Pestivirus seroprevalence in sheep populations from inland and coastal zones of Turkey

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SUMMARY

In this study, pestivirus seroprevalence was investigated by serum neutralisation test using the NADL strain of Bovine Viral Diarrhoea Virus (BVDV-NADL) in 2444 sheep blood serum samples collected from a total of 11 provinces in coastal (6) and inland (5) areas of Turkey. Overall pestivirus seropositivity was 18.94 % (463/2444) and the mean BVDV seroprevalence observed in the inland zone was significantly higher than in the coastal zone (p < 0.05). The distribution of antibody titres significantly differed between the coastal and inland sheep populations : proportions of intermediate titres (1:20 – 1:40) were markedly increased in the coastal area with a warm climate whereas low titres (1:5 – 1:10) were more frequently observed in the inland region with cold climate. Consequently, pestivirus infections would be enzootic in the inland area and climate, especially a cold climate, can play a major role in the virus incidence.

Keywords : seroprevalence, sheep, pestivirus, climate, Turkey.

RÉSUMÉ

Séroprévalence des pestivirus au sein de 2 populations de moutons, une en zone centrale et une en zone côtière en Turquie.

Au cours de cette étude, la séroprévalence des pestivirus chez le mouton a été établie dans 11 provinces turques situées en zone côtière (6 provinces) ou en zone intérieure (5 provinces) à partir de 2444 animaux par un test de séro-neutralisation utilisant la souche NADL du BVDV (Bovine Viral Diarrhoea Virus). La séroprévalence globale a été de 18.94 % (463/2444) et la valeur moyenne de la séroprévalence du BVDV observée dans la région intérieure (caractérisée par un climat froid) a été significativement plus élevée que celle observée dans la région côtière (avec un climat chaud) (p < 0.05). La distribution des titres en anticorps a différé significativement entre les populations ovines de ces 2 régions (p < 0.05) : les proportions des titres intermédiaires (1:20 – 1:40) ont été nettement augmentées au sein de la population côtière alors que les faibles titres (1:5 – 1:10) ont été plus souvent observées chez les ovins de la région intérieure. Par conséquent, les infections par les pestivirus pourraient être enzootiques dans cette zone et le climat, particulièrement un climat froid, serait un élément prépondérant dans le développement du virus.

Mots-clés : séroprévalence, mouton, pestivirus, climat, Turquie.

Introduction

Pestivirus are small enveloped, single-stranded, positive sense RNA viruses that are divided into the three virus species, including bovine viral diarrhoea virus (BVDV), Border disease virus (BDV) of sheep and classical swine fever virus (CSFV) [6, 8, 12, 19]. They are widespread throughout the world and are responsible for economically important effects [12]. Pestivirus in cattle and sheep have a wide clinical spectrum, ranging from a mild and moderate sub-clinical form to the highly fatal form known as mucosal disease (MD) with symptoms including reproductive failure, poor development and enteric diseases [1, 12]. Both BVDV and BDV can infect sheep and cattle. In addition, spread between the host species has been confirmed naturally and by experimental studies [6].

BVDV has two known biotypes and genotypes. The cytopathogenic (cp) and non cytopathogenic (ncp) biotypes of BVDV are described according to the presence or absence of visible cytopathic effects in the infected cell culture [4, 10, 20]. The two BVDV genotypes are defined as BVDV-1 and BVDV-2 according to their nucleotide and antigenic differences [9, 10]. Both genotypes of BVDV may occur with mild clinical symptoms or lead to sub-clinical courses in sheep [11, 16].

Turkey is a very important sheep-producing country with 25000000 sheep [2]. However, productivity is very low because of the widespread breeding of sheep in small flocks and various diseases. Pestivirus are one of the important infections reducing productivity because they decrease meat and milk production and reproductive rates.

