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*Original article*

# Serological evidence of lack of contact with caprine herpesvirus type 1 and bluetongue virus in goat population in Poland

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## Abstract

Investigation into herd-level seroprevalence of caprine herpesvirus type 1 (CpHV-1) and bluetongue virus (BTV) was conducted in 2007 in Poland. It involved the entire population of goats covered by a milk recording program in 2007, which included 49 goat herds. The number of goats examined in each herd was determined statistically in order to detect the presence of at least one seropositive animal in a herd with a 95% probability and simple random method of sampling was applied. No antibodies to CpHV-1 or BTV were detected. Further calculations were carried out to determine the herd-level true seroprevalence, taking into account sensitivity and specificity of the test as well as several other factors. It can be concluded that till the middle of 2007 population of Polish goats covered by the milk recording program remained negative with respect to CpHV-1 and BTV.

**Key words:** bluetongue virus, caprine herpesvirus type 1, seroprevalence, HerdAcc, goat

## Introduction

Data on seroprevalence of infectious diseases in small ruminants in Poland are very limited. Infection of goats with caprine herpesvirus type 1 (CpHV-1) has been reported from several countries all over the world but in Europe only in the Mediterranean Basin (Koptopoulos et al. 1988, Keuser et al. 2004, Thiry et al. 2008) and Switzerland (Plebani et al. 1983). CpHV-1 is considered an important cause of abortions and neonatal mortality in goats as well as infectious pustular vulvovaginitis in females and bal-

anoposthitis in bucks (Uzal et al. 2004, Piper et al. 2008). The virus is also capable of producing a generalized infection in kids and contributes to the development of respiratory tract disorders as well as enteritis (Roperto et al. 2000).

Recent emergence of bluetongue (BT) in Northern Europe has led to the intensification of studies on this disease in cattle and sheep. Even though bluetongue has never been detected in native population of ruminants in Poland, the highly virulent serotype 8 has been reported in neighbouring countries such as Germany and Czech Republic (Wilson and Mellor

2009). Moreover, Niedbalski and Kęsy (2008) revealed antibodies to BTV in 0,28% of cattle, goats and fallow deers imported to Poland from Germany and Denmark. Goats are fully susceptible to BTV infection but they rarely manifest clinical signs. If clinical signs of bluetongue emerge in goats they are usually unspecific (Backx et al. 2007). It is crucial to know the serological status of the Polish goat population at the beginning of the BT epidemic, which is now spreading over the northern Europe.

The objective of the study was to check whether the goat population in Poland has had a contact with CpHV-1 and BTV and, if so, to determine the prevalence of antibodies among herds (herd-level true seroprevalence).

## Materials and Methods

The survey was conducted in June and July 2007. It involved the entire population of goats covered by a milk recording program which counted 4685 adult females (at least 1 year-old) dispersed among 49 herds. Size of herds ranged from 12 to 450 adult females at the moment when the survey was planned. Median herd size equaled 73 females. Expected within-herd seroprevalence for both viruses was assumed to be 10% (Thiry et al. 2008, Conraths et al. 2009). The sample size taken from each herd was determined on the basis of the number of adult females in each herd and calculated according to methods applied in veterinary epidemiology, so that detection of at least one seropositive goat was possible at the level of confidence 95% (Thrusfield 2007). All 49 herds were checked.

The goats in each herd were selected in a simple random way to ensure representativeness of a sample. Finally from 12 to 28 goats from each herd were involved in the study, giving a total number of 1060 blood samples. The study design allowed evaluation of herd-level apparent seroprevalence.

Blood was collected to dry 10 ml-volume tubes and kept for 24 hours at room temperature. After centrifugation, serum samples were stored at -20°C until testing.

