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Longitudinal study on seroreactivity of goats exposed to colostrum and milk of small ruminant lentivirus–infected dams

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Abstract

Introduction: Small ruminant lentivirus (SRLV) causes caprine arthritis-encephalitis in goats and maedi-visna disease in sheep. Transmission is *via* ingestion of colostrum and milk from infected dams or long-term direct contact between animals. Lifelong seroconversion can occur several weeks after infection *via* ingestion. However, sub-yearling lambs that ingest contaminated colostrum may be able to clear the infection and become seronegative. Whether a similar phenomenon occurs in goats remains unknown. Therefore, the serological status of goats was studied longitudinally from the moment of natural exposure to colostrum and milk of SRLV-positive dams through the age of 24 months. **Material and Methods:** Between February 2014 and March 2017 a dairy goat herd was studied which had been infected with SRLV for more than 20 years and carried maedi-visna virus-like genotype A subtype A17. Thirty-one kids born to dams seropositive for SRLV for at least a year beforehand were followed. They ingested colostrum immediately after birth and then remained with their dams for three weeks. The goats were tested serologically every month using two commercial ELISAs. The clinical condition of the goats was also regularly assessed. **Results:** Out of 31 goats, 13 (42%) seroconverted at the age ranging from 3 to 22 months with a median of 5 months. Two goats seroconverted in the second year of life. The other eleven did so before the age of one year; two of these reverted to seronegative status. Only 9 out of 31 goats (29%) seroconverted in the first year of life and remained seropositive. They were early and stable seroreactors to which SRLV was transmitted lactogenically. The age at which they seroconverted ranged from 3 to 10 months with a median of 5 months. In 8 of the 18 persistently seronegative goats, a single isolated positive result occurred. No goats showed any clinical signs of arthritis. The level of maternal antibodies at the age of one week did not differ significantly between the stable seroreactors and the remainder. **Conclusion:** Seroconversion appears to occur in less than 50% of goats exposed to heterologous SRLV genotype A *via* ingestion of colostrum and milk from infected dams and is delayed by 3–10 months. The natural lactogenic route of transmission of SRLV genotype A in goats appears to be less effective than this route of genotype B transmission reported in earlier studies.

Keywords: caprine arthritis-encephalitis, colostral antibodies, humoral immunity, maternal antibodies, seroconversion.

Introduction

Small ruminant lentivirus (SRLV), classified in the *Lentivirus* genus and *Retroviridae* family, causes two diseases of small ruminants – caprine arthritis-encephalitis (CAE) in goats and maedi-visna disease (MV) in sheep. Both are chronic and progressive, and have a considerable impact on animal productivity and welfare (34). Pathological processes comprise predominantly arthritis in CAE and interstitial pneumonia in MV, occasionally indurative mastitis and very rarely leukoencephalomyelitis (43). The clinical form of CAE develops in only approximately one third of infected goats and usually several months to years after infection (64). Historically, the aetiological agents of CAE and MV were considered related but distinct viral species referred to as caprine arthritis-encephalitis virus (CAEV) and maedi-visna virus (MVV). However, in the

late 1990s, marked genetic overlapping between SRLV isolates from goats and sheep was observed (65), and over the first two decades of the 21st century the taxonomic classification was modified. At present, SRLV is classified into at least four genotypes (A, B, C, and E, with D being in question) and the genotypes are further divided into sub-genotypes (12). Most genotypes and sub-genotypes have been shown to infect both goats and sheep (22, 40, 61). Genotypes A and B are widespread all over the world, while genotypes C and E are geographically restricted to Norway and Italy, respectively (22, 53). As genotypes A and B comprise most strains formerly classified as MVV and CAEV, they are customarily referred to as MVV-like and CAEV-like genotypes, respectively.

The major routes of SRLV transmission are the ingestion of colostrum and milk from infected dams (vertical postnatal transmission) and long-term direct contact between animals (horizontal transmission) (9, 50). The infection may also spread horizontally *via* contaminated milking cups (3). Intrauterine transmission is generally regarded as possible but sporadic (55). However, recent studies have yielded positive PCR results in a considerable proportion of lambs and kids taken from their mothers immediately after birth, strongly indicating the occurrence of intrauterine transmission (6, 21, 25). Traditionally, ingestion of colostrum and milk has been considered the most efficient mode of SRLV transmission in both goats and sheep, with most kids and lambs seroconverting after 4–12 weeks, which results in an overlap between periods of passive and active humoral immunity (1, 55, 56). On the other hand, more recent studies carried out in sheep infected with genotype A imply that the non-maternal route may play the principal role in SRLV transmission (10) and lactogenic transmission may be much less effective in natural conditions (5, 6), perhaps due to an ability of lambs that have acquired passive colostral immunity to clear the lactogenic infection (27). Whether a similar phenomenon may be observed in goats remains unknown. Therefore, we carried out a longitudinal study investigating the serological status of goats from the moment of natural exposure to colostrum and milk of dams contaminated with SRLV genotype A (MVV-like) through the age of 24 months.

