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*Original article*

# Herd-level seroprevalence of pestivirus infection in goat population in Poland

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## Abstract

A disease survey was conducted between 2014 and 2018 in the Polish goat population to determine the seroprevalence of pestiviral infection. Blood samples from 910 goats (782 females and 128 males) were collected in 62 goat herds and tested for bovine viral diarrhea virus (BVDV) infection with a competitive ELISA in a serial fashion. Between 10 and 13 adult female goats were randomly selected from each herd, assuming individual-level seroprevalence of pestiviral infection in a herd of at least 30% and a level of confidence of 95%. In each herd, all males were tested. At least one seropositive goat was found in each of the 4 herds. However, in one herd, the only positive goat tested negative in serial retesting. Finally, 3 herds were considered as seropositive which yielded apparent herd-level seroprevalence of 4.8% (CI 95%: 1.7%, 13.3%). After adjusting for the ELISA herd-level sensitivity and specificity, the true herd-level prevalence was 3.9% (CI 95%: 1.2%, 12.0%). No males tested positive. In 2 out of 3 seropositive herds, goats regularly shared pastures with cattle.

**Key words:** *Pestivirus*, BVDV, goat

## Introduction

Pestiviruses are important pathogens which cause economic losses in livestock all around the world. The *Pestivirus* genus belonging to *Flaviviridae* consists of four species responsible for livestock diseases: bovine viral diarrhea virus-1 and 2 (BVDV-1 and 2), border disease virus (BDV) and classical swine fever virus (CSFV). A number of other pestiviruses considered to be distinct species have also been identified (Krametter-Froetscher et al. 2010). Cross-species infections are known to occur between different ruminant species, including the transmission of BVDV from cattle to goats and vice versa (Tegtmeier et al. 2000,

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Broaddus et al. 2007, Broaddus et al. 2009, Bachofen et al. 2013). Molecular techniques have demonstrated that both BVDV and BDV can infect small ruminants (Pratelli et al. 1999, 2001). Symptomatic pestivirus infections are uncommon in goats, with abortion being the main clinical sign (Nettleton et al. 1998). Even though pestiviruses are rarely isolated from naturally infected goats (Krametter-Froetscher et al. 2008), serological evidence of pestivirus infections in goats has been reported in many countries, including Poland.

In Poland, pestiviral infections appear to be widespread in the cattle population (Stefaniak et al. 2008, Kuta et al. 2013, Rypuła et al. 2020). Although BVDV-2 has recently been identified for the first time in Polish cattle (Polak et al. 2014), BVDV-1 remains a leading pestiviral species (Kuta et al. 2013). So far only a single epidemiological study regarding pestiviruses has been carried out in the Polish dairy goat population (Czopowicz et al. 2011). This survey revealed that in 2007 herd-level seroprevalence was roughly 10% (10.2%, CI 95%: 4.4%, 21.8%), and identified BVDV-1 as an infectious agent responsible for seroconversion. A recent meta-analysis showed that over the last few decades seroprevalence of BVDV infection in cattle in Europe had remained quite stable or even fell slightly (Scharnbock et al. 2018). Therefore, we decided to determine the herd-level seroprevalence of pestivirus infection in the Polish goat population several years after the first serosurvey so that we could pinpoint any up- or downward trend if present.

## Materials and Methods

### Study population

The study was conducted between 2014 and 2018. The study population consisted of dairy goat herds with at least 20 adult (>1 year-old) female goats. According to the Main Statistical Office of Poland, there were 239 such herds in this time period. The herds contained from 21 to 426 adult female goats (median herd size of 72 adult goats), 4824 heads in total.

### Method of sampling and sample size

The two stage cluster sampling method was applied (Thrusfield and Christley 2018). First, a representative sample of herds was selected so that the true herd-level seroprevalence could be determined. Then, in each of these herds a sample of adult female goats was drawn using a simple random method to detect at least one infected animal provided that the true within-herd seroprevalence was at least 30%. In each herd all adult males were also tested. The level of confidence was set at 95%. Epidemiological calculations (sample sizes, individual- and herd-level test accuracy parameters) were performed in EpiTools (Sergeant 2019).

