, hidranensefali, hidrosefalus ve serebellar hipoplazi en çok dikkati çeken bulgulardır. Makroskopik ve histopatolojik değişiklikler genellikle mavidil, akabane ve enzootik ataksi ile karışır. Ayırıcı tanı için, virolojik ve patolojik incelemelerde pestivirus antijen ve genomunu belirlemek gerekir. BD küçük ruminantlarda dünya genelinde yaygın bir hastalıktır ve önemli ekonomik kayıplara neden olur. Ayrıca, enfekte küçük ruminantlar, türler arası nakil nedeniyle, ruminant populasyonları arasında pestivirus enfeksiyonlarının yayılımında anahtar bir rol oynarlar. Pestivirus kontrol programlarında bu rezervuarların büyük önemi unutulmamalıdır.

Keywords: Border hastalığı, Pestivirus, Moleküler tiplendirme, Kontrol

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Introduction

Since the first definition of Border Disease (BD) infection in small ruminants by Hughes et al. (1959) in border regions of the UK, it has been reported as endemic in several European-Asian countries and in Japan (Arnal et al., 2004; Krametter-Froetscher et al., 2007; Dubois et al., 2008; Oguzoglu et al., 2009; Giangaspero et al., 2011). Both wild (Marco et al., 2009; Martin et al., 2011; Fernandez-Sirera et al., 2011; Kautto et al., 2012) and domestic (Becher et al., 1997) ruminant species have been reported in the spectrum of the infection. Due to the clinical signs in newborn animals, for instance, the disorders of the quality of hair fleeces and congenital abnormalities, it has also been referred to as "hairy shaker disease" or "fuzzy lamb syndrome" (Nettleton, 2000).

The agent of BD infection is a *Pestivirus,* which belongs to the *Flaviviridae* family and is closely related to Bovine Virus Diarrhoea Virus (BVDV), one of the ubiquitous pathogens for cattle, and Classical Swine Fever Virus (CSFV), an economically important pathogen for pigs (Thiel et al., 2005). *Pestivirus* species can be transmitted between animal species because these viruses are antigenically related. It is obvious that BVDVs, as well as BDVs, are able to infect small ruminants (Carlsson, 1991), while BDVs can also infect cattle (Cranwell et al., 2007) and pigs (Oguzoglu et al., 2001; Oguzoglu, 2002; Schirrmeier et al. 1999; Rasmussen et al., 2010) in mixed farms (bovine/ovine, ovine/porcine). Therefore, *Pestivirus* infections in herds should be identified by using differential diagnostic methods (OIE, 2009).

Transmission to susceptible animals usually requires direct contact (oro-nasal route) with infected animals. Another route, which is crucial for pregnant animals, is the vertical transmission via placenta, which may cause persistent infection in newborns because fetuses can be exposed to the virus while their immune system is still immature. The noncytopathogenic (ncp) biotype, one of the two *Pestivirus* biotypes, can cause persistent infection in fetuses, which are infected prenatally before the development of their immune system (maturation of the immune system for sheep is at 60–80 days and for goat 80–100 days of pregnancy) (Hewicker-Trautwein and Trautwein, 1994; Hewicker-Trautwein,1994; Oguzoglu, 2008). The ncp biotype does not cause lytic effects in infected cells. The other biotype is the cytopathogenic (cpe) biotype and it has importance concerning the pathogenesis of *Pestivirus* species.

Persistently infected (PI) animals are responsible for the consistency of the infection in the herds and these infected animals die in a certain period of their life from an erosive-ulcerative form, namely mucosal disease (MD). MD form develops in PI animals with the ncp biotype as a result of being affected by the cpe biotype, which can only be possible in consequence of superinfection or self-mutations. The persistent infection in small ruminants has been described especially in sheep; goats are not infected persistently in the natural route of the infection. MD, like in cattle, was only described for sheep (Hilbe et al., 2009).

