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*Original article*

# Antibodies to parainfluenza virus type 3 in goat population in Poland

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## Abstract

Respiratory diseases constitute a major health problem in small ruminant herds around the world, and parainfluenza virus type 3 (PIV-3) has been shown to play a vital role in their etiology. This cross-sectional study describes the serological status of the non-vaccinated dairy goat population in Poland with respect to PIV-3 infection and investigates the relationship between the presence of antibodies to PIV-3 and some basic herd-level and animal-level factors, including small ruminant lentivirus (SRLV) infection. Serum samples from 1188 goats from 48 herds were tested for the concentration of antibodies to PIV-3 using a quantitative immunoenzymatic assay. Specific antibodies were detected in all tested goats from all herds. The concentration of PIV-3 antibodies varied from 8.4 to >240 ng/ml (median 95.9 ng/ml) and was significantly higher in goats from larger herds and from these herds in which cough was often observed by farmers. Moreover, it was noted that female goats had higher antibody concentrations than males. On the other hand, the concentration of PIV-3 antibodies did not prove to be significantly linked to the presence of SRLV infection. This study shows that PIV-3 infection in the Polish goat population is widespread and appears to contribute to the occurrence of respiratory diseases in goat herds.

**Key words:** caprine arthritis-encephalitis, CAE, goats, parainfluenza, PIV-3, respiratory disease, small ruminant lentivirus, SRLV

## Introduction

Respiratory diseases number among the most common health problems of ruminants resulting in considerable economic losses throughout the world (Ackermann et al. 2000). Multiple etiological agents, viral and bacterial, play a role in their development, with a substantial influence of environmental conditions (Chakraborty et al. 2014). Many viruses are capable of crossing the interspecies barrier and producing the infection in various ruminant species (Yeşilbağ and Güngör 2009). Viral infections render sheep and goats vulnerable to bacterial complications mainly due to injuries they inflict on the epithelium of the airways (Brogden et al. 1998, Dassanayake et al. 2013). Stress and poor environmental conditions, such as overcrowding, low-quality ventilation, and transportation often contribute to the emergence of clinical signs (Kapil and Basaraba 1997, Chakraborty et al. 2014). Parainfluenza type-3 virus (PIV-3) is one of the most important respiratory pathogens of ruminants (Yener et al. 2005). This non-segmented negative-sense genome single-stranded RNA virus belongs to the *Paramyxoviridae* family. PIV-3 can cause pneumonia by itself, however more often is it one of the pathogens of the etiological complex which causes enzootic pneumonia (Anita et al. 2015). During the infection clinical signs such as nasal and ocular discharge, cough as well as dyspnea in severer cases, usually accompanied by fever and inappetence, have been observed (Contreras-Luna et al. 2017).

PIV-3 infection is rarely diagnosed using pathogen-specific laboratory methods, of which the most common are virus isolation in cell culture, PCR (Gafer et al. 2009) and immunohistochemical examinations of tissues from the lower respiratory tract (Ceribas et al. 2012, Jarikre and Emikpe 2017). Modern serological methods based on the immunoenzymatic technique offer a cost-effective and efficient diagnostic modality, especially in large-scale epidemiological surveys, provided that no vaccinations are used in the study population (Yeşilbağ and Güngör 2009, Mao et al. 2017). While inactivated and attenuated polyvalent vaccines against PIV-3 are widely available and extensively used in cattle, none are either registered or available on the market for small ruminants. In some studies attenuated vaccines developed for intranasal administration to cattle have been used in sheep (Rodger 1989, Thonney et al. 2008); however, no studies regarding vaccinations of goats against PIV-3 have so far been published.

Small ruminant lentivirus (SRLV) causes caprine arthritis-encephalitis (CAE) and maedi-visna disease – chronic progressive multisystemic diseases of goats and

sheep, respectively (Ramírez et al. 2013). SRLV belongs to the family of retroviruses. Some representatives of this family are able to compromise immunity of infected animals. Even though SRLV does not seem to have any immunosuppressive potential (Thormar 2005), its ability to cause chronic, slowly progressive inflammation of the lungs (Blacklaws 2012) might predispose the animal to secondary viral and bacterial respiratory infections.

SRLV infection results in the infiltration of tissues by mononuclear cells, both newly recruited lymphocytes or monocytes from the blood, and cells proliferating in the tissues. Accumulated lymphocytes organize into lymphoid follicles (Blacklaws 2012). The histopathological picture of the lungs from infected animals indicates the presence of interstitial pneumonia. The observed changes include thickening of intraalveolar septa, caused by infiltration of inflammatory cells, muscular hyperplasia and connective tissue growth, peribronchial and perivascular lymph nodules and proliferation of pneumocytes (Georgsson et al. 1971). Progression of lung lesions leads to dyspnea and emaciation.

