Presence of specific antibodies and proviral DNA of small ruminant lentiviruses in lambs in their first weeks of life

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Abstract

The aim of this study was to investigate the presence of proviral DNA and colostral antibodies in lambs born to and fed by ewes infected with small ruminant lentiviruses (SRLV). It was demonstrated that all 20 lambs tested 24 h after colostrum ingestion were serologically positive with high antibody titres. These gradually decreased with time, and at week 12 all lambs were seronegative. Twenty percent of lambs tested at the 2nd week postpartum were provirus positive by qPCR as a result of consumption of infected colostrum or in utero infection. When tested at three months of life, 95% of the lambs were provirus positive, probably as a result of horizontal transmission of the virus. Since these animals could play an important role in the early propagation of SRLV to susceptible herdmates, early removal of provirus-positive animals could help to prevent new infections.

Keywords: ewes, lambs, small ruminant lentiviruses, colostrum.

Introduction

Maedi-visna virus (MVV) and caprine arthritis encephalitis virus (CAEV) are retroviruses belonging to the genus Lentivirus and nowadays are termed small ruminant lentiviruses (SRLV). Infections with SRLV are widespread in sheep and goats in many countries including Poland (15, 16, 18, 23). Since evidence for cross-species transmission of lentiviruses from goats to sheep and vice versa was provided under field conditions, neither virus has yet been considered species-specific. Genetic analysis of field isolates of SRLV evidenced common cross-species transmission of these viruses between goats and sheep, with frequent identification of MVV-related viruses (group A) in goats and CAEV-related viruses (group B) in sheep. Recently two novel subgroups, A12 and A13, were found in sheep from infected flocks in Poland (22).

Infection with SRLV causes chronic multisystemic inflammatory lesions in many organs, leading to lower milk quality, production losses from early culling of infected animals, increased mortality in lambs, and a higher risk of opportunistic infections (24). Since the treatment or vaccination of infected animals is not available, disease control relies on the identification of infected individuals and their removal from the affected flock to prevent new infections (4, 26). In sheep, as in goats, ingestion of colostrum and milk from infected ewes is the main route of transmission of SRLV infection (4). However, several field observations have demonstrated that only approximately 20% of colostrum-fed lambs were serologically positive (1) and the overall contribution of ovine colostrum to seroprevalence in lamb was relatively low. It was also demonstrated that when lambs were removed from their ewes after birth, separated from the rest of the flock, and artificially reared, they remained seronegative and virus-free throughout their lives (9, 13). As the raising of virus-free animals is of primary importance in eradication programmes, it is crucial to know the dynamics of SRLV infection in lambs born to infected ewes. In this paper proviral DNA and antibodies to SRLV were detected and analysed in lambs during the first three months of their lives.
Material and Methods

Animals and blood sampling. Twelve seropositive ewes from a Polish Lowland sheep flock were selected on the basis of a previous serological survey. These ewes gave birth to a total of 20 lambs, which were allocated at birth to an experimental group and left free to suck maternal colostrum and milk for 12 weeks. After delivery, blood samples were taken from ewes and tested for antibody titres and the presence of SRLV proviral DNA. Blood samples were also collected from newborn lambs within 24 h after colostrum ingestion (time 0) and thereafter six times, approximately every two weeks, to test the presence of antibodies. Additionally, blood samples were collected for PCR analysis at 2 and 12 weeks after birth.

Estimation of antibody titres. Serum samples were serially diluted two-fold and tested in duplicate by ELISA (CAEV/MVV p28 Ab Verification Test, IDEXX Laboratories Inc., USA) according to the manufacturer’s recommendations. Measurements were made using ELISA readers (Multiskan EX, Thermo Scientific/Labsystems, USA).

PBL preparation. Peripheral blood leukocytes (PBLs) were isolated from 10 mL of blood with EDTA by centrifugation at 1500 g for 25 min. Erythrocytes were haemolyzed by osmotic shock with cold water and 4.5% NaCl. After two washes in PBS, the cell pellet was used for extraction of genomic DNA with the Dneasy Tissue Kit (Qiagen, USA), according to the manufacturer’s protocol. DNA concentration was calculated by measuring the optical density at 260 nm and the samples were stored at -20°C until PCR amplification.