BDV infection is occasionally seen in goats and it is likely to cause abortions in pregnant animals. Adult goats or sheep don't show obvious signs of the infection. The spread of *Pestivirus* infections in small ruminant flocks has been detected by the usage of live vaccines derived from cell cultures, which were produced with *Pestivirus* contaminated fetal calf serum (Thabti et al., 2005). BD virus causes immunosuppression and increases the vulnerability of the organism against the other agents; for instance, some studies have reported BD with peste des petits ruminants (PPR) (Kul et al., 2008; Toplu et al., 2012) and ruminant species of *Lentivirus* (Schaller et al., 2000).

Classification and Molecular Characterization of BDVs

Pestivirus species previously have been named according to the host animal species, subsequently referred to not according to their host origin but genetically related strains. It is known that *Pestivirus* species are not host specific, except for CSFV. With the advancement of molecular diagnostic techniques resulting in sequence analysis, some differences in the nomenclature for *Pestivirus* species have occurred. Recently, BDVs have been identified phylogenetically in seven numbered genogroups (Table 1) (Becher et al., 2003; Arnal et al., 2004; Thabti et al., 2005; Dubois et al., 2008; Oguzoglu et al., 2009; Giammarioli et al., 2011). Novel *Pestivirus* species from ovine species have been described based on their geographical origin (Thabti et al., 2005). This shows that BDVs are equal to or more variable than BVDVs genotype 1.

Pestivirus species have single-stranded positive sense RNA genomes about 12.3–12.5 kilobases in length (Meyers and Thiel, 1996). Genomic RNA encodes for a polyprotein with one large open reading frame flanked by two untranslated regions (UTR): 5'- N^{pro}, C, E^{rns}, E1, E2, p7, NS2-3, NS4A, NS4B, NS5A, NS5B - 3'

During recent years, the genetic heterogeneity of *Pestivirus* species has been determined through sequence analysis. Phylogenetic characterization of *Pestivirus* species for taxonomic status have been made by comparing 5'UTR, N^{pro}, and E2 gene region sequences in classifications studies. Although the N^{pro} sequencing is preferred in phylogenetic studies, the results of 5'UTR sequencing in grouping of *Pestivirus* isolates were found identical (Oguzoglu et al., 2009). Liu et al. (2009) analyzed 56 *Pestivirus* species through the Maximum likelihood and Bayesian approach.

Recently, the *Pestivirus* species have been identified

Table 1. Border Disease genogroups and their origin.
Tablo 1. Border Hastalığı genogrupları ve onların orijinleri.

Genogroups of BDVs	Host origin of isolates	References
BDV 1	Old classical ovine isolates	Becher et al., 2003
BDV 2	Reindeer, bison and sheep derived pestiviruses	Becher et al., 2003
BDV 3	Pig derived isolates	Becher et al., 2003
BDV 4	Pyrenean chamois	Arnal et al., 2004
BDV 5	Sheep isolates from France	Dubois et al., 2008
BDV 6	Sheep isolates from France	Dubois et al., 2008
BDV 7 from Turkey *	One sheep and one goat	Oguzoglu et al., 2009
BDV 7 from Italy *	4 sheep and one goat	Giammarioli et al., 2011
Tunesian	Sheep	Thabti et al., 2005

**It was not into same group.*

palindromically according to species differentiation in nine genomic groups (Giangaspero and Harasawa, 2011). This method provides opportunity for rapid classification by sequencing the variable loci of 5'UTR of the *Pestivirus* species based on the recurrent motifs in this region. BDVs have been classified palindromically in eight genogroups (a to h) (Giangaspero, 2011). This method has been reported as a convenient "genetic marker" for genotyping (Harasawa and Giangaspero, 1998; Giangaspero and Harasawa, 2007). As lineage identification is considerably important, along with the molecular diagnosis of field isolates, for the choice of vaccines.

Interactions of Pestivirus and Host

The studies on *Pestivirus* species indicate that the binding and entry of a *Pestivirus* into a cell is a multistep process (Neill, 2005; Lindenbach et al., 2007). To establish infections in vivo, viruses must replicate in the face of powerful innate and acquired immune response mechanisms. The innate immune response constitutes the first line of host defense against an invading virus and, therefore, plays a crucial role in the early recognition and subsequent triggering of a proinflammatory response. For this purpose, the innate immune response represents two main mechanisms (interferon -IFN- production and induction of apoptosis) to limit the infection at the cellular level (Bonjardim, 2005).