In humans parainfluenza infection may last longer in immunocompromised hosts (Fishaut et al. 1980), which suggests that lentiviral infections may increase the prevalence of parainfluenza in the goat population. SRLV infection is widespread in the Polish goat and sheep population (Kaba et al. 2013, Junkuszew et al. 2016), and has been shown to substantially affect milk and cheese productivity (Kaba et al. 2012, Nowicka et al. 2015a). PIV-3 has been shown to be present in cattle and sheep from many regions of Poland with the seroprevalence exceeding 50% (Saddour 1987, Larski and Wiśniewski 1972), however, no data on the occurrence of PIV-3 infection in Polish goats are available. Therefore, we decided to carry out the study to determine the serological status of the Polish goat population with respect to PIV-3 infection and to assess the relationship between PIV-3 and SRLV infection in goats.

## Materials and Methods

### Study population

This cross-sectional study was carried out in the Polish dairy goat population between 2014 and 2018. The study enrolled 48 herds counting at least 20 adult goats. Herd size ranged from 23 to 426 adult goats with a median of 82 goats (IQR from 56 to 111 goats). Herds were arbitrarily classed as small (21 to 50 adult goats; n=10), medium (50 to 100 adult goats; n=21), and large (>100 adult goats; n=17). In 45 herds a sample

of between 10 and 17 goats was randomly selected using a simple random method. Sample size was calculated using the following formula (Thrusfield 2018):

$$n = \{1 - (1 - P)^{1/d}\} \times (N - d/2) + 1$$

where:  $n$  – required sample size

$N$  – herd size

$d$  – minimum expected number of infected goats

$P$  – level of confidence

This allowed the presence of disease to be identified, assuming the minimum expected within-herd prevalence of at least 20%, and 95% level of confidence. In three large herds of 103, 171 and 184 adult goats all the animals were tested. In total, 1188 adult goats were blood-sampled. In each herd the occurrence of cough among adult goats and among kids was recorded as observed rarely or often based on a personal interview with the herd owners.

### Blood sampling and serological testing

Blood was collected from the jugular vein to a 10 ml dry vial and left overnight for clotting. The serum was then harvested (after centrifugation at 3000 rpm, if necessary) and stored at  $-20^{\circ}\text{C}$  until testing. Blood collection was approved by the 3<sup>rd</sup> Local Ethics Committee in Warsaw, Poland (Approval No. 31/2013, 22 May 2013).

The concentration of antibodies to PIV-3 (IgG class) was measured using quantitative immunoenzymatic assay (Goat PIV-IgG ELISA Kit, Abclonal, Woburn, USA). The concentration of antibodies to PIV-3 in a serum sample was determined by comparing the optical density of the sample to the standard curve and expressed in ng/ml. The assay had a limit of detection (laboratory sensitivity) of 1.0 ng/ml, however the manufacturer reported the assay range of quantification between 7.5 and 240 ng/ml. Therefore, serum samples with a concentration  $<7.5$  ng/ml were considered as seronegative to PIV-3, whereas concentrations  $>240$  ng/ml were replaced in further analyses with the figure of 240 ng/ml. The assay's intra- and interassay coefficients of variation were  $<10\%$  and  $<15\%$ , respectively.

The presence of SRLV infection was determined using a commercial indirect immunoenzymatic assay (ID Screen MVV-CAEV Indirect Screening test, IDvet Innovative Diagnostics, Grabels, France). This ELISA was 92% sensitive and 99% specific at the manufacturer's cut-off of sample-to-positive control ratio (S/P%) of 50% (Nowicka et al. 2014). Both tests were performed according to the manufacturers' manuals.