Material and Methods

Study design. The study was carried out between February 2014 and March 2017 in the research dairy goat herd of the Institute of Genetics and Animal Biotechnology at the Polish Academy of Sciences. The herd numbered approximately 50–60 adult Polish White Improved (PWI) and Polish Fawn Improved (PFI) dairy goats. The herd had been infected with SRLV for more than 20 years, and all adult goats had been regularly serologically tested twice a year (28). The diagnosis of CAE was also confirmed by SRLV isolation (29). The virus in this herd belonged to the MVV-like genotype A subtype A17 as demonstrated in several genetic studies (47, 48, 49). A harem mating system was practiced in this herd. In September, goats were divided into groups of 10–15 females of the same breed, and a male of the same breed was kept with them for 4 to 6 weeks. Hence, kids were usually born between January and March.

The study enrolled 31 kids of PWI (n = 22) and PFI (n = 9) breeds. Twenty kids were born in February 2014 and eleven in February (n = 7) and March (n = 4) 2015. All kids but one were male. The kids were born to 17 dams (15 dams in 2014 and 6 dams in 2015, 4 of those 6 had also borne a kid in the year before (Table 1). The dams were from 3 to 9 years old with a median age of 5 years (interquartile range (IQR) 4–6 years) when they gave birth to the first kid enrolled in the study. In all dams SRLV infection was confirmed by at least two positive results of a serological ELISA (ID Screen MVV-CAEV Indirect Screening test, ID.vet Innovative Diagnostics, Grabels, France) obtained six months apart, the latest result having been obtained shortly before the mating season (August 2013 and June 2014).

Immediately after birth, a blood sample was collected from each kid and ingestion of colostrum from its dam was ensured by careful monitoring and manual assistance if necessary. The kids were kept together with their dams and other SRLV-infected does for three weeks. During this period they could freely suckle their dams and other does. Then, they were moved to a separate pen where they stayed together until the study was completed. Kids born in 2015 were moved to the pen already inhabited by kids born the year before. The goats were fed with hay and oat grain, and supplemented with mineral licks. They were not grazed on the pasture; however, from April to October they were allowed access to an outdoor pen. Blood collection and serological testing was performed every week until the goats turned seronegative and then at one-month intervals up to the age of 24 months. The goats were also clinically examined for the development of lameness or joint swelling at the time of each blood collection. The carpal-to-metacarpal circumference (C/MC) ratio, calculated as the circumference of the carpal joint divided by the circumference of the middle part of the metacarpal region (both measured with a measuring tape), was determined in all goats at the age of two years. All but one kid (goat no. 5) tested seronegative right after birth, and at the age of one week all tested seropositive. During the first four months of life they all reverted to seronegative status. The results regarding the duration of maternal antibodies have been analysed and published elsewhere (14). The present study focused on the serological status of goats from the moment when the maternal antibodies vanished through the age of two years. The goat seropositive right after birth turned seronegative after three months, as described elsewhere (14), and did not seroconvert for the entire period of the study. This indicates accidental consumption of colostrum rather than intrauterine infection.

Table 1. Characteristics of dams and kids born to them and enrolled in the study

Dam	Number of kids born in a particular year with number of seroreactors in parentheses		Year the dam was born	Year the dam seroconverted or first tested seropositive
	2014	2015		
1	1 (0)	-	2005	2007
2	1 (0)	-	2009	2011
3	1 (0)	1 (0)	2009	2012
4	-	2 (2[a])	2009	2014
5	2 (0)	-	2010	2011
6	1 (1[a])	-	2010	2013
7	2 (1[c])	-	2006	2013
8	1 (0)	-	2011	2013
9	1 (1[c])	-	2011	2013
10	1 (0)	-	2011	2013
11	-	2 (1[a])	2012	2014
12	1 (1[b])	2 (2[a])	2008	2013
13	2	-	2007	2010
14	1 (0)	-	2008	2010
15	1 (0)	-	2008	2013
16	2 (1[b])	2 (1[a])	2008	2013
17	2 (1[a])	2 (1[a])	2009	2013
Total	20 (2[a], 2[b], 2[c])	11 (7 [a])		

[a] – early and stable seroreactor; [b] – intermittent seroreactor; [c] – late seroreactor

Serological tests. Blood sampling was approved by the Third Local Ethics Committee in Warsaw (approval no. 31/2013). The kids were manually restrained, and blood samples were collected by jugular venepuncture into 10 mL clot activator tubes (BD Vacutainer, Beckton Dickinson, Plymouth, UK), left overnight at +4°C for clotting, and centrifuged at 3,000 rpm (1,390 × *g*) for 10 min. The serum was harvested to 2 mL Eppendorf tubes and frozen at −20°C until testing. Serum samples were serologically tested using two commercial ELISAs. The first was an indirect assay coated with a panel of synthetic peptides from SRLV structural proteins comprised of surface glycoprotein (gp135, SU), transmembrane glycoprotein (gp46, TM), and capsid protein (p25/p28, CA) (ID Screen MVV-CAEV Indirect Screening test; ID.vet Innovative Diagnostics, Grabels, France; henceforth referred to as sp-iELISA). The second was a competitive ELISA coated with SU (Small Ruminant Lentivirus Antibody Test Kit, cELISA; VMRD, Pullman, WA, USA) (henceforth referred to as SU-cELISA). The assays were performed according to the manufacturers' instructions and the optical density (OD) was measured at wavelengths of 450 nm and 630 nm, respectively using an Epoch Microplate Spectrophotometer (BioTek, Winooski, VT, USA). Quantitative results of the ELISAs were expressed as corrected OD: the sample-to-positive control serum ratio (S/P%) in the sp-iELISA and the percent inhibition (PI) in the SU-cELISA. The cut-off values provided by the manufacturers were used: S/P% of 50% (39) and PI of 35% (23). At these cut-off values the ELISAs were shown to be highly sensitive at 91.7% (95% confidence interval (CI 95%): 85.0%–95.6%) and 100% (CI 95%: 94.0%–100%), respectively (26, 46). They were also proven highly specific at these cut-off values, achieving 98.9% (CI 95%: 96.2%–99.7%) and 96.4% (CI 95%: 91.9%–98.5%), respectively (26, 46).