Individual-level sensitivity (Se) and specificity (Sp) of the ELISA were assumed to be 92% and 95%, respectively (Evans et al. 2017, Kalaiyarasu et al. 2015). On the basis of the former serosurvey (Czopowicz et al. 2011) the herd-level prevalence of the infection in the goat population was expected to be low, so serological testing was performed in a serial fashion i.e. positive samples were retested with the same test to increase the predictive value of the positive result. Serial Se and Sp were calculated according to the following formulas (Thrusfield and Christley 2018):

$$Se_{serial} = Se^2$$

$$Sp_{serial} = 1-(1-Sp)^2$$

The individual-level $Se_{serial}$ and $Sp_{serial}$ were 84% and >99%, respectively. On this basis the number of goats randomly selected in each herd ranged from 10 to 13, depending on the herd size.

$Se_{serial}$ and $Sp_{serial}$ were then adjusted from individual- to herd-level ($HSe_{serial}$ and $HSp_{serial}$) according to Cameron and Baldock (1998), for a median herd size of 72 animals, herd cut-off value of one animal positive indicating a positive herd, 10-13 animals sampled per herd (median of 13), within-herd prevalence of 30% and sampling without replacement. $HSe_{serial}$ and $HSp_{serial}$ were 99% and 99%, respectively.

Assuming a true herd-level prevalence of 10% (Czopowicz et al. 2011), accepted precision of the estimation of 8% and the aforementioned test accuracy the number of herds to be randomly selected was at least 61.

True herd-level prevalence was calculated using the Rogan-Gladen estimator (Rogan and Gladen 1978):

$$\text{True herd – level prevalence} = \frac{\text{apparent herd – level prevalence} + HSp_{serial} - 1}{HSp_{serial} + HSp_{serial} - 1}$$

Ninety-five percent confidence intervals for proportions were calculated using the Wilson score method (Altman et al. 2000). Seroprevalences were compared between the present and the previous study (Czopowicz et al. 2011) using Pearson's chi-square test, with the significance level (α) set at 0.05.

### Blood collection and serological testing

Each year herds from a different part of Poland were visited so no overlapping between goat herds tested each year existed. Not more than 10 herds enrolled in this study had been screened a decade ago.

Blood was collected from the jugular vein to dry 10ml tubes, allowed to clot overnight, and centrifuged

Table 1. Detailed results of Innovative Diagnostics (ID) Screen® Bovine Viral Diarrhoea (BVD) p80 Antibody Competition assay.

| Herd | Total herd size | Herd location in Poland (name of province) | Goat | ELISA testing [a] | | | | Herd status |
|---|---|---|---|---|---|---|---|---|
| | | | | 1st | | 2nd | | |
| | | | | S/N% | Result | S/N% | Result | |
| **A** | 35 (1 male) | north-eastern (Podlasie) | 1 | 14.89 | pos | 13.46 | pos | POS |
| **B** | 171 (7 males) | western (Wielkopolska) | 1 | 7.82 | pos | 195.44 | neg | POS |
| | | | 2 | 34.94 | pos | 28.41 | pos | |
| | | | 3 | 14.33 | pos | 13.46 | pos | |
| | | | 4 | 14.78 | pos | 14.90 | pos | |
| | | | 5 | 8.14 | pos | 201.54 | neg | |
| **C** | 80 (2 males) | western (Wielkopolska) | 1 | 26.66 | pos | 17.89 | pos | POS |
| | | | 2 | 16.08 | pos | 14.69 | pos | |
| | | | 3 | 41.39 | pos | 35.39 | pos | |
| **D** | 184 (8 males) | southern (Dolny Śląsk) | 1 | 7.59 | pos | 190.17 | neg | NEG |

[a] A cut-off point according to the manufacturer's manual was set at a competition percentage ratio (S/N%) of ≤50%

at 1500 rpm for 10 min. After centrifugation, serum samples were stored in Eppendorf tubes at -20°C until testing.

The serum samples were tested using ID Screen® BVD p80 Antibody Competition assay (IDvet – Innovative Diagnostics, Grabels, France). This was a competitive ELISA for the detection of antibodies against the p80-125 protein (NSP2-3) of BVD/MD/BD virus in serum, plasma or milk from sheep, goats, cattle or other susceptible species. The ELISA was performed according to the manufacturer's manual (BVDC ver. 1117 GB). The plate was read at 450 nm using an ELISA reader (BioTek Instruments). A competition percentage (S/N%) of ≤50% was considered as a cut-off indicating the presence of antibodies.

## Results

Sixty-two dairy goat herds were visited, which accounted for 25.9% of the study population. Seven hundred and eighty-two adult dairy female goats (16.2% of the study population) and 128 adult male goats (all kept in herds) were enrolled in the study – 910 animals in total.

