A Flock Level Analysis of an Outbreak of Natural Border Disease in Sakiz Ewes and their Progeny

Kerem Ural¹, Bulent Ulutas¹, Pinar Alkim Ulutas², Mehmet Gultekin¹ & Abidin Atasoy¹

ABSTRACT

Background: Pestiviruses cause economically important diseases in domestic ruminants worldwide. Border Disease is a congenital viral infection of sheep, caused by a pestivirus, and is first reported in the Border region of Wales and England. The BDV has worldwide distribution in sheep with different prevalences among countries. Vertical transmission is an important route in the epidemiology of this virus. Infection of fetuses may cause birth of persistently infected lambs, that are viremic, antibody negative, and are excreting virus. The disease is characterized by abortion, barren ewes, stillbirth and persistently infected weak lambs showing neurological and dermatological signs. The economic importance of the disease is related to reproductive failure, abortions and significantly low survival rate of affected lambs. In the present study the aim was to describe clinical, hematological and seological aspects of natural Border disease virus (BDV) infection in a sheep flock in Cine, Aydin. Besides we sought to elucidate the relationship between natural (active infection) and persistent BDV disease and the serum concentration of haptoglobin (Hp) and serum amyloid A (SAA) in infected sheep

Materials, Methods & Results: Field observations were carried out in a sheep flock comprising 327 sheep with a history of an outbreak abortion in Cine town in Aydin, Turkey. Twenty-five out of 327 sheep in the flock was monitorized by blood sampling. The animals were selected among aborted ones, at least once, into two weeks preceeding period or with a history of weak lambing. BDV antigen (persistent infection) was detected in 8 out of 25 sheep (32%) while antiviral antibodies (active infection) were detected in 18 animals tested (68%). The disease was mainly characterized by abortions, stillbirth/weak lamb and abnormal brown/black fleece pigmentation, which occurred in an epidemic form. Twentyfive sheep were related to disease condition as detected serologically, and the ratio of the number affected to number at risk being was 17:8. The culling rate was 50% of the affected animals. Most of the affected animals were second lambing sheep (5/25, 20%). Hematological variables did not reveal statistical difference whereas serum concentrations of Hp ($P < 0.05$) and SAA ($P < 0.01$) were significantly higher in naturally infected sheep in contrast to persistently infected sheep with BDV.

Discussion: Clinical signs and detailed laboratory analysis related to natural Border disease outbreak have never been reported in Turkey, although previous epidemiological studies had shown that Border disease virus infection is relatively common in some parts of Turkey in sheep flocks and persistent Border disease virus infection had been described in apparently healthy sheep in Turkey. In the present study the disease was mainly characterized by abortions, stillbirth/weak lamb and abnormal brown/black fleece pigmentation, which occurred in an epidemic form. Besides bronchopeumonia, enteritis and conjunctivitis were detected in a limited population. Neurological signs were only observed in 2 animals. Besides persistent infection was detected in 32% of sheep enrolled. An acute phase reaction involving Hp and SAA has been identified in the present study. These results indicate that the monitoring of selected acute phase proteins may increase the diagnostic information available as a result of their analyses in naturally infected sheep and persistently infected sheep with BDV.

Keywords: Border disease, sheep, Sakiz ewes, flock, analysis, outbreak.
INTRODUCTION

Border Disease is a congenital viral infection of sheep, caused by a pestivirus [3,12,24]. The genus Pestiviruses, involving Bovine Viral Diarrhea Virus 1 (BVDV-1) and 2 (BVDV-2), Border Disease Virus (BDV), and Classical Swine Fever Virus (CSFV), are capable of infecting a wide range of animals [12,27,28].

The disease has been characterised within abortion, stillbirth and barren ewes [18]. Persistently infected lambs with Border disease virus (BDV) may also have an enteric disease within diarrhoea and illthrift [15]. Affected newborn small and weak lambs may present several abnormalities including low neonatal survival rate, abnormal body conformation, pigmented hairy fleeces, growth retardation, clonic tremors and skeletal problems [9,18]. Border disease in adult sheep may be transient, and in generally seroconversion may be detected within 30 to 40 days of post-infection [3,24]. Following exposure to BDV, the ewes develop immunity [3,24].