Seroconversion was defined as a quantitative result of at least one ELISA above the cut-off value in at least two consecutive months following the disappearance of colostral antibodies. Seroconversion was considered early if it occurred before the age of 12 months or late if it occurred between the 13th and 24th months of life. The seropositive status was classified as stable *(i.e.* the animal was a stable seroreactor) when a goat tested positive continuously in subsequent months after seroconversion until the study was completed (one seronegative result being allowed). Otherwise, the status was noted as intermittently seropositive (*i.e.* the animal was an intermittent seroreactor).

Statistical analysis. Categorical variables were presented as counts and percentages, and compared between groups using Fisher's exact test. The CI 95% values for the proportions were calculated using the Wilson score method (4). The assumption of normality of the numerical variable distribution was verified using the Shapiro–Wilk W test. As it was violated in most cases, the numerical variables were presented as the median, IQR and range. The age at seroconversion was compared between two ELISAs using the Wilcoxon signed rank test. The quantitative results of the ELISAs (S/P% and PI) were rank transformed with type RT-1 transformation (13) and compared between consecutive time points starting from the age of four months using repeated measures analysis of variance with Dunnett's post-hoc test. Comparisons of rank-transformed S/P% and PI between goats that did and did not seroconvert were performed using the unpaired Student's *t*-test. The significance level (α) was set at 0.05. Statistical analysis was performed in TIBCO Statistica 13.3 (TIBCO Software, Palo Alto, CA, USA).

Results

Seroconversion was evident in 13 out of the 31 goats (42%; CI 95%: 26%–59%) during the study; in age breakdown they were 6 out of 20 goats (30%) born in 2014 and 7 out of 11 goats (64%) born in 2015 (Fig. 1). This difference was not significant (P = 0.128). Four of them tested seropositive only in one ELISA – three in the sp-iELISA and one in the SU-cELISA. The age at which the goats seroconverted ranged from 3 to 22 months with a median of 5 months (IQR 4–7 months). In 11 goats seroconversion was early and in the other 2 goats (no. 13 and no. 20) it was late (occurring in the 22nd and 19th months, respectively). Two of the goats that seroconverted early became seronegative again after 2 (goat no. 9) or 5 (goat no. 18) months. The former goat reverted to seropositive status after a further 13 months, while the latter remained seronegative until the end of the study (Fig. 1). These two goats with intermittent seroreactivity and one goat with late seroconversion (no. 20) tested positive only in the sp-iELISA (Fig. 2).

Only 9 of 31 goats (29%; CI 95%: 16%–47%) seroconverted in the first year of life and remained seropositive until the end of the study (*i.e.* were early and stable seroreactors). Eight of them seroconverted demonstrably in both ELISAs and one (goat no. 30) only in the SU-cELISA (Fig. 3). Early and stable seroconversion occurred in only 2 out of 20 kids (10%) born in 2014 but in all kids born in 2015 that seroconverted (7 out of 11; 64%) (P = 0.003). The age at which they seroconverted ranged from 3 to 10 months with a median of 5 months (IQR 4–6 months) and did not differ significantly between the sp-iELISA and SU-cELISA (P = 0.263).

Eighteen goats remained seronegative for the entire study. In eight of them a single isolated seropositive result was obtained: in the sp-iELISA in two goats (nos. 12 and 26) and in the SU-cELISA in six goats (nos. 10, 16, 17, 19, 21, 23). In the sp-iELISA, the S/P% in seronegative goats ranged from 0% to 96% with a median of 3.6% (IQR 2.0%–7.4%). Compared to the S/P% at the age of 4 months, it was significantly higher at the ages of 6 months (P = 0.004) and 7 months (P = 0.029), and significantly lower at the age of 20 months (P = 0.017). In the SU-cELISA, the PI in seronegative goats ranged from 0% to 40.3% with a median of 13.7% (IQR 9.8%–20.3%) (Fig. 4). Compared to the PI at the age of 4 months, it was significantly higher at the ages of 11 months (P = 0.016), 12 months (P = 0.003), and 13 months (P <0.001), and then from the age of 20 months until the end of the study (P = 0.001) (Fig. 5).

None of the 31 goats showed any clinical signs of arthritis (joint swelling or lameness) during the observation period. The C/MC ranged from 1.5 to 1.7 with the median of 1.6.