Based on BVDV studies, *Pestivirus* species appear to inhibit IFN synthesis; however, the mechanism of inhibition of IFN synthesis by ncp *Pestivirus* strains in infected cells remains unclear. Ncp strains, but not cpe strains, possess a function that inhibits IFN production in response to infection (Schweizer and Peterhans, 2001). Schweizer and Peterhans (2001) demonstrated that interference with apoptosis and IFN synthesis was a general and important function of ncp BVDV strains. Interferon regulatory factor-3 (IRF-3), a transcriptional activator responsible for the increased transcription of IFN genes and closely associated with the induction of IFN-α/β, is ubiquitously expressed in the cytoplasm and it is activated in response to viral infection (Baigent et al., 2002; Kawai and Akira, 2006). Many viruses, including *Pestivirus* species, have mechanisms to interfere with the IRF-3 pathway, thus, inhibiting the induction of IFN-α/β. N^{pro}, from both BVDVs and CSFVs, is essential for evading the cellular antiviral defense system. It targets IRF3 for proteasomal degradation, significantly decreasing the amount of available IRF3 and, thus, disrupting the IFN-α/β response (Ruggli et al., 2005; Gil et al., 2006; Hilton et al., 2006; Bauhofer et al., 2007; Chen et al., 2007; Seago et al., 2007).

From a virological standpoint, apoptosis (programmed cell death) is an important aspect of the pathogenesis of viral infections. The primary purpose of apoptosis is to kill the virus-infected cell to prevent the virus from replicating, producing progeny, and spreading to neighboring cells. Apoptosis is also a means to kill the cell without inducing an inflammatory response that may damage the surrounding tissue (Hay and Kannourakis, 2002). Many viruses encode proteins to inhibit apoptosis until viral replication steps have been conducted, allowing production of maximal levels of progeny virus. Some studies on BVDVs have provided valuable information in understanding the differences in the interaction between virus and host cells (Zhang et al., 1996; Grummer et al., 1998; Schweizer et al., 2006). Although no inhibitor of apoptosis has been specifically identified in the *Pestivirus* genomes, apoptosis has been induced in cultured cells with the cpe strains (Bendfeldt et al., 2007; Bielefeldt-Ohmann et al., 2008). In contrast, the ncp BVDV does not cause any visible alteration or induce the synthesis of IFN-α/β in its host cells, however, it does inhibit apoptotic cell death in vivo, as well as in vitro (Charleston et al., 2001; Schweizer et al., 2006). The loss of the ability to prevent cell death in cpe BVDV strains appears to be related to the genetic changes that occur within the NS2/3 protein coding sequences that give rise to the formation of NS3 (Lambot et al., 1998; Bruschke et al., 1997; Vassilev and Donis, 2000).

Conventionally, apoptosis is induced in virus-infected cells by either the extrinsic pathway involving death receptors or the intrinsic pathway relevant to intracellular stimuli that transmit a signal to the mitochondria (Dockrell, 2001; Benedict et al., 2002). The intrinsic pathway results in the loss of mitochondrial membrane potential, leading

to the release of cytochrome c and the activation of caspase-9. When an extracellular signal is received and transduced into the cytoplasm, the extrinsic pathway is mediated by the interaction of a ligand with death receptors, such as Fas, to activate caspase-8. In both pathways, the activation of the executioner caspase-3 is the key inducer of downstream effectors of apoptosis (Hay and Kannourakis, 2002). An investigation of the mechanism apoptosis induction in cpe BVDV-infected cell culture indicates that cpe BVDV-infected cells induce the intrinsic apoptotic pathway (Grummer et al., 2002). However, Toplu et al. (2010) showed that apoptosis in BDV-infected neuronal and glial cells in aborted and neonatal small ruminants infected with BDV involved both the intrinsic and extrinsic pathways. Moreover, the expression of caspase-8 in glial and neuronal cells in BDV-infected kids is greater than that in the lambs. Since cpe BDV is generally responsible for BD in kids, it is possible that the elimination of cpe BDV-infected neurons by the extrinsic pathway of apoptosis is a defensive mechanism against viral infection in kids. The strong expression of neuronal bcl-2 in the infected areas in the lambs reveal that bcl-2, as an anti-apoptotic protein, may play an important role in protecting neuronal and glial cells infected with ncp BDV (Toplu et al., 2010). Taken together, this appearance supports that evasion of the innate immune system is crucial for the establishment of persistent infection.

