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Original article

Occurrence of emerging ruminant viruses in goats in Poland

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Abstract

Health status of Polish goat population in regard to the viral diseases remained mostly unknown. In order to determine serological status of Polish goats for selected emerging ruminant viruses, 365 serum samples collected between 2017 and 2019 in 36 districts within 10 of Polish provinces, were tested. No antibodies specific to Peste de Petite Ruminants Virus (PPRSV) and capripoxviruses (CaPV) were found in any of the tested animals. Only single individual (0.27%) was seropositive to Blutongue Virus (BTV). Antibodies directed to Schmallenberg Virus (SBV) were detected in 46 goats which represented 12.6% of the tested population. No association between seropositivity to SBV and year of sampling, province of origin, gender and age was found. In conclusion, among studied viral pathogens, currently only SBV seemed to be important for epidemiological status of Polish goats.

Key words: goat, seroprevalence, emerging viruses, BTV, PPRV, SBV, capripoxviruses

Introduction

The occurrence of viral infections in Polish goat population remains mostly unexplored. As the goat population in Poland is around 40,000 (CSO 2018), and goat breeding is still considered a marginal area of agriculture, the species is rarely covered by official monitoring, with the exception of Bluetongue virus (BTV) (Kaba et al. 2007, Anonymous 2010 and 2017). Therefore, in last two decades, the exposure to viral infections among goats was sporadical. The studies included Schmallenberg virus (SBV) (Kaba et al. 2013, Kesik-Maliszewska et al. 2021), Small Ruminant Lentiviruses (SRLVs) (Kaba et al. 1997, Olech et al. 2012, 2018), Bovine Viral Diarrhea Virus (BVDV) (Czopowicz et al. 2011), Caprine Herpesvirus (CpHV-1) and Bluetongue Virus (BTV) (Czopowicz et al. 2010). As for the other, especially those classified as emerging, current data on their occurrence among goats in Poland are mostly unavailable. The lack of studies concerning this subject, could stem from the fact that infections with some of those viruses have never been detected in Poland nor was their presence confirmed for the last time many years ago (Kaba et al. 2007). However, in the last two decades the risk of new pathogen emergence is increasing and new outbreaks are reported annually in many European countries. The most evident example of the rapid spread of viral pathogen into new territories

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Table 1. Specificities and sensitivities of ELISA tests used in the study.

Test	Sensitivity (Se) %	Specificity (Sp) %	Reference
ID Screen Schmallenberg virus Competition Multi-species (IDVet, Grabels, France)	97.6	100.0	Pejaković et al. 2018
ID Screen PPR Competition (IDVet, Montpellier, France)	94.5	99.4	Libeau et al. 1995
ID Screen Capripox Double Antigen Multi-species (IDVet, Grabels, France)	98.6	97.3	Ibrahim et al. 2022
INgezim BTV DR (Ingenasa. Madrid, Spain)	100.0	99.5	Niedbalski 2011

is BTV that until 1998 was present in Europe only in Mediterranean region, however, since that time it has emerged on the large scale in northern parts of the continent (Wilson et al. 2008). Another recent example is an outbreak of Peste des Petits Ruminants Virus (PPRV), a pathogen previously not isolated in Europe, which appeared in Bulgaria in 2018 (Kamel et al. 2019). As international animal trade is increasing and advancing climate changes affect the range and population of potential viral vectors (Baylis et al. 2017), outbreaks of infections caused by emerging viruses could be expected in Europe. As it was proven that many viruses which infect goats could equally infect cattle and sheep (Lievaart-Peterson et al. 2015, Tuppurainen et al. 2017), the importance of goats as reservoirs, transmitters or sentinels for various viruses should be monitored.

The aim of this study was to evaluate the current serological status of Polish goat herds for the selected emerging viral pathogens including SBV, BTV, PPRV and viruses from genus *capripoxviruses* (CaPV). The presence of those pathogens was either previously confirmed in goat population (Kaba et al. 2013, Larska et al. 2013, Kesik-Maliszewska et al. 2021), detected in other species of farm animals (Niedbalski et al. 2010, Orlowska et al. 2016) or could become threat to the local population of small ruminants in foreseeable future.