Neither the S/P% of the sp-iELISA nor the PI of the SU-cELISA when the goats were one week old differed significantly between the stable seroreactors and the rest of the goats (P = 0.966 and P = 0.481, respectively), which disqualifies these variables as potential predictors of stable seroconversion.

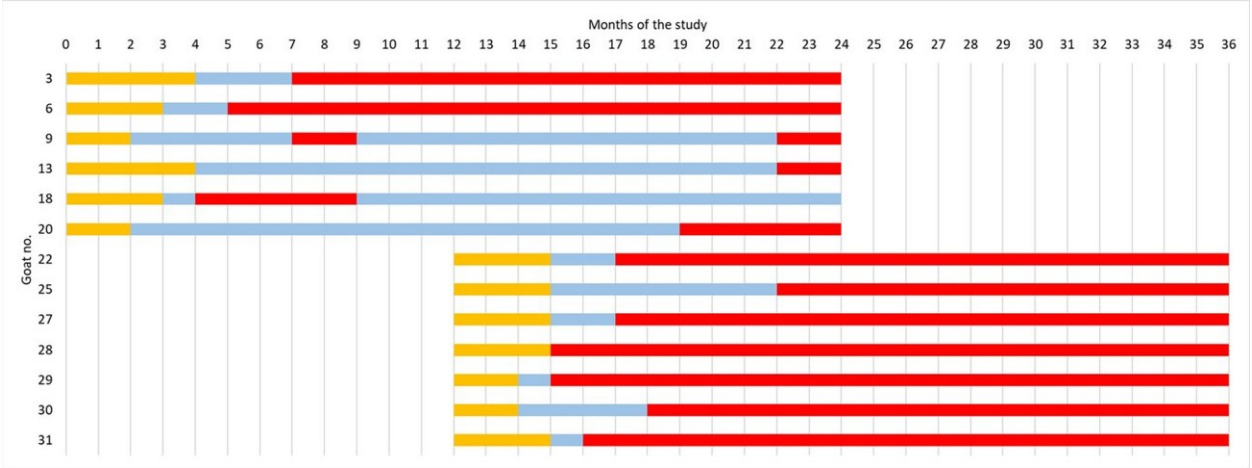

Fig. 1. Timeline of the serological status of 13 goats that seroconverted during the study. The orange band is the duration of maternal antibodies, the blue band is the time of seronegativity in both ELISAs, and the red band is the time of seropositivity in at least one ELISA. The direct transition from the period of maternal antibodies to the period of active seroreactivity by goat no. 28 results from the overlapping seropositivities of this goat in the two ELISAs used – see Fig. 3 for details