Diagnosis of BD Infection

Clinic and Postmortem Diagnosis

BD in small ruminants generally presents with clinical signs related to congenital type infection, characterized with abortion, stillbirth, unviable birth, and growth retardation (Hewicker-Trautwein and Trautwein, 1994; Oguzoglu, 2008; Garcia-Perez et al., 2009; Toplu et al., 2010). Infected newborn lambs and kids with small weak and paralysis (Figure 1) are unable to rise unaided and have involuntary muscular tremors. Such animals have difficulty standing up and walking, with ataxia or not, and show uncoordinated movements and posterior wobbling (Löken et al.,1991; Oguzoglu et al., 2009; Toplu et al., 2012). Additionally, prognathism and arthrogryposis with permanent flexion of carpal, tarsal, and genu can be observed. One of the most conspicuous findings in many sick lambs is abnormal fleece, consisting of long and straight birth coats, known as “hairy shaker” .

At necropsy, thymic hypoplasia and brain anomalies are important for macroscopical diagnosis of intrauterine infection. Porencephalic cysts, hydranencephaly (Figure 2), hydrocephalus, and cerebellar hypoplasia are some of the brain anomalies encountered (Wohlsein et al., 1992; Hewicker-Trautwein and Trautwein, 1994; Toplu et al., 2010). The tonsils and lymph nodes are usually edematous, enlarged, and hemorrhagic (Toplu et al., 2012). Less frequently, PI sheep can show erosive-ulcerative lesions with hemorrhage in oral and intestinal mucosa, which are analogous to mucosal disease-like lesions (Monies et al., 2004).

Virological Diagnosis

The lifelong persistence of *Pestivirus* species is important for the virological diagnosis. According to OIE criteria, virus isolation is essential and the “gold” standard for the BD identification, followed by immunohistochemical staining with panpesti-specific monoclonal antibodies. Although most BDV isolates are the ncp biotype, some BDV isolates, especially from goats, are cytopathogenic.

A single RT-PCR assay was developed for rapid typing and as a specific detection method of BDV by Vilcek and Paton (2000). The primer pair, named PBD1 and PBD2, flanked a 225 bp DNA fragment that was selected from the conserved 5’UTR of the genome to detect all *Pestivirus* strains. This rapid diagnostic method is very important for the differential diagnosis of small ruminant-originated *Pestivirus* infections of porcine, since serological differential diagnosis based on the neutralization test takes more time, this can delay the choice of CSF disease intervention method and this causes a problem in keeping pigs, because CSF is a notifiable disease.

We should remember that the sheep may be infected naturally with BVDV-1 and BVDV-2 (Giangaspero and Harasawa, 2004) and BVDV-infected pregnant sheep show similar clinical signs as BVDV-infected pregnant cattle. Julia et al. (2009) reported BVDV circulating in an Argentinean sheep herd that was in close contact with cattle infected with BVDV-1a. In another study investigating *Pestivirus* species obtained from BD-suspected ruminants (Giangaspero, 2011), 131 of 536 isolates were classified as BDV (grouped in palindromic genotypes a–h) and the rest of the isolates in this study were BVDV-1 and BVDV-2.

Serological Diagnosis

For the serological detection of BDV, specific neutralizing antibodies based on virus neutralization test (VNT) can be used, as well as in all *Pestivirus* infections. However, the choice of reference viruses used in this test is important. Because these reference strains should be genetically related with local strains. Some commercial AGID and ELISA kits are available to identify BDV-specific antibodies. The fact of cross-neutralization in serological tests should be noted. In addition, using BVDV specific antibody ELISA techniques for BDV serodiagnosis would not be suitable. Because, BVDV antibody ELISA kits might not recognize only BDV specific antibodies.