### Statistical methods

Categorical variables were presented as count and percentage in a group and compared between groups

using the chi-square test. The 95% confidence interval (CI 95%) for percentage was calculated using the Wilson score method. Numerical variables were expressed as the median, interquartile range (IQR) and range, as their distribution was significantly different from normal according to the Shapiro-Wilk test ( $p < 0.001$ ). The Mann-Whitney U test and Kruskal-Wallis H test were used for comparing the concentration of PIV-3 antibodies between groups of goats. A 3-level hierarchical linear model (HLM) was developed to evaluate the influence of SRLV infection on the concentration of PIV-3 antibodies. Herd-level variables (e.g. herd size, occurrence of cough) were entered in the model at the 1<sup>st</sup> level, individual-level variables (e.g. sex) at the 2<sup>nd</sup> level, and SRLV infection, as the main independent variable, at the 3<sup>rd</sup> level. A significance level ( $\alpha$ ) was set at 0.05. Statistical analysis was performed in TIBCO Statistica 13.3.0 (TIBCO Software Inc., Palo Alto, CA) except for HLM which was developed in IBM SPSS Statistics 26 (IBM Corporation, Armonk, NY, USA).

## Results

PIV-3 antibodies were detected in all goats which corresponded to the seroprevalence of 100% (CI 95%: 99.7%, 100%). Their concentration ranged from 8.4 to  $>240$  ng/ml with a median of 95.9 ng/ml (IQR 58.7 to 175 ng/ml). Concentration higher than 240 ng/ml was detected in 187 goats (15.0% of 1188 goats). In total 455 of 1160 goats (39.2%) tested positive for SRLV and they were found in 42 of the 46 tested herds (91.3%).

Cough was observed rarely by the owners of 24 herds (50%) and often in another 24 herds (50%). The occurrence of cough was significantly linked to the herd size ( $p = 0.006$ ), with cough observed in only one of 10 small herds (10%), 11 of 21 medium herds (52%), and 12 of 17 large herds (71%).

The concentration of PIV-3 antibodies differed significantly between goat herds ( $p < 0.001$ ). More specifically, it differed significantly between goats kept in herds of different size ( $p < 0.001$ ). Concentration of PIV-3 antibodies was the lowest in small herds (median 70.9 ng/ml, IQR 46.6 to 120 ng/ml), significantly higher in medium herds (median 82.2 ng/ml, IQR 53.8 to 167 ng/ml;  $p = 0.015$ ), and the highest in large herds (median 107.0 ng/ml, IQR 64.4 to 201 ng/ml;  $p < 0.001$ ). The concentration of antibodies to PIV-3 was significantly higher in goats from herds in which cough was observed often (median 109 ng/ml, IQR 65.4 to 205 ng/ml) compared to herds in which cough was observed rarely (median 69.0 ng/ml, IQR 47.7 to 120 ng/ml;  $p < 0.001$ ). When