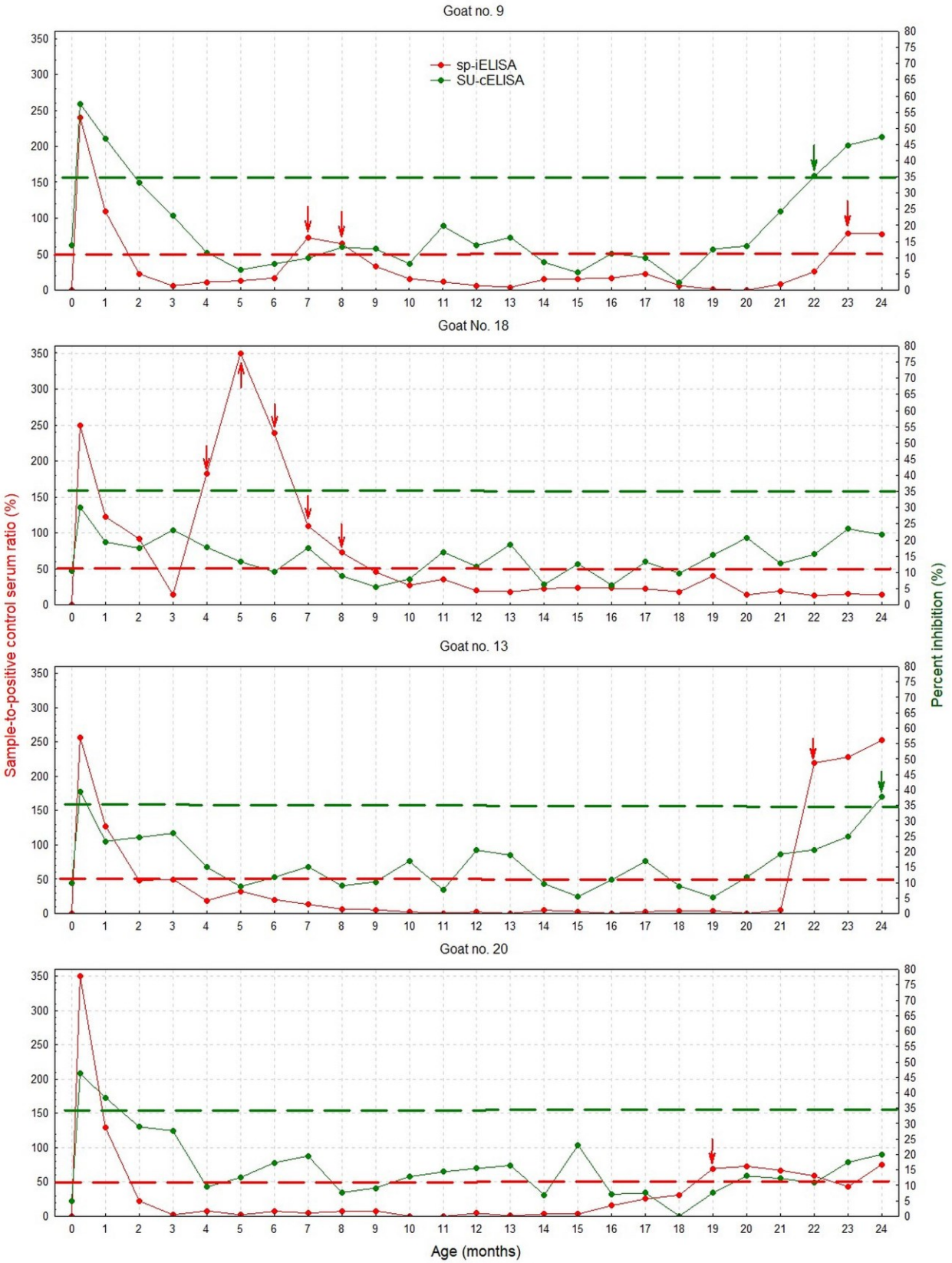

Fig. 2. Quantitative results of an indirect ELISA based on synthetic viral peptides (sp-iELISA; sample-to-positive control serum ratio, S/P%) and a competitive ELISA based on surface glycoprotein (SU-cELISA; percent inhibition, PI) in intermittent (goat no. 9 and goat no. 18) and late seroreactors (goat no. 13 and goat no. 20). Dashed lines indicate the manufacturer's cut-off value used to interpret the results of the sp-iELISA (red; S/P% = 50%) and the SU-cELISA (green; PI = 35%). The arrows indicate the first positive result of the sp-iELISA (red) and the SU-cELISA (green) or the times at which an intermittent seroreactor was seropositive