Histopathologic Diagnosis

Consistent with other studies (Wohlsein et al., 1992; Hewicker-Trautwein and Trautwein, 1994; Garcia-Perez et al., 2009), some studies (Oguzoglu et al., 2009; Toplu et al., 2010; Toplu et al., 2012) from

Turkey have shown that the microscopic findings are complementary to macroscopical lesions in the central nervous system. Nonsuppurative and/or necrotizing meningo-encephalomyelitis, often accompanied by hypomyelinogenesis, were in the periventricular areas, cerebellum, brainstem, cerebral hemispheres, and occasionally in the medulla spinalis. The most consistent finding was periventricular leukomalacia with gliosis and infiltration of mononuclear cells in ependyma throughout the cerebrospinal axis. Additionally, focal or multifocal malacia could be observed in the grey and white matter of the cerebral hemispheres, thalamus, hypothalamus, periventricular areas, and brainstem. Immunohistochemistry (IHC) with anti-GFAP antibody showed that the gliosis mainly consisted of astrocyte proliferations in the periventricular areas. The hypoplastic cerebella showed severe necrotic and dysplastic changes, with Purkinje cell loss and focal to diffuse depletion of granule cells and disorganization of the cellular layers of cerebellar cortex (Toplu et al., 2010).

The gross and histologic lesions described above were generally consistent with bluetongue, akabane, and enzootic ataxia of small ruminants. Thus, clinical, postmortem, and histopathologic diagnosis of BD may be difficult to make because of the wide variability of clinical signs and pathologic lesions. Therefore, labeling the *Pestivirus* viral antigen and genome was essential to clarify the differential diagnosis.

Immunohistochemistry (IHC) and In Situ Hybridization (ISH)

IHC includes methods for identifying cellular or pathogen constituents (antigens) via antigen-antibody interactions (the site of antibody binding being identified either by

