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Original Contribution

COMPARISON OF THE SEROPREVALENCE AGAINST SOME RESPIRATORY VIRUSES IN MIXED SHEEP-GOAT HERDS IN TWO REGIONS OF BULGARIA

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ABSTRACT

In the course of the study 199 blood samples from sheep and goats were tested. They were collected from 4 mixed herds, situated in 4 different settlements, divided in two regions (two settlements in each). The sera were tested for presence of virus-neutralizing antibodies against Parainfluenza-3 Virus (PI-3), Bovine Viral Diarrhea Virus (BVDV) and Bovine Herpesvirus -1 (BHV-1); they were also tested for group-specific antibodies against adenoviruses through Ovine adenovirus serotype 3 (OAV-3). For detection of virus-neutralizing antibodies a micro-virusneutralizing reaction (MVNR) was used. For detection of group-specific antibodies immuno -diffusion test (ID) was used. The average seroprevalence among sheep was 2.4% to BHV-1; 64 % to BVDV, 31.3 % to PI-3 and 37.3% to OAV-3. Among goats average seroprevalence was 3.4 % to BHV-1; 18.1 % to BVDV, 58.6 % to PI-3 and 69 % to OAV-3.

Key words: sheep, goats, seroprevalence, mixed herds, BHV-1, PI-3, BVDV, OAV-3

INTRODUCTION

The total number of sheep in our country in 2005 has been about 1 600 000 and the number of goats – approximately 608 000 (1). According to the data of Ministry of Agriculture and Foods in approximately 199 500 small household animal farms the average number of goats is only three, and about 60 % of sheep are kept in farms with ten or more animals (2). The two ruminant species are often gathered into mixed pasture herds or are bred together. The close contact between these two species predisposes the exchange of microorganisms, including viruses. Some of the most important economical losses in small ruminant farming are due to respiratory infections. This great importance is due to their rapid spreading and infection of many animals. The most often studied for their seroprevalence respiratory viruses are the virus of PI-3, BVDV, the adenoviruses (Ad) and BHV-1, with widely varying seropositivity worldwide. It is as follows:

For BHV-1 from total lack of seropositivity in both species to 12 % in sheep and 19.3 % in goats (3, 4).

For BVDV – from total lack of antibodies in both species to 48 % in sheep and 59 % in goats (5).

For PI-3 - from total lack of antibodies in sheep to 61.5 % seropositivity (6, 7), in goats from 0.3 % to 40.25 % (5).

For Ad – between 5% and 80.8 % among sheep (8,9) and between 0.02 % and 45.1 % among goats (10, 11).

Studies on the Balkans (including Bulgaria) reveal high values for PI-3 – prevalence in both species (61.5 % for sheep and 78.3 % for goats) (6, 11). High values have been detected also for Ad – 45.1 % (11). The aim of the present study was to determine the seroprevalence of antibodies against the abovementioned four prominent viruses, connected with respiratory diseases (BHV-1, PI-3, BVDV, OAV-3) in mixed herds of small ruminants in two regions of Bulgaria and to compare the data for both species in a same herd.

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MATERIALS AND METHODS:

Materials

A total of 199 serum samples from clinically healthy non-vaccinated animals originating from 4 mixed herds, situated in 4 villages in two regions (A and B) of Southeastern Bulgaria were studied. Eighty three serum samples originated from sheep and 116 were from goats aged between 1 and 10 years. The distribution of samples is as follows:

Region **A** a herd **1** – 20 sheep and 14 goats; herd **2** – 20 sheep and 20 goats.

Region **B** a herd **3** – 20 sheep and 13 goats; herd **4** – 23 sheep and 69 goats. The blood samples were collected during the summer season and the sera were frozen at -20 °C until tested.

Viral strains

The following viral strains were used:

An ovine isolate of Parainfluenza-3 Virus, strain 2H; Ovine adenovirus-3 (OAV-3) strain PD; cytopathogenic strain of Bovine Viral Diarrhea Virus (BVDV) strain Kableshkovo; Bovine Herpes Virus 1 (BHV-1) and strain Dolen Chiflik.

Methods

Micro – virusneutralizing reaction (MVNR).