Materials and Methods

Sampling

In total 365 serum samples collected from goats were obtained from regional labs participating in BTV, brucellosis and Q fever eradication and monitoring programmes in ruminants in Poland. The samples derived from 81 individual herds located in 35 districts found within 10 out of 16 Polish provinces, and were collected between September 2017 and January 2019. The sample size for the surveillance purposes are estimated using ruminant population data (Sergeant 2012, CSO 2019), however, goats in the BTV program are sampled after all sheep in a given district have been examined (Anonymous 2017). Therefore, the sample numbers could not be achieved representative for all provinces. The gender data was available for 340 individuals (290 females and 50 males). Data about the age of the animals were available for 339 individuals which age ranged from 6 months to 17 years. The number of samples collected from each province varied between 1 and 224.

Enzyme linked immunosorbent assays (ELISA)

Serum samples were tested for the presence of antibodies specific to different emerging viruses associated with infections in ruminants. For this four different ELISA kits specific to: SBV - ID Screen Schmallenberg virus Competition Multi-species (IDVet, Grabels, France), PPRV - ID Screen PPR Competition (IDVet, Montpellier, France), CaPV - ID Screen Capripox Double Antigen Multi-species (IDVet, Grabels, France) and BTV - INgezim BTV DR (Ingenasa. Madrid, Spain) were used. All assays were performed according to manuals provided by the manufacturers of the tests. The sensitivities (Se) and specificities (Sp) of the tests are given in Table 1.

Statistical analysis

The 95% or 97.5% (one-sided) confidence interval (CI) of the apparent prevalence of the antibodies (percentage of seropositive) to tested viruses was estimated using a binomial exact distribution. The true prevalences were calculated using the formula: $\frac{\text{apparent seroprevalence} + \text{Sp} - 1}{\text{Se} + \text{Sp} - 1}$.

Most explanatory variables such as: year of sample collection, origin (province, county, herd) and gender were nominal. The age was categorized by the 25th and 75th centiles onto three groups: 1) kids ($\leq$ 1 year), 2) young goats (2-3 year old), 3) adults ($\geq$ 4 year old) in order to estimate odd ratios between different levels in the multivariate model. Bivariate associations between SBV seropositivity and exposure variables were estimated using χ^2, Fisher exact test and standard logistic regression, where appropriate. In order to assess the risk factors of SBV seropositivity, a genera-

Table 2. Descriptive statistics: Schmallenberg virus (SBV) seroprevalence in goats according to the explanatory variables including bivariate associations using logistic regression.

Variable	Category (values)	*n*/*N*[2]	Seroprevalence		OR[4]	95% CI OR	p value[5]
			%	95% CI[3]			
Year of sampling (*n*=363[1])					0.73	0.49-1.09	0.122
	2017	7/45	15.6	4.5-26.6			
	2018	15/72	20.8	11.2-30.4			
	2019	27/246	11.0	7.0-14.9			
Province (*n*=363)					1.11	0.94-1.31	0.234
	Dolnośląskie	0/1	0	0-97.5[6]			
	Kujawsko-Pomorskie	1/8	12.5	0.3-52.6			
	Lubuskie	0/1	0	0-97.5[6]			
	Małopolskie	1/24	4.2	0.1-21.1			
	Podkarpackie	3/8	37.5	8.5-75.5			
	Pomorskie	2/7	28.6	3.7-70.9			
	Świętokrzyskie	26/223	11.6	7.8-16.6			
	Śląskie	1/5	20.0	5.0-71.6			
	Wielkopolskie	0/2	0	0-84.2[6]			
	Zachodniopomorskie	15/84	17.8	10.3-27.7			
Gender (*n*=338)					0.67	0.25-1.79	0.423
	female	41/288	14.2	10.4-18.8			
	male	5/50	10.0	3.3-21.8			
Age group (*n*=337)					1.18	0.75-1.85	0.474
	≤ 1 year	6/49	12.2	4.6-24.8			
	2-3 years	18/145	12.4	7.5-18.9			
	≥ 4 years	22/143	15.4	9.90-22.4			