At least one seropositive goat was found in 4 herds (1 goat in herd A and D, 5 goats in herd B, and 3 goats in herd C). However, in two herds (B and D) retesting of positive samples yielded negative results – since in one of these herds (D), this was the only positive sample, this herd was finally classified as seronegative (Table 1). Therefore, 3 herds (A, B, C) were eventually considered as seropositive which yielded an apparent herd-level seroprevalence of 4.8% (CI 95%: 1.7%, 13.3%). After adjusting for $HSe_{serial}$ and $HSp_{serial}$ of the ELISA the true herd-level prevalence was 3.9% (CI 95%: 1.2%, 12.0%). There was no significant difference in the apparent herd-level seroprevalence between the previous and the present study (p=0.278). In 2 out of 3 seropositive herds the goats regularly shared pastures with cattle.

In total, only 7 of 910 goats tested positive which yielded an overall individual-level apparent seroprevalence of only 0.8% (CI 95%: 0.4%, 1.6%). This turned out to be even lower when corrected by $Se_{serial}$ and $Sp_{serial}$ of the ELISA – 0.6% (CI 95%: 0.2%, 1.3%). No males tested positive.

## Discussion

Our study shows that the herd-level seroprevalence of pestiviral infection in the dairy goat population is low and appears not to have increased over the previous decade, which is consistent with the results of the meta-analysis conducted recently in European cattle (Scharnbock et al. 2018).

This herd-level prevalence is much lower than that found in other European countries where it was roughly 30% in Austria, the Netherlands, Italy, and Norway (Loken 1990, Loken 1995, Krametter-Froetscher et al. 2006, Orsel et al. 2009, Cirone et al. 2018), and even 50% in Switzerland (Danuser et al. 2009). This difference in seroprevalence between Poland and other countries is very likely to spring from the fact that in Poland contact between the goat and cattle population is limited. Those two species are rarely kept on one farm, and if they do, there are usually a few cows which very rarely commingle with the goats.In contrast, in countries such as Austria and Switzerland, domestic

ruminants and wild ruminants share Alpine pastures during the grazing season, which increases the risk of transmitting pathogens (Krametter-Froetscher et al. 2006, Danuser et al. 2009). Wildlife has been shown to play generally a limited role in the epidemiology of pestiviruses in domestic ruminant species (Krametter-Froetscher et al. 2006), and it seems to be similar in the Polish goat population. Although the infection has been reported in European bison in eastern and south-eastern Poland (Salwa et al. 2007) and from red deer in north-eastern Poland (Fabisiak et al. 2018), positive goat herds were not located in any of the these regions.

The epidemiological methodology of the present study is to some extent comparable with the study carried out several years earlier by Czopowicz et al. (2011). At first glance the expected within-herd seroprevalence of pestiviral infection for calculation of the sample size to be drawn from each herd is now much higher than in the previous study. However, this discrepancy is compensated by the fact that in this study adjustment for imperfection of the ELISA used was applied, whereas no such adjustment had been made in the previous study. As a consequence, the previous study could only inform about the apparent herd-level seroprevalence.

Clearly, we cannot fully exclude the possibility that the true within-herd prevalences in Polish herds were in fact much below the threshold of 30%, and as a result some positive herds were missed. Even though most studies show high BVDV seroprevalences within infected cattle herds (Sarrazin et al. 2013, Kovago et al. 2015, Moore et al. 2015, Barrett et al. 2018), data on typical within-herd seroprevalence of pestiviral infections in small ruminants indicate high diversity between herds (Evans et al. 2018, Fecknous et al. 2018).

In contrast to the present study, in the previous one (Czopowicz et al. 2011) the viral neutralization test, which is a gold standard for serological diagnostics of pestiviral infections (Hanon et al. 2018), was used to verify the status of positive samples and to identify pestiviral species. This allowed us to conclude that the goats were infected with BVDV-1 and not BDV. The lack of a confirmatory test is an important limitation of the present study. As herds seropositive in this and in the previous study did not overlap, we can only suspect that BVDV-1 was still responsible for the serological response. The fact that no reports on the presence of BDV infection in any Polish ruminant species have so far been published and the presence of cattle on two of three positive farms seem to strengthen this suspicion.

Our study confirms that even though pestiviral infection is present in the Polish goat population, it appears to remain at a low and stable level. Nevertheless, serological surveillance of small ruminants should be recommended as a part of any pestivirus control program.

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## References

Altman D, Machin D, Bryant T, Gardner M **(2000)** Statistics with Confidence: Confidence Intervals and Statistical Guidelines, 2nd ed., BMJ Books, Bristol, UK, pp 46-48.