Micro-virusneutralizing reaction was accomplished in 96-well PVC plates Limbro (Flow Laboratories™) following the method, prescribed by OIE (12). The antibody titer was determined as the highest serum dilution, preserving at least 50 % of the cells in a separate well from cytopathic effect (CPE). We used this reaction for detection of antibodies against the viruses BHV-1, BVDV and PI-3.

Immunodiffusion (ID)

Sera were tested for antibodies against a group-specific antigen, obtained from OAV-3, cultivated in "Calf Trachea" cell line. ID was carried out by the modified method of Haralambiev (13). It was used for detection of antibodies against the group-specific antigen of OAV-3. The precipitating viral antigen was prepared by addition of ammonium sulfate to a cell culture suspension, infected with OAV-3 and freeze-thawed four times. The mixture was stirred for 24 h at 4 °C with magnetic stirrer. The precipitate was separated by centrifugation at 12 000 rpm for 30 min. Then Tris-HCl buffer with pH 7.4 was added to it up to 1/100 of the initial volume. The sulfate ions were removed by dialysis through cellophane membrane against saline for 48 h. The antigen was concentrated again to 1/100 of the initial volume and mertiolate was added to a final concentration of 1:10 000. The purified antigen had final precipitating titer of 1:16.

A positive control hyperimmune serum against OAV-3 was obtained from rabbits after six fold intravenous inoculation of 1 ml semi-purified viral antigen according to the method of McFerran et al. (14). The obtained serum had a virus-neutralizing titer of 1:256 and precipitating titer of 1:16.

RESULTS AND DISCUSSION

The study data are shown in **Table 1**. Antibodies against all the 4 viruses, included in the study were detected in all the herds, except herd 2, in which no antibodies against BHV-1 were detected. Most positive reagents were detected against OAV (55.8 %), followed by PI-3 (47.2 %), BVDV (37.2 %) and least positive animals were detected against BHV-1 (3 %).

We found differences between the two animal species. The sheep had most seropositive reagents to BVDV (64 %), while the goats had three times less seropositive reagents to the same virus (18.1 %). The second most prevalent antibodies among sheep were those against OAV (37.3%). The goats displayed about twice more seroreagents to this virus – 69%, which makes them the most frequent for this species. The third place by antibody frequency with the sheep takes PI-3. Thirty one point three per cent (31.3 %) of the sheep had an antibody response to it. This ratio is nearly twice higher by the goats – 58.6 %, the second most frequent antibodies. With both species the least seropositive animals were to BHV-1 (2.4 % for the sheep and 3.4 % for the goats.).

There were considerable differences between the two regions as well. The greatest difference is related to BVDV – 95 % seropositive sheep in region A compared to 34.9 % in region B. There was also appreciable difference concerning OAV-3 but in favor of region B (48.8 % seroprevalence compared to 25 % in region A). With the goats the biggest difference between the two regions is concerning BVDV – 2.9 % seroprevalence in region A compared to 24.4 % in region B. We have found a lesser difference for this species concerning PI-3 (32.4 % seroprevalence in region A against 69.5 % in region B).

There were considerable differences between sheep and goats in the separate herds. The

biggest is the difference in region A concerning BVDV – 95 % seropositives among the sheep in both herds, compared to 0 % seropositives among the goats in herd **1**, and 5 % in herd **2**. A great difference between the two animal species in this region is observed with OAV, in favor of the goats – 79.4 % seropositive goats compared to averagely 25 % seropositive sheep average for the region.

Table.1 *A study for BVDV, BHV-1 and PI-3 through virus neutralization and for OAV through ID. Legend: * - SH-sheep, **- GT-goats*