The purpose of this study was to actualize pestivirus seroprevalences observed in sheep in rural areas and to research the effects of regional climate on pestivirus seroprevalences by comparing them in 11 provinces (5 inland countries characterized by a cold climate) and 6 coastal countries characterized by a warm climate in Turkey.
Material and Methods

SERUM SAMPLES

In this study, blood samples were collected from 2444 sheep stemming from 11 provinces in Turkey, six were coastal (C) and 5 were inland (I) regions (Figure 1). Sampling was performed on randomly selected 1 year of age (or older) healthy sheep that had not been vaccinated for BVDV and BDV, and had been bred by rural breeders in these regions.

After clotting (overnight at 4 °C) the blood samples were centrifuged at 1500 g for 15 min at 4 °C and sera were transferred into sterile tubes (Eppendorf, Germany), heat inactivated at 56 °C for 30 minutes and stored at -20 ºC until tested.

CELL CULTURE AND VIRUS

Madin Darby Bovine Kidney (MDBK) cell lines were used for the titration and serum neutralisation tests of NADL (National Animal Disease Laboratory) strain of BVDV and cells were grown in Dulbecco’s Minimal Essential Medium (DMEM, PAA, Austria) containing 10 % foetal calf serum (FCS, PAA, Austria).

The BVDV-NADL strain (obtained from the Department of Virology, Veterinary School of Ankara University, Turkey) was inoculated into MDBK cells grown to confluence on a 25 cm² cell culture flask (TPP, Switzerland). After 1h incubation at 37 °C, DMEM containing 1 % FCS was added and cells were incubated at 37 °C. Cells were monitored by daily microscopic examination until the cytopathic effects (CPE), i.e. the gathering up and fusion of infected cells to form multinucleated giant syncitia, were 80-100 % completed. After freezing and thawing three times, cell lysates were aliquoted and stored at -80 °C as virus stocks.

MICRO-NEUTRALISATION TEST

For investigation of serum antibodies against the BVDV-NADL strain, the serum neutralisation (SN) method was used, as described by FREY and LIESS [7]. In 96 micro plate wells (Grainer, Germany), 50 µl of each serum/well (in duplicate) were mixed with an equal volume of virus that had been diluted to 100 TCID₅₀ (Tissue Culture Infective Dose 50) whereas two wells containing 100 µl of 100 TCID₅₀ diluted virus were used as virus control and two wells containing 100 µl of DMEM were used as cell control. All of the test plates were then incubated at 37 °C for 60 minutes. Finally, MDBK cells (20,000 cells per well) were distributed into test plate wells and incubated at 37 °C in 5 % CO₂ for 3 days. Serum samples which inhibited cytopathic effect were deemed to be positive for anti-BVDV antibodies. Serum Neutralisation Index 50 values (SN₅₀, the dilution of serum needed to inhibit 50 % of the cytopathogenic effects) were determined and antibody titres equal or greater than 1 : 5 were considered as positive.

STATISTICAL ANALYSIS

The Chi-square test was used to analyse seroprevalence values in the 11 provinces and the parametric student test and Friedman’s non-parametric test was used to determine differences among the 11 locations [15]. Data were analyzed with the SAS statistic program [18]. Differences were considered positive when p values were less than 0.05.

Results

The overall percentage of sheep with anti-BVDV antibodies was 18.94 % (463/2444) and the rates of seropositivity varied from 0 to 52.63 % according to the location (Table 1). The global BVDV seroprevalence in the coastal area was 4.90 %. The proportions of seropositive animals remained relatively low in the 6 provinces of this zone, ranging from 0 % (Ordu and Rize provinces) to 15.43 % (Giresun province) : the mean value (± standard deviation) was 4.04 ± 5.37 %. By contrast, BVDV seroprevalence in the total inland area was high (24.67 %) and seropositivities according to the location were comprised between 2.30 % (Amasya province) and 52.63 % (Erzurum province), with a mean positivity of 31.18 ± 19.21 %. The difference of seropositivity rates between coastal and inland areas was significant (p < 0.05).