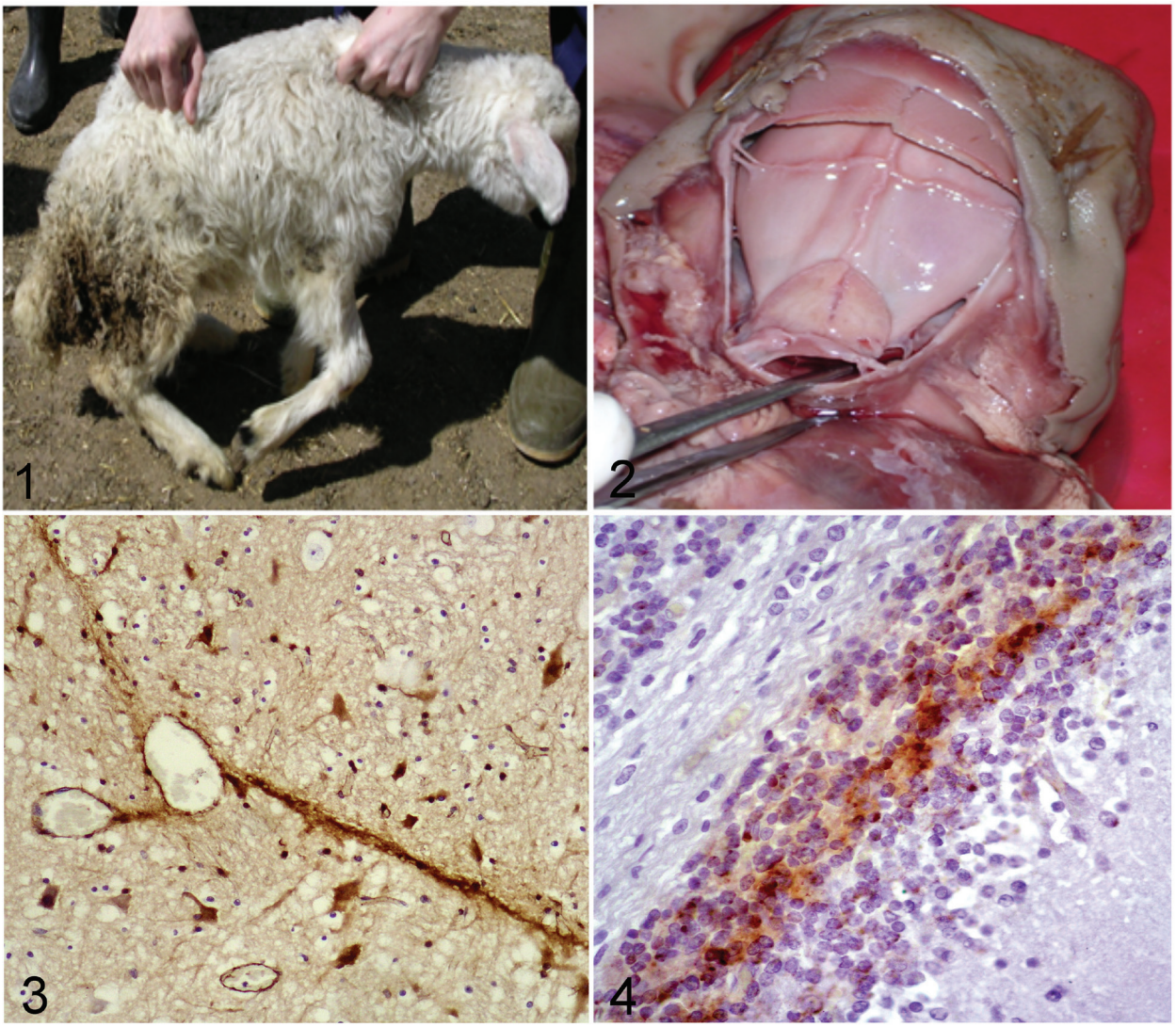

Figure 1. Paralysis in a lamb with BDV antigen positive. **Figure 2.** Hydranencephaly in an aborted lamb fetus. **Figure 3.** Neuronal and Linear labeling of BDV specific viral antigen (WS363) extended from parenchymal vessels to neuropil in the midbrain of a lamb. Avidin biotin immunoperoxidase method. 20X. **Figure 4.** Labeling of BDV RNA in granule cells of the cerebellum in a lamb. In situ hybridization method with BDV specific DIG-labelled probe. 20X.

Şekil 1. BDV antijen pozitif bir kuzuda paralizis. **Şekil 2.** Atık bir kuzuda hidranensefali. **Şekil 3.** Bir oğlağın orta beyininde BDV spesifik viral antijenlerin nöronlarda pozitifliği ve damarlardan nöropile doğru çizgisel dağılımı. Avidin biotin immunoperoksidaz metot. 20X. **Şekil 4.** Bir kuzuda serebellumunun granül hücrelerinde BDV RNA pozitifliği. BDV spesifik DIG-işaretli prob ile in situ hibridizasyon metodu. 20X.

direct labeling of the antibody or by use of a secondary labeling method) within tissue sections.

In viral diseases, immunofluorescence and immuno-peroxidase methods target specific viral antigen by means of polyclonal or monoclonal antibodies in the tissue sections. Both methods have been used for the diagnosis of *Pestivirus* infections (Choi and Chae, 2003; Lamm et al., 2009; Toplu et al., 2010). Moreover, these methods have been used to characterize cellular tropism of BDV in pathogenesis studies (Wohlsein et al., 1992; Hewicker-Trautwein and Trautwein, 1994). Some workers (Oguzoglu et al., 2009; Toplu et al., 2010; Toplu et al., 2012) in *Pestivirus* studies have used avidin biotin peroxidase methods. Firstly, used the more specific method with polyclonal antibody (*Pestivirus* group-specific antigen; WB103/105) to detect *Pestivirus* in suspected tissues. After detecting *Pestivirus* antigen, the discrimination between BDV and BVDV was carried out with monoclonal antibody (BDV-specific antigen; WS363). The results in naturally infected animals showed that the labeling of BDV antigens is localized particularly in the periventricular areas, brainstem, and cerebellum and to a lesser extent, in the cerebral hemispheres and spinal cord. Neuronal and glial cytoplasm and their processes are cell tropism of BDV (Figure 3). In the cerebellum, BDV antigens were observed in the cytoplasm and cell processes of Purkinje cells, in neurons of the molecular and granular layers, and in the glial cells of the substantia alba.

