Introduction

The Bluetongue disease is a viral, non-contagious disease of ruminants (domestic and wild) transmitted between its vertebrate hosts by Culicoides spp biting midges [9]. As these insect species are most active in the temperature range 18-29°C and are inactive below 10°C or above 30°C, the disease occurs most commonly in late summer when vectors are most numerous. The epidemiology and natural history of the disease depend on interactions between vectors, hosts, climate and virus [11, 12]. The distribution of bluetongue is effectively restricted to a band around the world between 50°N and 30°S in America and between 40°N and 35°S in the rest of the world, in which Culicoides midges are extremely abundant [11]. Although sheep are most severely affected, cattle are the main mammalian reservoir of the specific virus (BTV) and are very important in the epidemiology of the disease [9].

BTV is classified within the Orbivirus genus in the family Reoviridae. There are 24 known serotypes of the virus and other orbiviruses are closely related to the bluetongue virus [3]. A global evaluation, based on sequence analysis of the genome segment 10 (Seg-10) from 137 strains isolated in different regions of the world, identified two principal groups irrespective of their serotype or year of isolation. All of the viruses from Asia and Australia were grouped in one class, whereas those from other regions were present in both classes [2]. Seg-10 contains specific genes of BTV, notably the genes encoding the NS3/3A proteins which are essential for both virus assembly and release from infected cells [2]. The identification and the molecular analysis of the BTV strains circulating in some parts of Turkey during the infamous 1999-2001 epidemics in that part of the Mediterranean Region were performed using RT-PCR methods, sequence alignments and phylogenetic analyses of genome segment 10 [15]. These analyses were used to explore the epidemiological

SUMMARY

The present study aimed to investigate the seroprevalence of Bluetongue virus (BTV) serotypes 4, 9 and 16 in cattle in 3 North-eastern Anatolian provinces (Igdir, Kars and Ardahan). Using the virus neutralization test, serum antibodies specifically targeted each serotype were determined in 352 cattle. The overall BTV seroprevalence was very high (91.76%) and the specific frequencies were 72.16%, 42.05% and 36.93% for serotypes 4, 9 and 16 respectively. A high proportion of animals (46.31%) exhibited positive reactions for at least 2 serotypes, the major combinations being the association between the serotypes 4 and 9 (16.48%) and triple seropositivity (13.07%). Moreover, the overall BTV frequency as well as specific seroprevalences for the serotypes 16 and 4 and the association between serotypes 4 and 9 greatly varied among the 3 provinces investigated but not significantly, the cattle from the Igdir area appearing as the more exposed. These results demonstrate the high occurrence of the BTV serotypes 4, 9 and 16 in cattle from the North-East Turkey and emphasize the necessity to control animal transactions with neighbouring countries.

Keywords: Bluetongue, virus serotypes, cattle, seroprevalence, North-eastern Turkey.

RÉSUMÉ

Séroprévalence des sérotypes 4, 9 et 16 du virus de la fièvre catarrhale ovine chez les bovins de différentes provinces du Nord-Est de la Turquie

L’objectif de cette étude était de déterminer les séroprévalences des sérotypes 4, 9 et 16 du virus responsable de la fièvre catarrhale ovine (BTV) chez les bovins de 3 provinces du Nord-Est de l’Anatolie (Igdir, Kars et Ardahan). Les anticorps sériques dirigés spécifiquement contre chacun de ces 3 sérotypes ont été recherchés par séroneutralisation sur 352 bovins. La séroprévalence totale du BTV est apparue très élevée (91,76 %) et les fréquences spécifiques ont été respectivement de 72,16 % pour le sérotype 4, 42,05 % pour le sérotype 9 et de 36,93 % pour le sérotype 16. Une proportion importante d’animaux (46,31 %) ont présenté des réactions positives pour au moins 2 sérotypes, les principales combinaisons étant l’association des sérotypes 4 et 9 (16,48 %) et celle des 3 sérotypes (13,07 %). En outre, la fréquence totale des anticorps sériques anti-BTV ainsi que les séroprévalences spécifiques des sérotypes 16 et 4 et de l’association des sérotypes 4 et 9 ont grandement varié au sein des 3 provinces mais de façon non significative, les bovins de la région d’Igdir apparaissant comme les plus fréquemment infectés. Ces résultats démontrent la forte prévalence des sérotypes 4, 9 et 16 du BTV chez les bovins du Nord-Est de la Turquie et soulignent la nécessité de contrôler les transactions d’animaux avec les pays limitrophes.