Among positive sheep for anti-BVDV antibodies, most of them presented low titers (59.61 % for 1:5 - 1:10 antibody titers) while high titers (1 : 80 and more) were more rarely observed (in 8.01 % of cases) (Table 2). Although the proportions of low titers of anti-BVDV titers were always elevated in the 2 populations of sheep (coastal vs. inland areas) and were 42.56 % and 60.98 % respectively, the distribution of the antibody titers has significantly differed between these two regions (chi-square test : p < 0.05), the frequencies of intermediate SN₅₀ values being greater in the coastal area (1:20 – 1: 40 titers : 42.86 % in the coastal zone and 31.54 % in the inland zone) (figure 2). However, in 2 provinces of the inland area (with subsequent high number of positive sheep) (Sivas and Erzurum provinces), the proportions of intermediate titers were markedly elevated, 41.70 % and 46.70 % respectively.
Field and experimental studies have shown that both BVDV and BDV can infect sheep and cattle, causing similar clinical and pathological symptoms [4, 12, 17]. The BVDV seropositivity rate among sheep is worldwide between 4 % and 11 % [5]. Pestivirus prevalence in sheep has been investigated by many researchers using the cytopathogenic BVDV - NADL strain. With it, LAMONTAGE et al. [13] found a seropositivity rate of 10.9 % in Quebec, Canada, and LOKEN et al. [14] found a seropositivity rate of 4.5 % in Norway. In Turkey, BURGU et al. [3, 4] found a constant seropositivity rate in sheep of 21.5 % between 1984 and 2001. The BVDV seroprevalence observed in the present study (18.94 %) was in agreement with these previous studies, and showed the incidence of pestivirus in sheep across 11 provinces, 6 in the coastal area and 5 in the inland area. This rate is lower than the seropositivity values documented by previous researchers in Turkey but higher than for many other studies performed worldwide. Accordingly, pestivirus infections in sheep in Turkey tend to be enzootic.

### Discussion

Field and experimental studies have shown that both BVDV and BDV can infect sheep and cattle, causing similar clinical and pathological symptoms [4, 12, 17]. The BVDV seropositivity rate among sheep is worldwide between 4 % and 11 % [5]. Pestivirus prevalence in sheep has been investigated by many researchers using the cytopathogenic BVDV - NADL strain. With it, LAMONTAGE et al. [13] found a seropositivity rate of 10.9 % in Quebec, Canada, and LOKEN et al. [14] found a seropositivity rate of 4.5 % in Norway. In Turkey, BURGU et al. [3, 4] found a constant seropositivity rate in sheep of 21.5 % between 1984 and 2001. The BVDV seroprevalence observed in the present study (18.94 %) was in agreement with these previous studies, and showed the incidence of pestivirus in sheep across 11 provinces, 6 in the coastal area and 5 in the inland area. This rate is lower than the seropositivity values documented by previous researchers in Turkey but higher than for many other studies performed worldwide. Accordingly, pestivirus infections in sheep in Turkey tend to be enzootic.
When the huge economic losses caused by pestiviruses are considered, their control becomes even more important for sheep production in Turkey.

The mean seroprevalence was significantly higher in the inland zone with cold climate characteristics ($p < 0.05$). Consequently, climatic factors can play a major role in virus spread, and warm climate conditions would reduce virus transmission by altering viral membrane integrity and the natural resistance of the virus in the environment.

Albeit low titres were always in majority and high titres scarcely evidenced in the two populations of sheep, the partition of serum anti-BVDV antibody titres significantly differed according to the sheep location ($p < 0.05$), intermediate SN50 values being more frequently encountered in the population from the coastal zone. In the two inland provinces closely related to the coastal areas (Sivas and Erzurum), intermediate titres were also elevated. These results indicate that BVDV infections in inland regions are probably more severe and occur more recently, in an enzootic way. Two important factors may favour high BVDV occurrence in zones with a cold climate: firstly, a cold climate compared to a warm and moist climate would improve virus resistance in the environment and secondly, interspecies transmission due to closed breeding in these rural regions would be exacerbated.

In conclusion, the serological data generated by this study indicate that pestiviruses are widespread in sheep bred in rural areas in Turkey, particularly in the inland provinces. This situation increases the risk of spread of pestivirus which already causes huge economic losses in sheep farming. When the risk of spread between species is also considered, the importance of pestivirus infection control in ruminants becomes a priority in rural zones in Turkey.

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References