Settlement, species and nuber of the animals studied		Number of positives (%) BVDV	Number of positives (%) BHV-1	Number of positives (%) PI-3	Number of positives (%) OAV-3
Region A	Herd 1 SH* 20	19 (95 %)	-	8 (40 %)	3 (15 %)
	Herd 1 GT** 14	-	1 (7,1 %)	3 (21.4 %)	12 (85.7 %)
	Herd 2 SH 20	19 (95 %)	-	6 (30 %)	7 (35 %)
	Herd 2 GT 20	1 (5 %)	-	8 (40 %)	15 (75 %)
	Total for the region SH	38 (95 %)	-	14 (35 %)	10 (25 %)
	Total for the region GT	1 (2.9 %)	1 (2.9 %)	11 (32.4 %)	27 (79.4 %)
Region B	Herd 3 SH 20	12 (60 %)	-	6 (30 %)	9 (45 %)
	Herd 3 GT 13	7 (53.8 %)	2 (15.4 %)	4 (30.8 %)	6 (46.2 %)
	Herd 4 SH 23	3 (13.04 %)	2 (8.7 %)	6 (26.1 %)	12 (52.2 %)
	Herd 4 GT 69	13 (18.8 %)	1 (1.4 %)	53 (76.8 %)	47 (68.1 %)
	Total for the region SH	15 (34.9 %)	2 (4.7 %)	12 (27.9 %)	21 (48.8 %)
	Total for the region GT	20 (24.4 %)	3 (3.7 %)	57 (69.5 %)	53 (64.6 %)
Total SH		53 (64 %)	2 (2.4 %)	26 (31.3 %)	31 (37.3 %)
Total GT		21 (18.1 %)	4 (3.4 %)	68 (58.6 %)	80 (69 %)
Total animals		74 (37.2 %)	6 (3 %)	94 (47.2 %)	111 (55.8 %)

In region B a significant difference was detected only among the animals in herd **4** relatively to PI-3 – 26.1 % seropositive sheep compared to 76.8 % seropositive goats.

As a whole the seroprevalence rates of the studied viruses are relatively high compared to the data of similar studies across the world. Only the BHV-1 result is not considerably different from the worldwide data. For goats they vary from 5.52 % (15) and 6.3 % (16) to 11.3 % (5); for sheep they are even lower – 1.74 % in Turkey (17), 0 % in Canada (3), 1.2 % in USA (10). Other studies in Bulgaria also yield similar results – 1.8 % for goats (11) and 1.33 % (18). The seroprevalence to PI-3 among goats, determined in this study is the highest among all similar researches around the world – 40.25 % in Senegal (19); 35.3 % in Turkey (20); 28.3 % in Canada (16); 0.3 % in Zaire (5). The seroprevalence to this virus among sheep found by us (31.3 %) however is relatively low – 61.5 % in former Yugoslavia (6); 53 % in Northern Ireland (8); 39 % in Senegal (19). As for the BVDV there is a reverse correlation – the sheep seroprevalence detected by us (64 %) is relatively higher than those, found abroad – 48 % in Senegal (19), 25.6 % in Canada (16), 27.5 % in Egypt (21); 32 % in Northern Ireland (8). Among the goats the BVDV seroprevalence results (18.1 %) do not differ from the average world values – 16.7 % in Austria (22), 31,4 % in Egypt (21), 15.1 % in Canada (16), 1 % in Indonesia (7). The high levels of OAV-3 seroprevalence (37.3 % in sheep and 69 % in goats) are close to those detected earlier in our country (11) and are

higher than those, determined in other countries. There are values relatively close to ours only in Turkey – 33.04 % seropositivity to OAV-1 and 29.56 % seropositivity to OAV-3, detected by virus neutralization (23), which implies a regional spreading of this viral group on the Balkans. There has been conducted a similar study through ID with group antigen from Bovine Adenovirus – 1 (BAV-1) (9), in which the author has detected 5 % seroprevalence for sheep and 34 % for goats. Adair et al. (8) in Northern Ireland have detected 80.8 % seropositivity to BAV-1 and BAV-8 in sheep, but this research has utilized an immunofluorescence test.

We found the highly uneven interspecies distribution of the studied viruses between the two regions of the country. The differences between the seropositive animals in the separate herds were probably due to the different species susceptibility to the viruses as a result from their evolutionary adaptation to different hosts.

CONCLUSIONS

- Four studied viruses were found in both regions and in both species of animals, with exception of one herd without seroreagents against BHV-1 (herd 2). OAV was most widely spread, followed by PI-3, BVDV and BHV-1, which was least widely spread.
- In three viruses (BVDV, PI-3 и OAV) there were differences in distribution between two species - in sheep number of seroreagents against BVDV was threefold higher, while in goats number of seroreagents against PI-3 and OAV was twofold higher.
- There was prevalence of seroreagents from the two species in the different regions - in region A in sheep number of seroreagents against BVDV was threefold higher as compared to region B; in region B in goats number of seroreagents against BVDV was eightfold higher as compared to region A. In goats number of seroreagents against PI-3 in region B was twofold higher as compared to region A.

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