Mots clés : Fièvre catarrhale ovine, sérotypes viraux, bovins, séroprévalence, Turquie du Nord-Est.
PREVALENCE OF THE BTV SEROTYPES 4, 9 AND 16 IN CATTLE

background of individual isolates from both a regional and global perspective. In the regional analysis, the Turkish virus isolates were localized in the class 1 which contains two subgroups. The neighbour-joining analysis revealed that Seg-10 of majority of the Turkish viruses was closely related to certain other virus strains allocated in the eastern lineage. The majority of the Turkish isolates identified by RT-PCR were BTV-9 whereas the serotype 16 was more scarcely evidenced [15]. In 2005, the seroprevalence of the BTV-4 infection was investigated in cattle in 8 provinces (Igdir, Agri, Kars, Erzurum, Bayburt, Gumushane, Artvin, and Ardahan) from Northeast Anatolia [18]. The determined BTV-4 seropositivity rate was 48.02% in the surveillance population. It was concluded that BTV infection are common in cattle housed in private farms from Northeast Anatolia Region. Therefore, serious control measures should be taken in herds with similar epidemiologic features.

The aim of this study was to define, in cattle, the seroprevalence and the distribution of bluetongue virus (BTV) serotypes 4, 9 and 16, which have been shown to be active in western Turkey, in three provinces in North-eastern Anatolia. In this way, we expected to obtain seroepidemiological data authorizing some hypotheses on the possible BTV circulation pathways among the neighbouring regions.

Materials and Methods

ANIMALS AND SERUM SAMPLES

Blood serum samples were collected by jugular vein puncture into vacuum tubes with clot activator from randomly selected 352 cattle reared in private small scale production units from three provinces, i.e. Igdir, Kars and Ardahan (figure 1). After clotting at room temperature for 15-30 minutes and centrifugation at 3000 g, at 4°C for 10 minutes, sera were carefully harvested, then inactivated at 56°C for 30 minutes and stored at –20°C until analysis.

DETERMINATION OF THE BLUETONGUE VIRUS SEROTYPES

Presence of serum antibodies against Bluetongue virus serotypes (BTV-4, BTV-9, BTV-16) were screened using virus neutralization assay [5]. The serum samples (50 μL) in duplicate were mixed with an equal volume of 100 TCID50 diluted BTV suspensions in 96-well plates. After incubation at 37°C for 1 hour, the Vero cell suspension (50 μL) was added to each well, thereafter the plates were again incubated under 5% CO2 atmosphere at 37°C for at least 4 days. The results were assessed microscopically when 100% cytopathic effect was appeared in virus control wells.

STATISTICAL ANALYSIS

The Chi-square test was used to compare seroprevalence of BTV serotypes determined in the 3 provinces studied. Differences were considered as significant when P value was less than 0.05.