Border disease is widely distributed and has been reported from several regions in Spain [4,14,26], and Border Disease virus (BDV) was detected in 16% of the flocks with problems of abortions in the Basque Country, Northern Spain [19].

As aforementioned above BDV infection may result with a wide range of clinical signs, from symptomatic to a life threatening fatal disease [9,15,17,18]. Therefore it must be mentioned that the disease caused significant economic losses to the livestock industry due to the reproductive failure by abortions, low survival rate and low carcass scores in affected lambs [9,17,18]. Border disease is also relatively distributed and has been reported from some selected regions in Turkey, and BDV was detected with ranges of 8.4 to 100% of the flocks with problems of abortions [2,11,13]. All of the studies mentioned above were solely based on serological evidence of BDV, and none of them reported the clinical outcomes within detailed laboratory analysis involving acute phase response of the disease in Turkey. Therefore the aim of this study was to evaluate and analyse the clinical features of a natural BDV outbreak detected in a sheep flock with abortions, with special reference to clinical, haematological and serological findings and acute phase response.

MATERIALS AND METHODS

Animals and management

Field observations were carried out in a sheep flock comprising 327 sheep in Cine town in Aydin Province. The flock included 60 lambs, 250 sheep and 17 rams. The herd was certified as Brucella sp., Campylobacter sp., Salmonella sp., Chlamydia sp., Toxoplasma sp., Leptospira sp. and Blue tongue virus negative prior to study.

Within the herd there was a history of an outbreak abortion affecting up to 60% of the sheep, with at least 3 years noticed in some cases with no known apparent age predisposition.

Sampling

Twenty-five out of 327 sheep in the flock was monitorized by blood sampling. The animals were selected among aborted ones or with a history of weak lambing. Blood was withdrawn by double blinded investigators. Sheep at least having abortus into two weeks preceding period were enrolled. The blood samples were withdrawn into the tubes containing EDTA and silicone. Clotted blood samples were immediately tested in the day of sampling. The animals, antigen positive, were sampled again four week later to confirm the persistent viremia. The sera samples were centrifuged at 3000 × g for 10 min. Sera fractions were separated, inactivated at 56°C for 30 min and stored at -20°C until to the test.

Antigen detection

A commercially available antigen capture ELISA kit (BVD/MD Antigen Mix Screening1 was used for detection of the the blood samples for the possible presence of Pestivirus antigens. The test procedure was performed according to the manufacturer’s instructions.

Antibody detection

In a same manner sera samples were tested for specific antibodies to the BDV by use of a commercially available ELISA kit1. The test was also performed according to the manufacturer’s instructions.

Haematological analysis

Hematology was performed at the Adnan Menderes University, Faculty of Veterinary,
Department of Internal Medicine within 4 h of blood collection. EDTA anticoagulated blood was measured on Abacus Junior Vet 5 automatic blood cell counter with species specific software to provide automated results for total red blood cell count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin concentration, and total platelet count.

**Acute phase protein analysis**

The levels of haptoglobin (Hp) and serum Amyloid A (SAA) in sera were determined by use of the commercial kits Tridelta Phase™ Range Hp Assay² and SAA Assay³. Haptoglobin level in sera samples was detected by the haemoglobin binding method using micro-titre plates and SAA was measured by sandwich ELISA using phase SAA kits within ELISA reader³ regarding the manufacturer’s instructions.

**Statistical analysis**

For haematological analysis and detection of Hp and SAA, a mean value and standard deviation for each parameter was calculated for persistently and active infected Sakiz sheep. Student’s *t* test was used to assess for significant differences between gorups.