Polymerase chain reaction (PCR). Two fragments, V4V5 (608 bp) and V1V2 (394 bp), and one selected from the variable part of env gene as well as the fragment of gag gene encoding capsid protein CA (624 bp), were amplified by nested PCR. The V4V5 and V1V2 fragments were amplified with Ptat and Penv primers (21), in the first round of PCR and primers A51/B31 and 567/564 in the second run (21, 10). The primer GAGf1/P15 was used for the first amplification and CAGAG5/CAGAG3 for the second when PCR amplified the Gag fragment (22, 27). For each amplification, a positive and a negative control was run in parallel with the tested samples. After amplification, PCR products from each sample were separated by electrophoresis on 1.5% agarose gel containing ethidium bromide (1 µg/mL) in 1 × Tris base, acetic acid, and EDTA (TAE) buffer.

Real-time SYBR Green PCR. Primers were designed based on conservative sequences within the gag gene, typical for the A12 subtype of SRLV (22). Infection with this subtype was confirmed following the sequencing of PCR products and subsequent genotyping of strains circulating in infected ewes (data not shown). Proviral load was quantified using a QuantiTect SYBR Green I PCR kit (Qiagen) with 1 µg of genomic DNA. The qPCR was performed on a Rotor-Gene Q machine (Qiagen). The cycling conditions included an initial denaturation step at 95°C for 15 min followed by 45 cycles at 94°C for 15 s, at 50°C for 30 s, and at 72°C for 45 s. The degenerative primers (0.3 µM) were as follows: primer 1, 5’-CTCTRTTCCAGGC ATCATGGC-3’, and primer 2, 5’-GATAGAMTCRAG TAGTCTTCCAACA-3’. The amplified fragment was cloned into the plasmid (In-Fusion™ Ready pEcoli- Nterm 6xHN, Clontech, USA) to generate a standard curve. All samples were analysed in duplicate. To confirm the specificity of PCR products, a melting curve analysis was performed immediately after the amplification. The concentration of recombinant plasmid DNA was measured with a NanoPhotometer Pearl spectrophotometer (Implen, Germany). The copy number of recombinant plasmid per microlitre was calculated using a DNA copy number calculator (http://www.finnzymes.fi/java applets/copy number calculation.html). Serial 10-fold dilutions of recombinant plasmid, in a range of 1 × 10⁰–1 × 10⁵ copies/µL, were prepared in nuclease-free water.

Results

Infectious status of ewes. All ewes were confirmed to be infected based on the presence of serum antibody titres (1 - 32) in ELISA. The overall number of positive ewes by nested PCR was 11 and the CA fragment was detected in all of them, whereas the V4V5 and V1V2 fragments were detected in six (50%) and two (16.6%) animals respectively. Four ewes were positive for both V4V5 and CA while two were positive for all genome fragments. Real-time PCR confirmed the presence of provirus in all animals and the copy numbers varied from 1.2 to 23 per µg of DNA.

Duration and antibody titres in lambs. Fig. 1 shows that all lambs were positive by ELISA and they had the highest antibody titres (1-32) within 24 h after colostrum ingestion. Two and four weeks after parturition, 90% and 80% of lambs were still seropositive and the titres varied from 1 to 8. Then the number of seropositive animals and antibody titres gradually decreased with time, and at week 10 after parturition the percentage of seropositive animals was 10% with antibody titre close to 1. At week 12, all lambs were seronegative.

To test for the possible relationship between antibody titres in ewe and lamb sera, the Pearson correlation coefficient was calculated (Fig. 2A). The analysis revealed a clear correlation (R = 0.7401) between both groups of samples tested 24 h after parturition. However, there was only medium correlation (R = 0.575) between the antibody titre in ewe sera and the duration of antibodies in lamb sera.
Fig. 1. Kinetics of antibody titres in lamb sera at different times after parturition. The upper and lower edges correspond to the range of the titres. The central point corresponds to the average values.

Fig. 2. Relationship between the antibody titres (A) and provirus load (B) in ewes and their offspring.
Evidence of provirus in lambs. When nested PCR was carried out there were no positive amplifications of gag and env gene fragments in any lambs examined at the 2\textsuperscript{nd} week after birth. At the 12\textsuperscript{th} week, 17 (85\%) lambs were positive with gag primers, however, the PCR failed to amplify V1V2 and V4V5 fragments. When real time PCR was undertaken 4 (20\%) lambs were positive when tested at the 2\textsuperscript{nd} week after birth and 19 (95\%) were when tested at the 12\textsuperscript{th} week. The number of provirus copies in blood samples taken at the 12\textsuperscript{th} week varied between 0.8 and 12.6 per µg of DNA. As shown in Fig. 2B, there was no relationship between the number of provirus copies in samples collected from ewes and their progeny at the 12\textsuperscript{th} week after birth (R = -0.0998).