Bachofen C, Vogt HR, Stalder H, Mathys T, Zanoni R, Hilbe M, Schweizer M, Peterhans E **(2013)** Persistent infections after natural transmission of bovine viral diarrhoea virus from cattle to goats and among goats. Vet Res 44: 32

Barrett D, Parr M, Fagan J, Johnson A, Tratalos J, Lively F, Diskin M, Kenny D **(2018)** Prevalence of Bovine Viral Diarrhoea Virus (BVDV), Bovine Herpes Virus 1 (BHV-1), Leptospirosis and Neosporosis, and associated risk factors in 161 Irish beef herds. BMC Vet Res 14: 8.

Broaddus CC, Holyoak GR, Dawson L, Step DL, Funk RA, Kapil S **(2007)** Transmission of bovine viral diarrhea virus to adult goats from persistently infected cattle. J Vet Diagn Invest 19: 545-548.

Broaddus CC, Lamm CG, Kapil S, Dawson L, Holyoak GR **(2009)** Bovine Viral Diarrhea Virus Abortion in Goats Housed with Persistently Infected Cattle. Vet Pathol 46: 45-53.

Cameron AR, Baldock FC **(1998)** A new probability formula for surveys to substantiate freedom from disease. Prev Vet Med 34: 1-17.

Cirone F, Cirone S, Trerotoli P, Buono R, Ciappetta G, Di Summa A, Gaggiano G, Pratelli A **(2018)** Cross sectional study for pestivirus infection in goats in southern Italy. Small Ruminant Res 166: 12-16.

Czopowicz M, Kaba J, Schirrmeier H, Bagnicka E, Szaluś-Jordanow O, Nowicki M, Witkowski L, Frymus T **(2011)** Serological evidence for BVDV-1 infection in goats in Poland – short communication. Acta Vet Hung 59: 399-404.

Danuser R, Vogt HR, Kaufmann T, Peterhans E, Zanoni R **(2009)** Seroprevalence and characterization of pestivirus infections in small ruminants and new world camelids in Switzerland. Schweiz Arch Tierheilkd 151: 109-117.

Evans CA, Lanyon SR, O'Handley RM, Reichel MP, Cockcroft PD **(2018)** Seroprevalence of antibodies to Pestivirus infections in South Australian sheep flocks. Aust Vet J 96: 312-314.

Evans CA, Lanyon SR, Reichel MP **(2017)** Investigation of AGID and two commercial ELISAs for the detection

of Bovine viral diarrhea virus-specific antibodies in sheep serum. J Vet Diagn Invest 29: 181-185.

Fabisiak M, Salamaszynska A, Stadejek T **(2018)** Detection of seroconversion to bovine herpesvirus 1 related alphaherpesvirus and bovine viral diarrhea virus in Polish free-living deer. Pol J Vet Sci 21(3): 437-440.

Feknous N, Hanon JB, Tignon M, Khaled H, Bouyoucef A, Cay B **(2018)** Seroprevalence of border disease virus and other pestiviruses in sheep in Algeria and associated risk factors. BMC Vet Res 14: 339.

Hanon JB, De Baere M, de la Ferté C, Roelandt S, Guillot G, Van der Stede Y, Cay B **(2018)** Serological monitoring on milk and serum samples in a BVD eradication program: A field study in Belgium showing antibody ELISA performances and epidemiological aspects. Prev Vet Med 160: 136–144.

Kalaiyarasu S, Mishra N, Rajukumar K, Nema RK, Behera SP **(2015)** Development and evaluation of a truncated recombinant NS3 antigen-based indirect ELISA for detection of pestivirus antibodies in sheep and goats. J Immunoassay Immunochem 36: 312-323.

Kovago C, Forgach P, Szabara A, Mandoki M, Hornyak A, Duignan C, Pasztine - Gere E, Rusvai M **(2015)** Seroprevalence of bovine viral diarrhoea virus in Hungary - Situation before launching an eradication campaign. Acta Vet Hung 63: 255-263.

Krametter-Froetscher R, Benetka V, Duenser M, Bago Z, Theiner A, Preyler B, Moestl K, Vilcek S, Baumgartner W **(2008)** Descriptive study of a pestivirus infection in an Austrian goat. Vet Rec 163: 192-194.

Krametter-Froetscher R, Duenser M, Preyler B, Theiner A, Benetka V, Moestl K, Baumgartner W **(2010)** Pestivirus infection in sheep and goats in West Austria. Vet J 186: 342-346.