[1] excluding doubtful results, [2] number of SBV seropositive animals/all ruminants tested in the category, [3] 95% confidence interval, [4] odds ratio, [5] significance level at $p<0.05$ considered significant; [6] one-sided, 97.5% confidence interval.

lized linear mixed model (GLMM) was fitted by backward selection of insignificant (with $p>0.05$) predictor nominal and continuous variables one-by-one. The collinearity (Spearman $|\rho| > 0.5$; $p<0.05$) between the predictors was considered when building up the multivariable model. Possible confounding and clustering were analysed as previously described by Dohoo et al. (2010). To account for clustering, models including random intercept were assessed by checking the variance of the component and other variables. The multivariable models were compared by Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC), and the model with the lowest and highest values, respectively, was considered better fitting. Data were analysed in STATA v.13.0 software (StataCorp LP, Texas, USA).

Results

None of the animals were identified as seropositive for PPRSV or CaPV (0%, 97.5% CI: 0-1%) and only single individual (0.27%; 95% CI: 0.007-1.5%), a goat from Małopolskie province, sampled in 2019, was seropositive for BTV. Therefore, the true prevalences were 0%, 0% and 0.5% for PPRSV, CaPV and BTV, respectively. SBV specific antibodies were found in 46 (12.6%) of goat sera, whereas 2 samples (0.54%) were inconclusive and those were excluded from the statistical analysis. No association between SBV seroprevalence and year of sampling, province of origin, gender and age were found (Table 2).

Discussion

Although all of the emerging viruses analysed in the study have already been detected in goats in Europe in the last decade, only antibodies specific for one virus – SBV, were identified. This was in accordance with previous studies which reported the presences of this virus is goats in Poland (Kaba et al. 2013, Kesik-Maliszewska et al. 2021). As for PPRV, capripoxviruses and BTV, they were either not detected or no tests for their presence was ever performed in a caprine population.

PPRV is a virus from *Morbilivirus* genus causing acute severe diarrhea and a bronchointerstitial pneumonia in infected animals. It is characterized by the very high morbitidity and mortality rate as well as by the highly contagious nature, which make it a serious threat for small ruminant breeding in many countries of Africa, Asia and Middle East (Banyard et al. 2010). Some may argue whether testing notifiable diseases with acute or hyperacute outcome in Europe is justified. However, we should consider different susceptibility of indigenous ruminant breeds and different pathogenicity of viruses, which emerge in a different environment. The environment which changes is the main driver of pathogen transmission and disease emergence. Halecker et al. (2019) have observed, for example, only subclinical outcome with short period of elevated body temperature in one animal after experimental infection of German goats with Indian PRRSV stain. In our study, for the first time a population of Polish goats was tested for the presence of antibodies specific to PPRV. As until recently, no cases of PPRV infections were described in Europe, the lack of the seropositive individuals was not surprising. Nevertheless, the outbreak of the disease was for the first time detected in Bulgaria in 2018 within the borders of the European Union. That means that the risk of the spread of the virus to our region exists and freedom-of-infection status of small ruminants should be monitored (Kamel et al. 2019).

A similar epidemiological scenario applies to capripoxviruses including lumpy skin disease virus (LSDV), sheeppox virus (SPPV) and goatpox virus (GTPV), which have an important economic impact on cattle, goat and sheep farming in endemic regions. Infections with SPPV and GTPV are associated with potentially very high mortality and morbidity in small ruminants and both can be equally dangerous to goats, while LSDV mainly infects cattle causing relatively low mortality rarely exceeding 5% of infected animals (Babiuk et al. 2008, Machado et al. 2019). Although, infections with capripoxviruses have been previously restricted mainly to Africa and Asia, outbreaks of LSDV as well as SPPV and GTPV had appeared in Balkans and Russia in the recent years (Tuppurainen et al. 2017, Mercier et al. 2018). In Poland, until now infections with capripoxviruses have not been registered and the country was regarded as free from those viruses, although, no field studies on this subject have been done (Rola et al. 2016). Our study is the first one to investigate and confirm this status, at least regarding Polish goat herds.