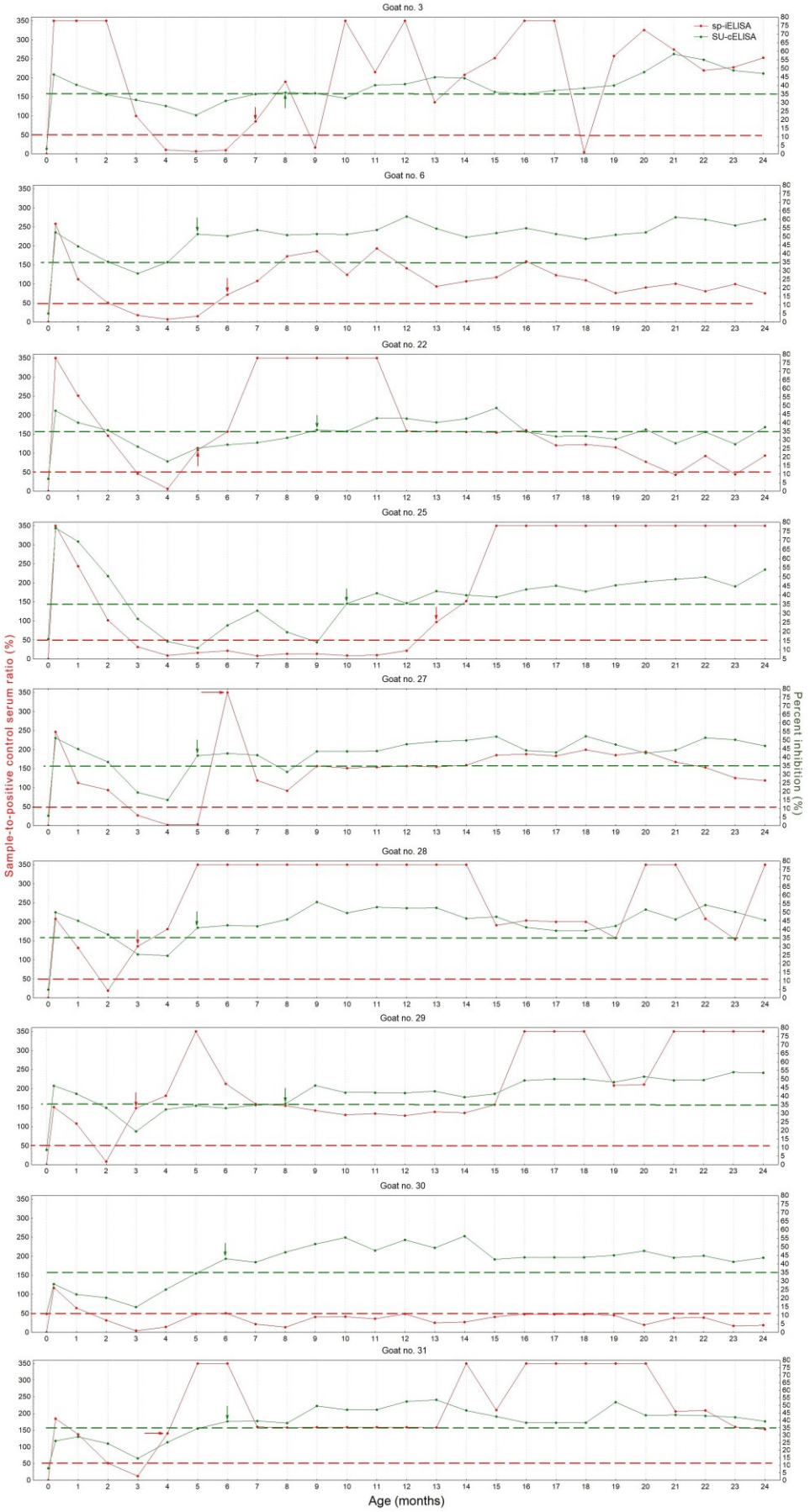

Fig. 3. Quantitative results of an indirect ELISA based on synthetic viral peptides (sp-iELISA; sample-to-positive control serum ratio, S/P%) and a competitive ELISA based on surface glycoprotein (SU-cELISA; percent inhibition, PI) in early and stable seroreactors. Dashed lines indicate the manufacturer's cut-off values used to interpret the results of the sp-iELISA (red; S/P% = 50%) and the SU-cELISA (green; PI = 35%). The arrows indicate the first positive result of sp-iELISA (red) and SU-cELISA (green)

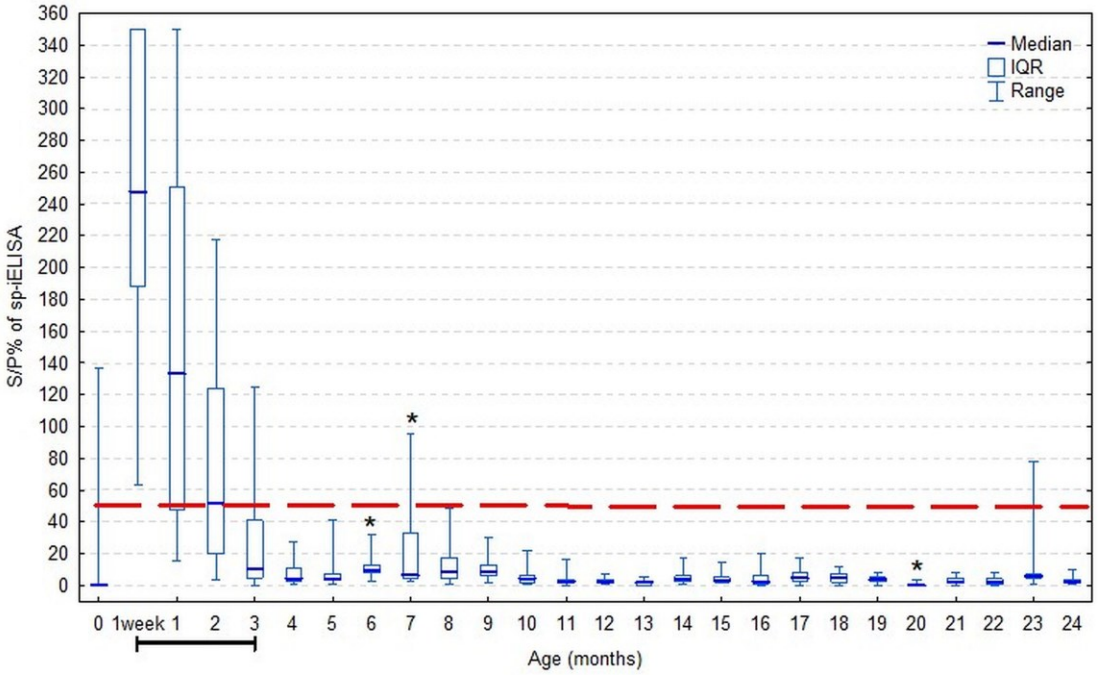

Fig. 4. Quantitative results of an indirect ELISA coated with the mixture of synthetic viral peptides (sp-iELISA; sample-to-positive control serum ratio, S/P%) of 18 seronegative goats in the first 24 months of their lives. The red dashed line indicates the manufacturer's cut-off value of S/P% = 50%. The dark solid line under the x axis indicates the time period in which maternal (colostral) antibodies were detected. The asterisk (*) indicates significant difference (α=0.05) compared to the age of 4 months