Krametter-Froetscher R, Loitsch A, Kohler H, Schleiner A, Schiefer P, Moestl K, Golja F, Baumgartner W **(2006)** Prevalence of antibodies to pestiviruses in goats in Austria. J Vet Med B Infect Dis Vet Public Health 53: 48-50.

Kuta A, Polak MP, Larska M, Zmudzinski JF **(2013)** Predominance of bovine viral diarrhea virus 1b and 1d subtypes during eight years of survey in Poland. Vet Microbiol 166: 639-644.

Løken T **(1990)** Pestivirus infections in Norway. Epidemiological studies in goats. J Comp Pathol 103: 1-10.

Løken T **(1995)** Ruminant Pestivirus Infections in Animals other than Cattle and Sheep. Vet Clin North Am: Food Anim Pract 11: 597-614.

Moore SJ, O'Dea MA, Perkins N, O'Hara AJ (**2015)** Estimation of nasal shedding and seroprevalence of organisms known to be associated with bovine respiratory disease in Australian live export cattle. J Vet Diagn Invest 27: 6-17.

Nettleton PF, Gilray JA, Russo P, Dlissi E **(1998)** Border disease of sheep and goats. Vet Res 29: 327-340.

Orsel K, Antonis AF, Oosterloo JC, Vellema P, van der Meer FJ **(2009)** Seroprevalence of antibodies against pestiviruses in small ruminants in the Netherlands. Tijdschr Diergeneeskd 134: 380-384.

Paton DJ **(1995)** Pestivirus diversity. J Comp Pathol 112(3): 215-236.

Polak MP, Kuta A, Rybaltowski W, Rola J, Larska M, Zmudzinski JF **(2014)** First report of bovine viral diarrhoea virus-2 infection in cattle in Poland. Vet J 202: 643-645.

Pratelli A, Bollo E, Martella V, Guarda F, Chiocco D, Buonavoglia C **(1999)** Pestivirus infection in small ruminants: Virological and histopathological findings. New Microbiol 22: 351-356.

Pratelli A, Martella V, Cirone F, Buonavoglia D, Elia G, Tempesta M, Buonavoglia C **(2001)** Genomic characterization of pestiviruses isolated from lambs and kids in southern Italy. J Virol Methods 94: 81-85.

Rogan WJ, Gladen B **(1978)** Estimating prevalence from the results of a screening test. Am J Epidemiol 107: 71-76.

Rypuła K, Płoneczka-Janeczko K, Czopowicz M, Klimowicz-Bodys MD, Shabunin S, Siegwalt G **(2020)** Occurrence of BVDV Infection and the Presence of Potential Risk Factors in Dairy Cattle Herds in Poland. Animals (Basel) 10(2): 230.

Salwa A, Anusz K, Arent Z, Paprocka G, Kita J **(2007)** Seroprevalence of selected viral and bacterial pathogens in free-ranging European bison from the Bialowieza Primeval Forest (Poland). Pol J Vet Sci 10: 19-23.

Sarrazin S, Veldhuis A, Meroc E, Vangeel I, Laureyns J, Dewulf J, Caij AB, Piepers S, Hooyberghs J, Ribbens S, Van Der Stede Y **(2013)** Serological and virological BVDV prevalence and risk factor analysis for herds to be BVDV seropositive in Belgian cattle herds. Prev Vet Med 108: 28-37.

Scharnbock B, Roch FF, Richter V, Funke C, Firth CL, Obritzhauser W, Baumgartner W, Kasbohrer A, Pinior B **(2018)** A meta-analysis of bovine viral diarrhoea virus (BVDV) prevalences in the global cattle population. Sci Rep 8: 14420

Sergeant ESG **(2018)** Epitools Epidemiological Calculators. Ausvet. Available at: http://epitools.ausvet.com.au.

Smith MC, and Sherman DM **(2009)** Goat Medicine, 2nd ed., Wiley-Blackwell, pp 196-198.

Stefaniak T, Janeczko K, Jawor P, Błach J, Pytloch P (**2008**) Occurrence of BVDV antibodies in cattle herds showing reproductive pathologies. Med Weter 64: 431-433.

Tegtmeier C, Stryhn H, Uttentha I, Kjeldsen AM, Nielsen TK **(2000)** Seroprevalence of border disease in Danish sheep and goat herds. Acta Vet Scand 41: 339-344.

Thrusfield MV, Christley R **(2018)** Veterinary epidemiology, 4th ed., Wiley-Blackwell, Oxford, UK, pp 227-284, 444-446.