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Seroprevalence and risk factors related to small ruminant lentivirus infections in Belgian sheep and goats

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ABSTRACT

Maedi-Visna virus (MVV) and caprine arthritis encephalitis virus (CAEV) are two prototype members of the group of small ruminant lentiviruses (SRLVs). Both result in progressive and persistent infections of sheep and goats that impact animal health and cause economic losses. In Belgium, the sheep and goat sector is small and consists mostly of hobbyist farmers keeping few animals. A voluntary control program however exists, but less than 2% of the farmers participate to the program. The current lack of SRLV seroprevalence data and knowledge on risk factors related to SRLV seropositivity in this hobbyist sector makes it difficult to evaluate the risk of SRLV transmission from non-certified to SRLV free certified farms.

We performed a nationwide SRLV seroprevalence study based on a stratified sampling proportional to the number of sheep and goat holders per province. Randomly selected sheep and goat owners were invited to participate and subject to a short questionnaire to collect information about flock size, animal health condition, age, flock constitution and housing conditions. Samples were collected from maximum 7 animals per farm and tested in a commercial ELISA.

In total, we received samples from 87 sheep and 76 goat farms. Sheep flocks showed an overall seroprevalence of 9% (CI $_{95\%}$: 5–15) and a between-herd seroprevalence of 17% (CI $_{95\%}$:11–27). Seroprevalence at animal level in goat flocks was 6% (CI $_{95\%}$: 3–12) and the between-herd seroprevalence was 13% (CI $_{95\%}$: 7–23). Multiple sheep and goat breeds were found SRLV seropositive. Answers provided during the questionnaire confirmed the mostly hobbyist nature of the sector and showed that more than 65% of sheep and goat farmers had never heard of the disease. The only risk factor found to be related to SRLV seroprevalence was flock size. Herds of more than 10 goats had significantly higher chance to harbor seropositive animals (OR: 4.36; CI: 1.07; 17.73).

In conclusion, it was shown that participants to the SRLV free certification program are at risk for re-introduction of the disease in their herds since SRLVs are present on about 15%–20% of non-certified farms. Except from flock size, no clear risk factors were found that are helpfull to identify flocks at risk. Greater effort should be made to inform sheep and goat farmers about the existence and consequences of this disease in order to promote the voluntary control program and further reduce the disease prevalence.

1. Introduction

Maedi-Visna virus (MVV) and caprine arthritis encephalitis virus (CAEV) are two prototype members of the group of small ruminant lentiviruses (SRLVs) belonging to the family of Retroviridae (Blacklaws, 2012). MVV was the first lentivirus to be discovered and was isolated from sheep in Iceland in the 1960s (Sigurdsson et al., 1957). Twenty years later, CAE virus was isolated from goats (Craword et al., 1980). Recent epidemiological studies have shown that natural inter-species transmission can occur in mixed flocks (Leroux et al., 2010). Both viruses are therefore grouped together as SRLVs and are currently divided into five phylogenetic groups; from A to E. Genotype A in sheep consists of MVV-like strains and genotype B corresponds to CAEV-like isolates while the others three genetic groups include genotypes from more restricted geographical areas (Ramirez et al., 2013). Recent reports on SRLV seroprevalence in European countries are limited. Only

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Poland, Spain and Switzerland reported seroprevalence rates of 72%, 53% and 9%, respectively (Schaller et al., 2000; Lago et al., 2012; Kaba et al., 2013).

The primary route of SRLV transmission is via the ingestion of infected colostrum and milk by lambs. Also horizontal transmission via the inhalation of infected secretions has been described and is regarded mainly as a transmission route in intensive and indoor production systems (Leginagoikoa et al., 2006; Villoria et al., 2013; Junkuszew et al., 2016).

SRLV infection results in progressive and persistent disease in sheep and goats leading to neurological disorders and chronic lesions in lungs, joints and mammary glands (Minguijon et al., 2015). Aside from their impact on animal welfare and their fatal outcome in the long term, SRLVs have become a worldwide problem with substantial economic losses in the small ruminant industry due to reduced lamb weight, decrease of milk production, early culling and restrictions to animal trade (Reina et al., 2009; Perez et al., 2010). To our knowledge, no exact quantification of the economic impact due to SRLV infections is however available.