The testing of exposure to two further viruses, BTV and SBV, was performed from quite different perspective, since those pathogens are considered re-emerging or even endemic in some parts of Europe. Bluetongue virus (BTV) is a vector-borne infectious agent infecting both domestic and wild ruminants. Infection could vary in the outcomes from lack of clinical signs to high mortality, depending on the serotype of the virus and species involved (Courtejoie et al. 2018). Although, BTV has only recently been detected in native population of Polish ruminants (Orlowska et al 2016, Krzysiak et al. 2017), in the last 15 years epizootics caused by this virus were responsible for serious economic losses in ruminants in a number of European countries (Wilson et al. 2008). Seropositive individuals have been identified also among animals imported to Poland from Germany and Netherlands since 2006 (Niedbalski et al. 2010). Since 2009, active monitoring of BTV in sheep and goats is carried out in Poland, but according to outlines of the programme samples from goats are only collected when the number of the sheep available for testing is insufficient (Anonymous 2017). Most recent study on BTV seroprevalence in Polish goat herds, that included large number of samples, was conducted in 2007 and did not result in detection of any seropositive animals (Czopowicz et al. 2010). In our study, for the first time, a goat with antibodies to BTV was detected in one of the herds from Małopolska region. Unfortunately, data about the origin of this particular animal were not available. It was not possible to establish whether it was introduced to the herd from abroad or became infected in Poland. If that second possibility is true, the source of infection could be herd of other species more prone to infections such as cattle or wild ruminants. We have shown that BTV-14 infections were present in Polish wild species of ruminants in the eastern part of Poland including European bison population from the Bieszczady Mountains, the region neighbouring with Małopolskie between 2013 and 2014 (Krzysiak et al. 2017). Alternatively, as no other animal from the same herd was found to be seropositive it is also possible that this single positive result could be attributed to non-specific reaction of the test.

The most prevalent among tested goats Schmallenberg virus (SBV), together with BTV are examples of climate sensitive infections, which risk is growing recently with the environmental changes including

climate alterations. Infections with SBV were first identified in Germany in 2011 and since then the novel orthobunyavirus has spread to the majority of countries in Europe infecting both wild and domestic ruminants (Lievaart-Peterson et al. 2015). In non-pregnant adult ruminants, clinical signs of infections are usually mild or remain absent but the virus may cause abortions and congenital malformations in offspring in pregnant ruminant females (Wernike et al. 2014). Studies on SBV seroprevalence in different countries have shown that compared to cattle and sheep, goats are relatively less frequently exposed to SBV (Helmer et al. 2016). In Poland, first cases of goats seropositive to SBV were identified in 2012 (Kaba et al. 2013) when 21 from 897 (2.3%) tested animals from western provinces of Poland were found to be seropositive. Next year, on the peak of epizootics, when the total percentage of reported seropositive ruminants reached over 34%, seroprevalence in goats remained low (3.5%) (Larska et al. 2014). Higher percentage of SBV seropositive goats (19.7%) was observed in the most recent study that included samples collected between 2013 and 2018 (Kesik-Maliszewska et al. 2021). This was close to the results of our study, where antibodies to SBV were found in 12.6% of tested goats. It is hard to determine if the differences observed between all this studies stem from the variability in spread of the virus among the goat population in relation to sampling time or from differences in regions of origin of the tested animals. Although in our study the percentage of seropositive animals differed depending on year of sampling with peak in 2018 (20.8%), observed relationship remained insignificant. The majority of the samples collected in our study, originated from Małopolska, Świetokrzyskie and Zachodnio-Pomorskie provinces. This last province was previously analysed by Kaba et al. (2013) for the presence of SBV antibodies in goats. Since 2013, the percentage of SBV seropositive animals decreased there from 16% to merely 4.7%. This could be explained by the lack of new infections with SBV since that time, resulting in a gradual decrease in the number of serocompetent animals in this region. It is believed that SBV become endemic in Europe and new epizootics will appear in regular intervals when the majority of the animals will be exchanged by new generations of seronegative individuals or serocompetent animals will lose their immunity (Kęsik-Maliszewska et al. 2016, Larska et al. 2018).

In conclusion, this study has revealed that among analysed viral pathogens, currently only SBV seems to be important for the health status of Polish goats. Nevertheless as the geographical range of pathogens such as Capripox and PPRV is expanding in Europe, their future emergence in Poland cannot be excluded.

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