**RESULTS**

**Sampling and serological results**

BDV antigen (persistent infection) was detected in 8 out of 25 sheep (32%) while antiviral antibodies (active infection) were detected in 18 animals tested (68%). The disease was mainly characterized by abortions, stillbirth/weak lamb and abnormal brown/black fleece pigmentation, which occurred in an epidemic form. Twenty-five sheep were related to disease condition as detected serologically, and the ratio of the number affected to number at risk being was 17:8. The culling rate was 50% of the affected animals in previous years. Most of the affected animals were secondlambing sheep (5/25, 20%).

**Clinical findings**

Abnormal clinical signs and relevant findings were shown in Table 1. All 25 sheep enrolled in the study had had abortion at least 1 period. Some of the sheep were aborted 3 times. Among the population enrolled abnormal brown/black fleece pigmentation (Figure 1) was observed in 11 of the animals Seventeen of the animals had stillbirth/weak lambs (Figure 2a-d). Respiratory and gastrointestinal signs (Figure 3) were detected with a lowering frequency. Conjunctivitis and neurological signs were determined in 3 and 2, respectively, of the animals.

**Haematological analysis and acute phase response**

Haematological results were shown in Table 2. Hematological variables did not reveal statistical

### Table 1. Clinical signs of Sakiz sheep with Border disease.

<table>
<thead>
<tr>
<th>No of animals</th>
<th>Clinical signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>Abortion</td>
</tr>
<tr>
<td>17</td>
<td>Stillbirth/weak lambs</td>
</tr>
<tr>
<td>11</td>
<td>Abnormal brown / black fleece pigmentation</td>
</tr>
<tr>
<td>4</td>
<td>Bronchopneumonia</td>
</tr>
<tr>
<td>3</td>
<td>Enteritis</td>
</tr>
<tr>
<td>3</td>
<td>Conjunctivitis</td>
</tr>
<tr>
<td>2</td>
<td>Neurological signs</td>
</tr>
</tbody>
</table>
difference whereas serum concentrations of Hp ($P < 0.05$) and SAA ($P < 0.01$) were significantly higher in naturally infected sheep in contrast to persistently infected sheep with BDV (Table 3).

Figure 1. Hairy shaker syndrome in a Sakiz sheep. Hairy fleece (abnormal black/brown pigmentation) is typical for Border disease due to thyroid hypofunction.

Figure 2. Weak lambs naturally infected with Border disease virus. Affected lambs showed: (a) growth retardation. (b) ocular and nervous signs. (c) nervous signs and (d) locomotor disturbances.
Figure 3. Haemorrhagic enteritis in a Sakiz sheep infected with Border disease.

Table 2. Haematological analysis [mean (SD)] of Sakiz sheep with Border disease.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acute Infection [Ab+] (n=17)</th>
<th>Persistent Infection [Ag+] (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (103/µL)</td>
<td>6,684 ± 1,785</td>
<td>7,794 ± 2,27</td>
</tr>
<tr>
<td>RBC (106/µL)</td>
<td>8,909 ± 1,275</td>
<td>9,089 ± 1,19</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>11,52 ± 1,571</td>
<td>11,84 ± 1,1</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>28,49 ± 4,633</td>
<td>28,88 ± 3,33</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>32 ± 3,24</td>
<td>32 ± 2,39</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>41 ± 2,13</td>
<td>41,46 ± 2,72</td>
</tr>
<tr>
<td>PLT (103/µL)</td>
<td>703,4 ± 210</td>
<td>609,3 ± 102</td>
</tr>
</tbody>
</table>


Table 3. Acute phase response within mean (SD) values of Haptoglobin (Hp) and Serum Amyloid A (SAA) in Sakiz sheep with Border disease.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Acute Infection [Ab+] (n=17)</th>
<th>Persistently Infection [Ag+] (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hp [µg/L]</td>
<td>0.9 ± 0.93</td>
<td>54.7 ± 17.60</td>
</tr>
<tr>
<td>SAA [µg/L]</td>
<td>34.4 ± 2.78</td>
<td></td>
</tr>
</tbody>
</table>

P < 0.05 < 0.01
DISCUSSION

Clinical signs and detailed laboratory analysis involving acute phase response as a consequence of Border disease outbreak have never been reported in Turkey, although previous epidemiological studies had shown that Border disease virus infection is relatively common in some parts of Turkey in sheep flocks [2,10,11,13] and persistent Border disease virus infection had been described in healthy sheep in west part of Turkey [11]. In the present study the disease was mainly characterized by abortions, stillbirth/weak lamb and abnormal brown/black fleece pigmentation, which occurred in an epidemic form. Besides bronchopeumonia, enteritis and conjunctivitis were detected in a limited population. Neurological signs were only observed in two animals.