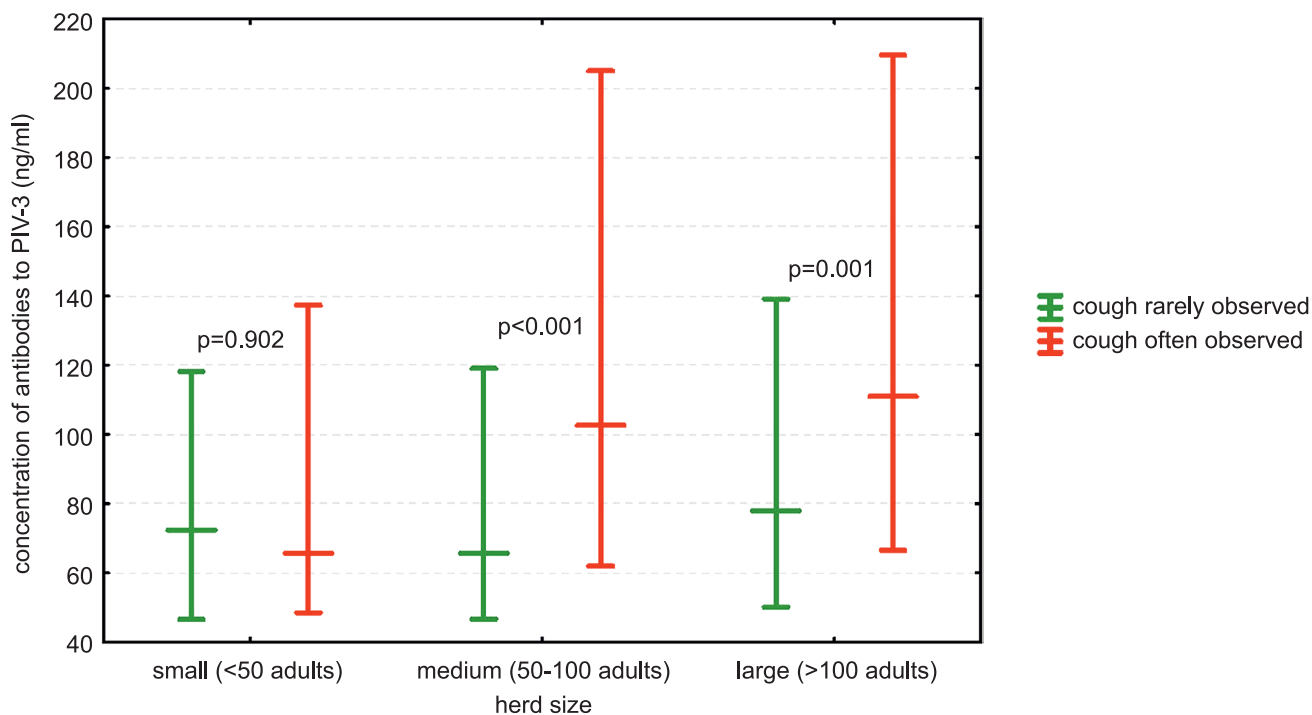


Fig. 1. Concentration of antibodies to parainfluenza virus type 3 (PIV-3), presented as the median and interquartile range (IQR), in goat herds of various size depending on the occurrence of cough.

both factors (herd size and the occurrence of cough) were analyzed together, the concentration of antibodies to PIV-3 was significantly higher in goats from medium ( $p < 0.001$ ) and large ( $p = 0.001$ ) herds in which cough was often observed, while no significant difference was found between goats from small herds irrespective of the occurrence of cough ( $p = 0.902$ ) (Fig.1).

Having included two confounding factors (size of the herd from which a goat came, and the occurrence of cough in this herd) female goats proved to have a significantly higher concentration of PIV-3 antibodies (median 98.6 ng/ml, IQR 59.5 to 183 ng/ml) than males (median 78.4 ng/ml, IQR 48.0 to 138 ng/ml;  $p = 0.019$ ).

ELISA for SRLV was performed in 1160 goats from 46 herds. Goats seropositive to SRLV were found in 6 of 9 small herds (67%), 17 of 20 medium herds (85%), and in all 17 large herds (100%), and herd size was significantly linked to the prevalence of SRLV infection ( $p = 0.027$ ).

Having included three confounding factors (two herd-level: size of the herd from which a goat came, and the occurrence of cough in this herd, and one individual-level: the goat's sex) SRLV infection did not prove to be significantly linked to the concentration of antibodies to PIV-3 (Table 1). Median concentration of PIV-3 antibodies was 97.5 ng/ml (IQR 58.8 to 193 ng/ml) in SRLV-seropositive and 97.1 ng/ml (IQR 59.4 to 172 ng/ml) in SRLV-seronegative goats.

## Discussion

In this study we demonstrated the presence of PIV-3 antibodies in all tested goats from all herds in Poland. This indicates that there are many occasions on which goats may be exposed to this virus. Serological evidence of PIV-3 infection has been reported in several domestic and wild ungulate species such as cattle (Betancur Hurtado et al. 2010, Albayrak et al. 2019), goats (Elazhary et al. 1984, Obi and Ibu 1990, Li et al. 2014), sheep (Elazhary et al. 1984, Gonçalves et al. 2011, Giangaspero et al. 2013, Contreras-Luna et al. 2017), bighorn sheep (Rudolph et al. 2007), and camels (Intisar et al. 2010). The pathogen was reported in the vast majority of Polish dairy cattle herds with an overall individual-level seroprevalence of 53% (Larski and Wiśniewski 1972). Similar seroprevalence was also observed in sheep (Saddour 1987); however, both these studies date back to the second half of the 20<sup>th</sup> century. PIV-3 was also detected in free-ranging and captive European bison in Poland (Salwa et al. 2007, Krzysiak et al. 2018). Due to the fact that respiratory diseases constitute a serious problem in cattle farming, many breeders in Poland use commercially available vaccines against respiratory tract pathogens containing attenuated PIV-3. No such vaccine is available for goats on the Polish market and no farmers reported extra-label use of cattle vaccines in their goats so serological status appears to reflect natural exposure and is a reliable proxy of the distribution of PIV-3 infection in Poland.

Table 1. Results of hierarchical linear multivariable analysis of the relationship between small ruminant lentivirus (SRLV) infection and the concentration of antibodies to parainfluenza virus type 3 (PIV-3) in goat serum.