To date, no vaccines or therapies are available. Therefore the control of SRLVs mostly relies on the early detection and removal of infected animals from the flock. Preventive measures such as snatching progeny from infected ewes at birth, artificial rearing of lambs and separating seropositive and seronegative animals have been proposed to minimise spread of the disease in flocks (Reina et al., 2009; Perez et al., 2010; Minguijon et al., 2015). In Belgium, a voluntary program with the possibility to obtain a SRLV-free certification (Royal Decree 24-03-1993 for MVV and 27-11-1997 for CAE) has been implemented and combines measures to prevent contact with potentially SRLV infected animals and to detect SRLV infections in an early stage. To enter this voluntary program, farmers have to show the negative SRLV serological status of all their animals over 1 year and 2 consecutive negative ELISA tests on serum collected within a six to twelve month interval. This allows farmers to get a first certified MVV/CAEV-free flock status for a period of 12 months. After a yearly screening, this one-year certificate can be renewed two times before leading to a 24 months free flock status. In case of positive or doubtful results during the ELISA screening, the certification is suspended and extra confirmatory ELISA, immunodiffusion and PCR tests are performed to define the infection status of the suspected animal. Obtaining the “SRLV free” certification allows farmers to sell, show and export their animals and generates extra value to the animals as breeding and replacement stock (Perez et al., 2010).

As in other countries with a voluntary eradication program of SRLV infections, Belgian participants are always at risk for a potential re-introduction of the virus via contacts with animals from non-certified farms. The current absence of SRLV seroprevalence data in Belgium makes it difficult to assess the extent of this risk and we therefore performed a nationwide SRLV seroprevalence study in the Belgian sheep and goat population. In addition, we also aimed to identify risk factors associated with SRLV seropositivity in order to more easily identify flocks at risk.

2. Material and methods

2.1. Sampling strategy

Participating farms were selected from the Sanitel database, the national Belgian information system that identifies, registers and collects information about animals including ovines and caprines. Farms participating in the national voluntary MVV/CAEV control program were excluded, as well as farms with less than 3 goats or 5 sheep to avoid an excess of small holdings. A stratified random sampling approach proportional to the number of goat and sheep holders per province was applied (Table 1). One database comprising all Belgian sheep holders and another comprising all Belgian goat holders was used to select ‘sheep farmers’ and ‘goat farmers’, respectively. When it became clear during the questionnaire (see further) that some farmers had mixed flocks, and were thus present in both databases, they were nevertheless enrolled in the group they were initially selected from and could only submit samples from that animal species.

According to the methodology described by Thrustfield (Thrusfield, 2005) as implemented on the website ‘http://epitools.ausvet.com.au/’, about 320 herds should be sampled to estimate the between herd SRLV seroprevalence with 95% confidence and a precision of 5% when its prevalence is estimated to be 30%. To remain within the budgetary limits, however, we could only collect samples from 100 sheep and 100 goat farms. This decreases the precision of the 95% confidence interval to about 10%.Only limited data is available in literature on efficiency of SRLV transmission and the prevalence of SRLV to be expected at farm level during an outbreak. Based on i) preliminary data estimating the within herd SRLV seroprevalence in Belgium at 30% and ii) publications indicating that efficient vertical and horizontal SRLV transmission can occur upon introduction of seropositive animals in a herd, sometimes leading to an increase in seroprevalence of 30–50% within a short period (Woodard et al., 1982; Adams et al., 1983; Robinson and Ellis, 1986; Blacklaws et al., 2004; Alvarez et al., 2006, Leginagoikoa et al., 2006), we considered a design prevalence of 30%. In combination with an estimated average farm size of 50 animals, 7 samples per farm were collected, allowing to predict with 95% confidence that the SRLV prevalence is lower than the design prevalence of 30% if all test negative (http://epitools.ausvet.com.au/content.php?page=FreedomSS3). For the calculation of the between herd seroprevalence, such farms where considered as negative. Since SRLV can however exist at a farm at a prevalence below 30%, this assumption might result in an underestimation of the true between herd seroprevalence. As will be discussed later, the actual average farm size in Belgium is however below the initial estimate of 50, making that sampling of 7 animals often meant sampling of all animals present (Supplementary Table 1), and ensuring that those herds were certainly SRLV free.

2.2. Sample collection

After a first series of telephone calls, 100 sheep and 100 goat farmers were enrolled for the study. Three months after having sent them all the documents, it became clear that only a part of them had sent in serum samples from their animals via their veterinarian. A second round of phone calls was launched, recontacting those from the first round that had not yet participated and contacting new potential candidates for those who were no longer willing to participate. Again sufficient people were enrolled to reach 100 sheep and 100 goat farmers. Unfortunately, also after this second round, not all participants sent in serum samples. In total, we received sera from 87 sheep farms (555 samples) out of 149 farmers that confirmed their participation and from 76 goat farms (401 samples) out of 129 that had engaged themselves to participate. Samples were collected between November 2015 and May 2016 by veterinarians designated by the participants. These veterinarians received an instruction letter to streamline the sample collection. They were asked to collect serum from 7 randomly selected animals older than 1 year. Only when less than 7 animals were present in this age category, younger animals could be included. Age information on the sampled animals was provided by the veterinarians.

2.3. Questionnaire

At the moment of enrollment of the participants over the phone, a short questionnaire was submitted to participating farmers. We collected data on their awareness of the disease, their motif to keep sheep