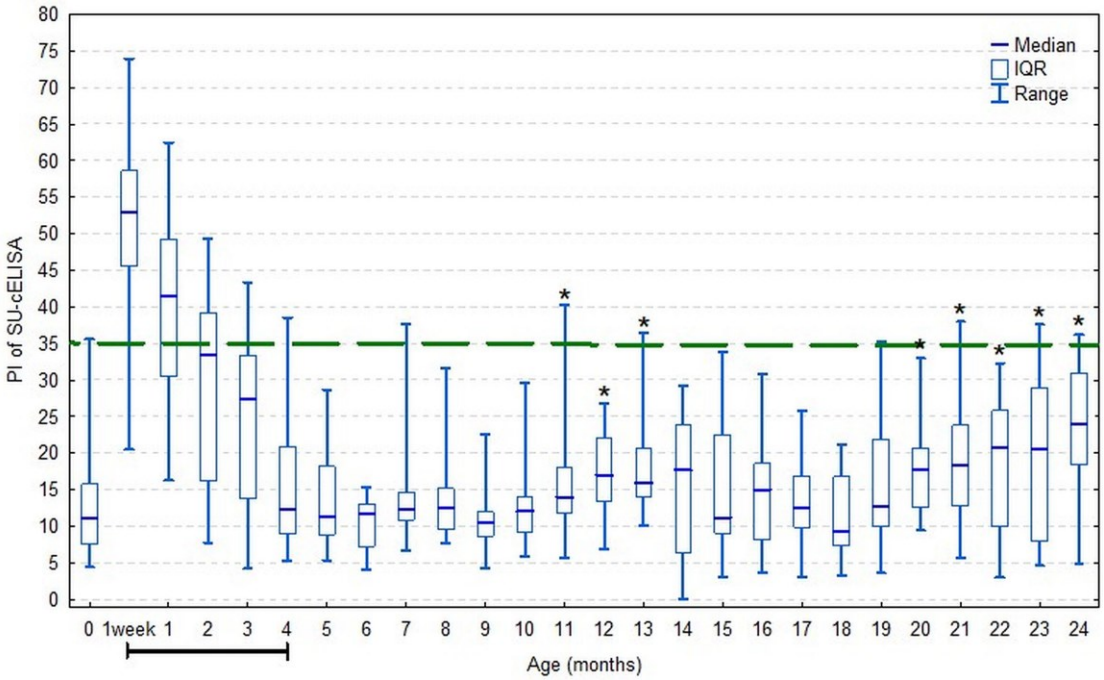

Fig. 5. Quantitative results of a competitive ELISA coated with viral surface glycoprotein (SU-cELISA; percent inhibition, PI) of 18 seronegative goats in the first 24 months of their lives. The green dashed line indicates the manufacturer's cut-off value of PI = 35%. The dark solid line under the x axis indicates the time period in which maternal (colostral) antibodies were detected. The asterisk (*) indicates significant difference (α=0.05) compared to the age of 4 months

Discussion

Our study showed that seroconversion was an uncommon and delayed event in goats exposed to oral infection with heterologous SRLV genotype A (sub-genotype A17) *via* ingestion of colostrum and milk from infected dams. Only approximately one third of goats seroconverted and it took them 3 to 10 months from the moment of first colostrum ingestion to mount an antibody response with levels detectable in commercial ELISAs. These observations are at odds with many older studies on SRLV-infected goats which showed that ingestion of SRLV-contaminated colostrum and milk is an effective mode of SRLV transmission in goats and that most of them seroconvert three weeks to five months after oral infection with CAEV-like genotype B SRLV (1, 9, 17, 18, 56).

There are several possible explanations for this phenomenon. First, the colostrum and milk of dams enrolled in this study could have been a poor source of infective SRLV. It cannot be directly excluded, as we

neither confirmed the presence of infective SRLV in colostrum or milk ingested by the kids nor made any attempt to detect SRLV in the kids. This is undoubtedly an important limitation of our study. On the other hand, such a scenario is very unlikely as all the dams enrolled in the study had been shown to be infected with SRLV genotype A (47, 48, 49) and seropositive for at least a year preceding the start of the study. Both the colostrum and milk of does infected with SRLV were shown to be potent and equally effective sources of infection (2, 18, 19, 36). The kids in our study certainly ingested colostrum from their infected dams and were freely consuming milk from infected does for three weeks in total. Such a time period has been shown to be more than enough to ensure lactogenic infection with SRLV. Therefore, we think that infection of the kids did occur.

The second possible explanation is that seroconversion may have in fact occurred in all kids, and the diagnostic sensitivities of the two ELISAs used were too low to detect it. This scenario is virtually impossible, as both ELISAs have been shown to be highly sensitive (42, 46, 60). Moreover, goats which did not seroconvert in our study had consistently low quantitative results in the ELISAs. Even lowering the cut-off value of the sp-iELISA to for example 20%, which, according to Nowicka *et al.* (46) ensures >95% diagnostic sensitivity, would not considerably change the interpretation of the results – goat no. 30 positive in the SU-cELISA would become positive also in the sp-iELISA for most of the time and goats no. 7 and 16 would be classified as intermittent seroreactors; however, no additional goats would be classified as stable seroreactors.

The third reason to consider for the low seroconversion rate and extended time in the present study is that seroconversion or lactogenic infection may have not occurred in some goats because they were exposed to the heterologous (MVV-like, A) SRLV genotype. Genotypes of SRLV have been shown to vary in terms of their potential for lactogenic transmission (51) as well as antigenic properties (11, 22, 45). As a result, the diagnostic performance of serological assays varies depending on the degree of sameness of the genotype responsible for the infection and the genotype from which the antigens used in the assay were derived (15, 33, 42, 52, 58, 60). Although very few studies have investigated the differences in antibody response between goats infected with genotype A and those infected with genotype B, the humoral response after infection with the homologous SRLV genotype has been shown to be stronger than that which followed the heterologous genotype infection in both goats and sheep (33, 41). Moreover, genotype A generally appears to stimulate slower development of the humoral response in both species (41). Notwithstanding the possible weaker lactogenic transmissibility and antigenicity of genotype A, the less pronounced humoral response associated with genotypic heterology, and the less rapid initiation of the same by genotype A, the goats were followed for two years and during this time seroconversion should have occurred, even if it was markedly delayed (54). Although the three goats that seroconverted at the age of >12 months (one of them, goat no. 9, after a temporary seroconversion) might have represented animals with a delayed humoral immune response following colostral or lactogenic infection, they were more likely to have been horizontally infected through sustained direct contact with their seropositive companions.