The sera were examined using two commercial immunoenzymatic tests (ELISA) – one blocking ELISA – Serelisa® BHV-1 gB Ab Mono Blocking (Synbiotics) and one competitive ELISA – Pourquier® ELISA Bluetongue Serum (Institut Pourquier). Sensitivity (Se) and specificity (Sp) of CpHV-1 blocking ELISA were 93,5% and 100%, respectively when confronted with seroneutralization as a gold standard (Thiry et al. 2008). Se of BTV cELISA was 84,5% and Sp was 99,1% when gold standard was real-time RT-PCR (Vandenbussche et al. 2008).

For calculating the herd-level true seroprevalence,

the test Se and Sp were adjusted from individual-level to herd-level according to Jordan and McEwen (1998), using the HerdAcc program, for a median herd size of 73 animals, herd cut-off value of one animal positive indicating a positive herd, 12-28 animals sampled per herd and sampling without replacement. Then herd-level Se (HSe) and Sp (HSp) were used to calculate the herd-level true seroprevalence according to the following equation: true prevalence = (apparent prevalence + HSp - 1) / (HSe + HSp - 1) (Noordhuizen et al. 2001).

## Results

Herd-level apparent seroprevalence of CpHV-1 or BTV was 0%. HSe and HSp for CpHV-1 blocking ELISA remained almost the same – 95% and 100%, respectively, whereas for BTV cELISA they changed significantly – HSe increased to 96% and HSp dropped to only 60%. Nevertheless, abovementioned modifications did not change the final results – herd-level true seroprevalence for both CpHV-1 and BTV remained 0%.

## Discussion

Statistical methods commonly used in veterinary epidemiology allow to draw conclusions on the prevalence basing on relatively small proportion of a population. Nevertheless it is imperative to remember that such result is only an estimate and many efforts shall be applied to reduce risk of mistake. When herd-level prevalence is evaluated not only Se and Sp of a diagnostic test at the individual animal level but also the number of animals tested, the total herd size, the true within-herd prevalence in infected herds and the herd cut-off value used to classify the herd as positive have to be taken into account (Baldock 1998). In our calculation Sp of cELISA changed considerably after adjustment as herd cut-off value of one positive animal per herd significantly increases HSe and decreases HSp. Nevertheless herd-level true seroprevalence remained unchanged as HSe in both cases was very high, what led to very high herd predictive value for a negative result (Christensen and Gardner 2000).

No commercial assays invented exclusively for the detection of antibodies to CpHV-1 in goats are available. On the other hand many tests for the detection of antibodies to bovine herpesvirus type 1 (BHV-1) have been developed. These are immunoenzymatic assays for antibodies to viral glycoproteins B (gB) or E (gE). Recent studies have shown that antibodies to CpHV-1 cross-react with gB of BHV-1 and appropri-

ate blocking ELISA tests intended for cattle may be applied to study goat sera (Thiry et al. 2008).

Our results on CpHV-1 seroprevalence are consistent with commonly accepted epidemiological situation in Europe where the infection has been detected only in Mediterranean Basin apart from one report from Switzerland (Plebani et al. 1983).

Serological investigation into BTV prevalence was conducted with standard competitive ELISA (cELISA) detecting specific antibodies to the VP7 protein – a major core protein of the virus. According to the manufacturer's instruction it is intended for antibody detection in goats. The test is unable to specify the serotype of BTV but it was not the objective of the study. cELISA was chosen for the study since it is more accurate in detecting BTV antibodies than other commonly applied techniques like dotELISA, indirect ELISA or agar gel immunodiffusion and is comparable to seroneutralization (Afshar et al. 1987, Reddington et al. 1991, Naresh and Prasad 1995).

Our study confirmed that till the middle of 2007 the Polish goat population covered by the milk recording program was free from BTV infection but the bluetongue epidemic in Northern Europe started in August 2006 and has been spreading since that time (Saegerman et al. 2008). Considering the presence of BTV in neighbouring countries and the dissemination of vectors able to spread the pathogen over long distances, the emergence of the disease in Poland may be expected soon.

It can be concluded till the middle of 2007 population of Polish goats covered by the milk recording program remained negative with respect to both CpHV-1 and BTV. The study constitutes the background for tracking the spread of ongoing bluetongue epidemic in Europe.

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