Only a limited numbers of studies had been performed among sheep in Turkey regarding the presence of Border Disease in Turkey. In a previous dissertation thesis BDV antibodies were detected in 31 out of 75 pregnant ewes with a seropositivity of 41.3% [5]. Another study reported from East and Southeast Anatolia, a seropositivity rate of 63.6% was determined in goats [1]. In Afyonkarahisar, Western part of Turkey, a total seroprevalence of 78.5% was determined in a total of 568 Akkaraman sheep [11]. Another study performed in Kirikkale region showed a seroprevalence rate as 8.4 to 100 % among flock basis, and a total of 74.51 % antibody seropositivity [2]. In South Marmara of Turkey, 95 out of 500 sheep (19%) showed seropositivity against Border disease based on ELISA [13]. In our study based on a limited portion of a sheep flock consisting 327 sheep, a total of 25 aborted sheep were enrolled and BDV antigen (persistent infection) was detected in 8 out of 25 sheep (32%) while antiviral antibodies (active infection) were detected in 18 animals tested (68%).

Acute Phase Proteins (APP) have been proposed as significant indicators of several pathological conditions in humans and animals, and as markers of herd health in cattle and sheep [8,16,20]. In sheep SAA and haptoglobin are recognized as major APPs [6,16,20,22]. Several studies reported an association between the severity of the disease and the circulating level of APP [6]. Haptoglobin (Hp) levels may be elevated during bacterial infections [22,25] and yeast infections [21]. Both Hp and SAA were assessed and showed elevations in an experimental model of caesous lymphadenitis in ovine [7].

In sheep with naturally occurring bacterial infection elevated levels of haptoglobin had 85% sensitivity and specificity compared to those of 52% sensitivity and 75% specificity of WBC counts in those animals [22]. In a retrospective dog study evaluating inflammatory conditions, APP showed significant changes in the absence of changes in WBC count [23]. In the present study hematological variables did not reveal statistical difference whereas serum concentrations of Hp ($P < 0.05$) and SAA ($P < 0.01$) were significantly higher in naturally infected sheep in contrast to persistently infected sheep with BDV. In summary these findings may be explained with the expected delay in marked WBC changes due to the necessity for generating cells in the bone marrow, whereas APP concentrations may increase within hours. Increased levels of Hp and SAA in naturally infected sheep may be associated with increasing activity indices of disease.

CONCLUSION

Several studies reported that a single APP may not be used exclusively to monitor an infectious disease condition. However, an APP index may be used in both human and veterinary medicine. APP may be elevated rapidly and/or slowly, by forming a comprehensive index might be in association with the severity of the inflammatory process. A recent APP interpretation in sheep summarized Hp and SAA levels as major indicators.

An acute phase reaction involving Hp and SAA has been identified in Border disease in the present study. These results indicate that the monitoring of selected acute phase proteins may increase the diagnostic information available as a result of their analyses in naturally infected sheep and persistently infected sheep with BDV.

To the present authors’ knowledge hematological and serological analysis and acute phase response in Sakiz sheep with naturally occurring active and persistent Border disease has not been previously evaluated in Turkey.

**SOURCES AND MANUFACTURERS**

1. BVD/MD Antigen Mix Screening, Institut Pourquier, France.
2. Tridelta Phase™, Tridelta Development Limited, Greystones, Ireland.
3. ELISA reader, Anthos 2010, Anthos Labtec Instruments, Salzburg, Austria.

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

**REFERENCES**