Discussion

In this study we followed the SRLV infection in lambs born to naturally infected ewes during the first three months of their lives to measure the duration of maternal antibodies in blood serum and the presence of proviral DNA. It was well documented that lambs can become infected readily following ingestion of SRLV-containing colostrum and milk (4), however, we demonstrated that colostrum from seropositive ewes was a low risk factor when lambs were kept with their mothers and left free to suck colostrum and milk for 12 weeks after parturition. This conclusion comes from the fact that only 4 out of 20 lambs tested at 2 weeks after birth were positive for proviral DNA by qPCR. One possible explanation is that the virus concentration in ewe colostrum or milk was too low to induce a detectable infection in lambs. Concomitantly passively transferred maternal antibodies could block development of infection in new born animals. We can suppose good colostrum uptake since the lambs had the highest ELISA titres within 24 h after colostrum ingestion and these titres were positively correlated with those noted in ewe sera. High amounts of SRLV antibodies in lamb sera may provide a preventive barrier to the establishment of persistent infection in the lamb, leading to the clearance of cell-associated SRLV (12). The presence of provirus noted in four lambs tested at the 2\textsuperscript{nd} week after birth could be the result of lactogenic infection, despite the presence of colostral antibodies. Presumably, maternal SRLV antibodies present in colostrum could bind to the virus forming macromolecules, which may be easily absorbed by epithelial cells, facilitating viral entry. It was also shown that intestinal epithelial cells might play an important role as the first site of SRLV replication (25).

Detection of the provirus early after birth could be also related to an infection acquired in utero. Indeed, transmission of SRLV from mother to foetus has been reported incidentally (4) or noted in 5%-8\% of offspring from seropositive ewes (6). Our data also indicated that 19 out of 20 lambs were provirus positive but serologically negative when tested at the 12\textsuperscript{th} week after birth. It is most likely that the decay of maternal antibodies coincided with an early viraemic phase, which is typical for lentiviral infection and which promoted the horizontal spread of SRLV among lambs.

Numerous studies on maedi-visna infection demonstrated that cell-associated viraemia occurred 2-6 weeks after infection (14). We can speculate that viral replication occurred in the first few months of life in infected lambs, allowing rapid transmission of the virus to susceptible animals. The significance of horizontal transmission of SRLV was demonstrated when lambs were left with their SRLV infected dams for different periods and the percentage of seropositive lambs increased even up to 80\% (8, 12). Some authors suggested that horizontal transmission of SRLV may be more important in virus spreading (1-3, 7). In this study, the average number of provirus copies in ewe’s blood was almost three times higher than in lambs examined at the 12\textsuperscript{th} week after birth and there was no relationship between provirus load in samples from ewes and their progeny (R = -0.0998). In many retroviral infections like HTLV, BLV, and FIV a high provirus load in pregnant mothers was associated with a greater probability of transmission of the infection to their progeny by oral consumption of infected colostrum or milk (11, 19, 20). Therefore, the lack of any correlation between proviral load in the blood of ewes and lambs in this study might be an additional argument that infection in lambs emerged from contact or horizontal transmission.

In this study, SRLV infection was evidenced by the detection of proviral DNA using nested PCR and qPCR. While both nested PCRs failed to detect provirus in lambs at 2 weeks after birth, qPCR revealed a low copy number in 4 out of 20 lambs. Due to the high heterogeneity of the lentiviral genome, it was essential to adapt a diagnostic PCR to the viral strains circulating in a particular area. Thus, the development of qPCR in this study was followed by genotyping of SRLV strains isolated from infected ewes. Using degenerative primers specific for subtype A12, which is typical for Polish isolates of SRLV, the newly developed qPCR showed high specificity and sensitivity, comparable to those noted for other qPCR assays developed for detection of SRLV infection (5, 17). There was a positive correlation between the number of lambs which were positive by qPCR and gag nested PCR at 12 weeks after the birth; however, none of the samples was positive when tested by env nested PCR. This is a quite disappointing outcome of this study, since many studies described the useful application of env-based PCR for the detection of SRLV DNA. In all likelihood, the high variability of env gene can be considered a possible reason for the negative result.

In conclusion, our data demonstrated that 20\% of lambs born to SRLV-infected ewes and tested at the 2\textsuperscript{nd} week after birth were infected, probably as a result of
in utero infection or consumption of infected colostrum. When tested after three months of life, most of them were provirus positive but serologically negative, probably as a result of horizontal transmission of the virus. Since these animals could play an important role in the early propagation of SRLV their early removal could help to prevent further virus transmissions.

References