Interestingly, significantly more goats born in 2015 seroconverted than goats born a year before. It is possible that transferring these goats to the pen already inhabited by adult males resulted in a stress reaction which promoted faster infection and seroconversion. Obviously, it is impossible to definitively state whether seroconversion in those 7 of the 11 goats placed in the pen already inhabited by goats born in the previous year was the consequence of colostrum and milk ingestion or horizontal transmission. The latter cannot be excluded, as several studies have shown that horizontal transmission plays a very important role, possibly even more important than vertical transmission, and little benefit results from preventing lactogenic transmission unless strict segregation from infected individuals can be ensured (5, 8, 57). Nevertheless, the relatively short time in which most of the seroconversions of goats born in 2015 took place (between the ages of 3 and 6 months in six out of the seven goats that seroconverted) implies that they resulted from the point-source exposure corresponding to colostrum and milk ingestion in the first days of life (59).

The fourth possible explanation of the low seroconversion rate in our study is that the infection did occur, but was cleared by the immune system. Such a phenomenon has been described in sheep (27): 21 lambs born to SRLV-infected ewes were positive for proviral DNA in PCR at 8 weeks of age, but by week 24 proviral DNA became undetectable. Moreover, their serum antibody titres, high a week after birth, had fallen drastically by nine months of age. It was proposed that colostral antibodies could have activated natural killer cells through antibody-dependent cellular cytotoxicity (ADCC). This cytotoxicity and the neutralising activity of anti-lentiviral antibodies have been described in some studies in cattle and humans. Bovine colostral immunoglobulins of class G have been shown to have strong ADCC and neutralising activity against human immunodeficiency virus (HIV) type 1 (31, 32). High serum titres of ADCC-mediating anti-HIV antibodies are also negatively correlated with plasma viremia (3). However, no evidence of such activity of colostral antibodies exists in goats. On the contrary, Ellis *et al.* (18) showed that four out of six kids exposed to SRLV-contaminated colostrum became infected and this proportion was the same as in the group of kids deprived of colostral antibodies and exposed to SRLV-contaminated milk. Therefore, our results are the first such observation in goats, which should be treated with the highest caution and verified in further studies involving molecular and virological diagnostic methods.

We must firmly stress that the lack of molecular testing precludes drawing the conclusion that most of the goats enrolled in our study remained uninfected despite extensive exposure to the natural lactogenic source of SRLV. We may only state that most goats remained seronegative in two different serological assays of high diagnostic accuracy for two years after exposure, which is strong evidence that they did not mount a humoral immune response at all or that it was markedly delayed. Whether it was because they remained uninfected, cleared the infection, or were infected but remained seronegative, and in the latter scenario, whether they were about to seroconvert later or not, remains unknown. Several studies based on molecular testing have shown that both goats and sheep may be infected with SRLV without detectable seroreactivity (7, 16, 24, 25, 37, 54, 62). In one study, as many as 6 out of 12 goats with presumed intrauterine SRLV infection were positive in a nested-PCR and negative in an SU-cELISA and agar gel immunodiffusion test (25). Usually, the proportion of molecular test–positive and seronegative individuals does not exceed 10–20%. Therefore, we think that it is very unlikely that all goats that remained seronegative in our study were in fact infected at any moment of the study. However, even if one quarter of them were truly infected, it still means that the rate of infection did not exceed 50%, which is lower than expected and indicates that the natural lactogenic route of transmission of SRLV genotype A in goats may be less effective than the same route transmission of genotype B reported in early studies. Several studies in sheep have shown that the respiratory route of transmission appears to be much more efficient than the lactogenic route in this species (8, 10, 63). Recent studies have also shown that goats seroconvert 2–8 weeks after intratracheal inoculation with SRLV (41, 52, 53), which is a considerably shorter period than observed in older studies which arranged natural lactogenic transmission (1, 9, 17, 18, 56). Obviously, an experimental intratracheal inoculation only partially reflects the mode of SRLV transmission *via* the respiratory route in field conditions. The pathogenesis of SRLV infection in sheep, in which the lungs are the main target organ, naturally promotes the respiratory route of transmission in this species (38, 39). Nevertheless, some studies indicate that subclinical interstitial pneumonia also develops in SRLV-infected goats (20, 44). These observations give rise to the suspicion that the respiratory route may play a more important role than the lactogenic route in SRLV transmission in goats as it does in sheep.

Concluding, seroconversion appears to occur in less than 50% of goats exposed to oral infection with heterologous SRLV genotype A *via* ingestion of colostrum and milk from infected dams and may be delayed by 3 to 10 months. The natural lactogenic route of transmission of SRLV genotype A in goats appears to be less effective than the transmission by this route of genotype B observed in investigations carried out previously.

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Animal Rights Statement: Blood sampling was approved by the Third Local Ethics Committee in Warsaw (Approval No. 31/2013).

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