Results

The overall BTV seroprevalence for the serotypes 4, 9 or 16 was 91.76% in the North-Eastern Turkey (Table I) but it did not significantly vary (P > 0.05) according to the province investigated, although the BTV seroprevalence appeared maximal in the Igdir province and minimal in the Ardahan province. In the all 3 provinces, circulating antibodies against each BTV serotype investigated were detected and the specific seroprevalences for BTV serotypes 4, 9 and 16

<table>
<thead>
<tr>
<th>Provinces</th>
<th>BTV</th>
<th>BTV-4 (%)</th>
<th>BTV-9 (%)</th>
<th>BTV-16 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Igdir (n = 113)</td>
<td>105 (92.92%)</td>
<td>84 (74.34%)</td>
<td>42 (37.17%)</td>
<td>47 (41.59%)</td>
</tr>
<tr>
<td>Kars (n = 133)</td>
<td>122 (91.73%)</td>
<td>94 (70.68%)</td>
<td>52 (39.10%)</td>
<td>39 (29.32%)</td>
</tr>
<tr>
<td>Ardahan (n = 106)</td>
<td>96 (90.57%)</td>
<td>76 (71.70%)</td>
<td>54 (50.94%)</td>
<td>44 (41.51%)</td>
</tr>
<tr>
<td>Total (n = 352)</td>
<td>323 (91.76 %)</td>
<td>254 (72.16 %)</td>
<td>148 (42.05 %)</td>
<td>130 (36.93 %)</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td>X² = 0.4</td>
<td>X² = 0.4</td>
<td>X² = 5.0</td>
<td>X² = 5.3</td>
</tr>
</tbody>
</table>

X² chi square; NS: Not significant

Table I: Numbers of positive sera and seroprevalences (%) of the BTV serotypes 4, 9 and 16 in cattle from 3 provinces (namely Igdir, Kars and Ardahan) of the North-Eastern Turkey.
were 72.16%, 42.05% and 36.93%, respectively (Table I). Moreover, albeit the proportion of BTV-16 seropositive cattle was the highest in the Igdir region and the lowest in the Ardahan area, it did not significantly differ according to the considered province (P > 0.05) (Table I). In the same way, the BTV-4 seroprevalence in cattle tended to be elevated in the Igdir province and relatively weak in Ardahan but not significantly, and no significant difference was evidenced for the BTV-9 serotype repartition according to the 3 areas investigated.

Data was also evaluated in respect to single and/or multiple-serotype seropositivity (Table II). Only 29 of 352 (8.24%) animals were found negative for serum antibodies against the 3 selected serotypes whereas 13.07% (46 / 352) of them exhibited seropositive reactions against the 3 serotypes and 33.24% (117 / 352) were seropositive for 2 serotypes, the main BTV serotype association being BTV-4/BTV-9. Furthermore, the main BTV serotype association being BTV-4/BTV-9 was mainly encountered in the Igdir province (P < 0.01).

### Table II: BTV serotype associations (number and frequencies in %) determined in cattle from 3 provinces (namely Igdir, Kars and Ardahan) of the North-Eastern Turkey.

<table>
<thead>
<tr>
<th>Serotype associations</th>
<th>Igdir</th>
<th>Kars</th>
<th>Ardahan</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Triple seropositivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BTV-4 + / BTV-9 + / BTV-16 +</td>
<td>12 (10.62%)</td>
<td>8 (6.02%)</td>
<td>26 (24.53%)</td>
<td>46 (13.07%)</td>
</tr>
<tr>
<td>Double seropositivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BTV-4 + / BTV-9 + / BTV-16 -</td>
<td>20 (17.70%)</td>
<td>25 (18.80%)</td>
<td>13 (12.26%)</td>
<td>58 (16.48%)</td>
</tr>
<tr>
<td>BTV-4 + / BTV-9 - / BTV-16 +</td>
<td>15 (13.27%)</td>
<td>10 (7.52%)</td>
<td>5 (4.72%)</td>
<td>30 (8.52%)</td>
</tr>
<tr>
<td>BTV-4 - / BTV-9 + / BTV-16 +</td>
<td>9 (7.96%)</td>
<td>12 (9.02%)</td>
<td>8 (7.55%)</td>
<td>29 (8.24%)</td>
</tr>
<tr>
<td>Single seropositivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BTV-4 + / BTV-9 - / BTV-16 -</td>
<td>37 (32.74%)</td>
<td>51 (38.35%)</td>
<td>32 (30.19%)</td>
<td>120 (34.09%)</td>
</tr>
<tr>
<td>BTV-4 - / BTV-9 + / BTV-16 -</td>
<td>1 (0.88%)</td>
<td>7 (5.26%)</td>
<td>7 (6.60%)</td>
<td>15 (4.26%)</td>
</tr>
<tr>
<td>BTV-4 - / BTV-9 - / BTV-16 +</td>
<td>11 (9.73%)</td>
<td>9 (6.77%)</td>
<td>5 (4.72%)</td>
<td>25 (7.10%)</td>
</tr>
<tr>
<td>No seropositivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 (7.08%)</td>
<td>11 (8.27%)</td>
<td>10 (9.43%)</td>
<td>29 (8.24%)</td>
<td></td>
</tr>
</tbody>
</table>