Variable	Regression coefficients (CI 95%)	Standardized regression coefficients	t-statistics (df <sup>b</sup> = 240)	p-value
Intercept	74.62	-	-	-
Herd size				
Small (<50 adult goats) <sup>a</sup>	-	-	-	-
Medium (50-100 adult goats)	32.32 (18.66, 45.98)	0.21	4.64	<0.001
Large (>100 adult goats)	23.49 (10.01, 36.97)	0.16	3.42	<0.001
Occurrence of cough reported by farmers				
Rarely <sup>a</sup>	-	-	-	-
Often	30.39 (20.87, 39.92)	0.18	6.26	<0.001
Goat's sex				
Male <sup>a</sup>	-	-	-	-
Female	16.11 (2.63, 29.58)	0.07	2.34	0.019
Serological status to SRLV infection				
Negative <sup>a</sup>	-	-	-	-
Positive	2.32 (-6.11, 10.75)	0.02	0.54	0.590

<sup>a</sup> baseline category

<sup>b</sup> df – degrees of freedom

As far as we know our study is the first to report the occurrence of this infection in the goat population in Poland.

The concentration of antibodies varies considerably from very low to very high values. On one hand, it seems to be associated with herd characteristics. In large herds there is a high density of animals and frequent contacts between individuals are possible. Reasons for a positive association between herd size and disease include a higher risk of introduction of pathogens from outside the herd and transmission of pathogens within herds (Gardner et al. 2002). The association between herd size and presence of antibodies to PIV-3 has been shown in cattle (Solís-Calderón et al. 2007) as well as the analogical relationship has been evidenced for CAE in goats (Kaba et al. 2013). On the other hand, in our study the concentration of antibodies to PIV-3 is higher in goats from herds in which cough is observed by farmers more often. This indicates that PIV-3 infection may account for at least a part of the respiratory diseases in Polish goat herds. The positive association between the presence of PIV-3 antibodies and respiratory signs in cattle has also been reported from Mexico (Figuroa-Chávez et al. 2012).

Interestingly, female goats turned out to have significantly higher antibody concentrations than males. This may result from the fact that female goats are usually kept in much more crowded barns and have more opportunities to contact other individuals during grazing or milking. Moreover, densely populated buildings usually have worse ventilation. This results in a high

concentration of toxic gases which injure the airways and promote viral and bacterial infections. Male goats are usually housed in individual pens, separated from females for most of the time except for the mating season (Nowicka et al. 2015b). The results of relevant studies in cattle are contradictory, with the risk of PIV-3 infection significantly higher in cows than in bulls in one study (León et al. 2019), and no such relationship observed in the another study (Betancur et al. 2010). This corroborates our suspicion that the conditions of animal housing play a more important role in the spread of PIV-3 infection than the animal's sex itself.

Our study unambiguously shows that no link between SRLV and PIV-3 infection exists. A small-scale molecular and histopathological study carried out previously in sheep resulted in the same conclusions regarding the severity of microscopic lesions in the lungs (Lyon et al. 1997). This adds to the growing body of evidence that CAE has no immunosuppressive potential (Thormar 2005) and does not seem to predispose goats to secondary respiratory tract infections (Moroz et al. 2020).

There are several drawbacks to our study. Firstly, only a few studies have been carried out with the use of PIV-3 ELISA which is the main limitation of our study. Most serological surveys have employed haemagglutination inhibition assay (Larski and Wiśniewski 1972, Saddour 1987). Secondly, environmental conditions may play important role in the intensity of PIV-3 infection. Unfortunately, sufficiently reliable data to investigate their role in the PIV-3 distribution in the

Polish goat population were not at our disposal. Therefore, it is possible that, not the herd size itself, but rather the conditions in which the goats are kept, which are usually worse in larger herds, are truly responsible for the higher concentrations of PIV-3 antibodies. Thirdly, even though the detection of specific antibodies indicates that a goat has been infected with PIV-3 during its life, it does not necessarily correspond to an ongoing infection. Precise data on the persistence of antibodies to PIV-3 after infection are lacking, however antibodies to other members of the *Paramyxoviridae* family such as measles virus or canine distemper virus have been shown to persist very long after infection (Prydie 1966, Olson et al. 1997, Griffin 2016). Therefore, it is difficult to determine the sequence of infections with PIV-3 and SRLV, with a high likelihood that the former infection took place earlier in the goat's life, given that the virus is widespread in the Polish goat population and has high potential to enter the animal's body.

In conclusion, PIV-3 infection is widespread in the Polish goat population and concentration of specific antibodies considerably varies between animals and between herds. PIV-3 infection appears to contribute to the occurrence of respiratory signs in goats; however, there is no association between PIV-3 and SRLV infection.

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