ISH is another method that targets the localization and detection of specific nucleic acid sequences through the application of a complementary strand of a nucleic acid probe to which a reporter molecule is attached. This method allows the presence or absence of the specific nucleic acid (DNA or RNA sequences) within fixed tissue sections or cell preparations. Entrican et al. (1991) used ISH in lymphocytes for BD diagnosis. Toplu et al. (2011) first used the ISH method for detecting nucleic acid sequences of BDV in tissue sections. The results of ISH with both Digoxigenin (DIG)-labeled cDNA probes and IHC with BDV-specific antigen (WS363) indicate that there is a good correlation between the intensity and distribution of viral nucleic acid and viral antigen in the brain sections, localizing in neuronal and glial cytoplasm and their processes (Figure 4). However, ISH show more positive cells in some areas compared to IHC. The reason for this discrepancy can be explained. Virus-infected cells in the early stages of viral infection, when the genome appears earlier than viral protein, may express no or very little viral antigen on the cellular surface. In addition, formalin fixation can denature antigenic structures, and ISH is less affected from structural changes caused by formalin fixation than IHC is (Coi and Chae, 2003). ISH and IHC are useful for the detection and discrimination of BDV in tissues obtained from naturally infected animals, and they may be valuable methods for studying the pathogenesis of BDV infection.

Control and Measures

Unlike for BVDV, any systematic control for BD infection has not been declared yet. Therefore, a similar control and eradication program that prevailed for BVDV in cattle in Europe is urgently needed, considering the prevalence of BD infection in the countries that reported the infection (Giangaspero, 2011).

Ideally, the control programs of *Pestivirus* infections should consist of identification and elimination of PI animals within the herds. However, no antigen ELISA kits for BDV for routine, diagnostic, serological screening could be recommended in terms of antibodies to determine BD infection in the herd. Generally, PI animals produced no antibodies; therefore, seronegative animals, especially ewes, in the flocks would be suspect. Brun et al., (1993) were described the experiment about a BD vaccine, but there is no available commercial ovine vaccine for BD; however, *Pestivirus* vaccines in cattle could be used for BD infection.

Status Quo for BD Infection in Turkey

The first detection of *Pestivirus* infections of small ruminants in Turkey was reported by Burgu et al. (2001). The first serological data of BDV infection in small ruminants was notified by Ataseven et al. (2006). They reported the presence of BD infection at a rate higher than that for the other infectious agents, as estimated by the abort cases in small ruminant flocks. The first molecular characterization of BD agents obtained from field isolates in Turkey was accomplished by Oguzoglu et al. (2009). The antigenic relation of these isolates was found to be closer to CSFVs than to worldwide BDV strains and they were declared a novel (BDV-7) subgenotype, which is characteristic for certain geographic regions.

In Turkey, BD infection as congenital infections are clinically characterized by abortions, malformations, stillbirths, the birth of small weak lambs, and persistent infections of the offspring. Affected lambs via congenital infection can show tremors, abnormal body conformation, and hairy fleeces. At necropsy, porencephaly, hydranencephaly, hydrocephalus, and cerebellar hypoplasia are the most marked lesions in aborted fetuses and stillbirth lambs. Less frequently, PI sheep can show erosive-ulcerative lesions with hemorrhage in the digestive system, which has some features analogous to the experimentally produced mucosal disease-like lesions (Oguzoglu et al., 2008, Toplu et al., 2010).

Recently, PPR infection with BD infection has been reported in abort cases in small ruminant flocks (Toplu et al., 2012) and the virus strains have been characterized as BDV-3. Interestingly, the results of both the first molecular characterization study of BDVs in Turkey by Oguzoglu et al. (2009) and the dual infection report in Turkey by Toplu et al. (2011) are in concordance, with the BD isolates being more closely related to CSFVs than to

the known BDV-3 subgenotype that contains pig-derived *Pestivirus* obtained from sheep. It is also interesting to note that in mixed infections, the immunosuppressive effects of *Pestivirus* infection increase the influence of the other infectious agents.

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