### Discussion

The Bluetongue disease is classified in communicable disease list of International Office of Epizootics and is widespread worldwide [6, 9]. It is an economically important infection of ruminants that causes deleterious effects on the reproductive function with abortions, early embryonic loss and congenital malformations [9, 14]. The Bluetongue virus (BTV) was evidenced in Turkey for the first time in 1944. Since, the prevalence of four BTV serotypes (BTV-2, BTV-4, BTV-9 and BTV-16) has been reported in Turkey [4, 7, 9, 18]. Some recent studies reported seropositivity rates between 2.30% and 69.04% in cattle and between 1.00% and 36.04% in sheep [4, 7, 8, 18]. Besides, serological evidence of BTV infection has been reported with various rates ranging from 7.10% to 83.30% in Belgium, Iran, Pakistan and Indian [1, 10, 13, 16, 17]. Between 1999 and 2001, the severe Bluetongue episode in Turkey was caused by two serotypes (namely, 9 and 16). The majority of these viruses were found to be closely related to those presented in neighbouring European countries [15]. Molecular epidemiological evidence obtained by phylogenetic analysis of two southern isolates supports the hypothesis that virus incursions have entered Turkey from neighbouring countries to the East [15].

In the current study, the proportion of cattle with circulating anti-BTV antibodies was very high (91.76%), suggesting that cattle was a very important reservoir for the BTV, as serologically there is no cross reaction with cattle specific viruses. Furthermore, the 3 main BTV serotypes were well represented in the North-Eastern Turkey with sero-prevalence ranged from 36.93% for the serotype 16 to 72.16% for the serotype 4. Furthermore, the frequency of associated seropositivities (mainly with BTV-4 and BTV-9) was markedly elevated, 50.46% of the infected animals (163/323) exhibiting serum antibodies targeted to at least 2 different BTV serotypes in the present study. These data may well represent the seroprevalence of the serotypes in the region as there is little cross reaction between BTV serotypes [12] that might lead to overestimation of the results. Detection of single serotype specific viral antibodies was very common which was followed by double and triple combinations. Furthermore, the overall BTV seroprevalence and the specific seroprevalences for the serotypes 16 and 4 at a lesser extend varied among the 3 provinces tested, although not significantly, cattle from the Igdir province close to the South-Western Armenia appearing as the most infected whereas animals from the Ardahan area near Georgia appeared to be less exposed to BTV. Considering the Turkey’s geographical location, this country could form a bridge between Asia-Europe for transition of many contagious diseases, such as the BTV infection. Further transversal epidemiological sero-survey would be necessary for exploring the BTV infection occurrence in this world area regrouping all borderline Provinces of Turkey, Georgia, Armenia, Azerbaijan and Iran and for evidencing the preferential virus circulation ways.

As a conclusion, the results obtained in the present study showed that BTV serotypes 4, 9 and 16 are common in North-eastern Anatolia region, which is the most important...
cattle production area in Turkey. Consequently, sanitary programs for preventing and controlling the blue tongue disease may be based not only on vaccination plans and vector eradication but also on control of cattle movement between neighbouring countries.

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References


