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Short communication

Fall in antibody titer to small ruminant lentivirus in the periparturient period in goats

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ABSTRACT

The prospective study was carried out to document the change of antibody level to small ruminant lentivirus (SRLV) in chronically infected pregnant does. Thirteen dairy goats of Polish White Improved and Polish Fawn Improved breeds, asymptomatically infected with SRLV for at least a year, were enrolled. The goats were blood-sampled at mating, then four times during pregnancy, 2 weeks before kidding, at kidding and monthly for three months postpartum. Antibody titers to SRLV were determined by screening sera in increasing dilutions with three different commercial ELISAs: indirect whole-virus antigen (wELISA), indirect p28-transmembrane antigen (p28-TM-ELISA) and competitive gp135 (SU-ELISA). Then, the reciprocal of the greatest dilution at which a serum yielded the result greater than the cut-off of the test was considered the end-point antibody titer. Compared to the level at mating antibody titers significantly fell at kidding in all three tests. Significant decrease in antibody titer was observed for the longest time in SU-ELISA and for the shortest time in p28-TM-ELISA. At kidding false negative results were observed in two ELISAs (p28-TM-ELISA and SU-ELISA) and 3 of 13 goats became seronegative at kidding in at least one ELISA. At least four-fold fall in antibody titer between mating and kidding was observed in wELISA in 6 goats, in p28-TM-ELISA in 4 goats and in SU-ELISA in 5 goats. None of goats showed at least four-fold decrease in all three tests. Fall in antibody titer to SRLV in the periparturient period can interfere with the results of serological screening of pregnant goats.

1. Introduction

Caprine arthritis-encephalitis (CAE) caused by a small ruminant lentivirus (SRLV) from the family Retroviridae, spreads insidiously in a herd with no symptoms apparent in infected goats for a long time following infection. Therefore, control of the disease requires early identification of infected individuals, which may only be achieved by laboratory testing. Even though PCR was first used for diagnosing SRLV infection over twenty years ago (Herrmann-Hoesing, 2010) and several highly accurate real-time PCRs for detection of SRLV have recently been developed (De Regge and Cay, 2013; Kuhar et al., 2013) none of them has so far been commercialized and they remain hardly available in routine veterinary practice. Hence, serological ELISA tests remain the mainstay of field CAE diagnostics, mostly owing to their high accuracy and wide commercial availability. Nowadays, various ELISA kits detecting antibodies to surface (SU), transmembrane (TM), capsid (CA) proteins as well as whole-virus antigen are available. Despite the variability of antibody types detected, the main pitfall of serological testing for SRLV results from a delayed seroconversion (Rimstad et al., 1983), fluctuations of antibody levels in chronically infected seropositive goats (Hanson et al., 1996), and genetic diversity of the virus (Lacerenza et al., 2006). One of the circumstances which may potentially interfere with humoral immune response is pregnancy (Herr et al., 2011) and antibody levels were shown to fall in the periparturient period in cows infected with another retrovirus – bovine leukemia virus (BLV) (Burridge et al., 1982). This has also been observed in cows infected with Bovine viral diarrhea virus (Bachofen et al., 2013) and

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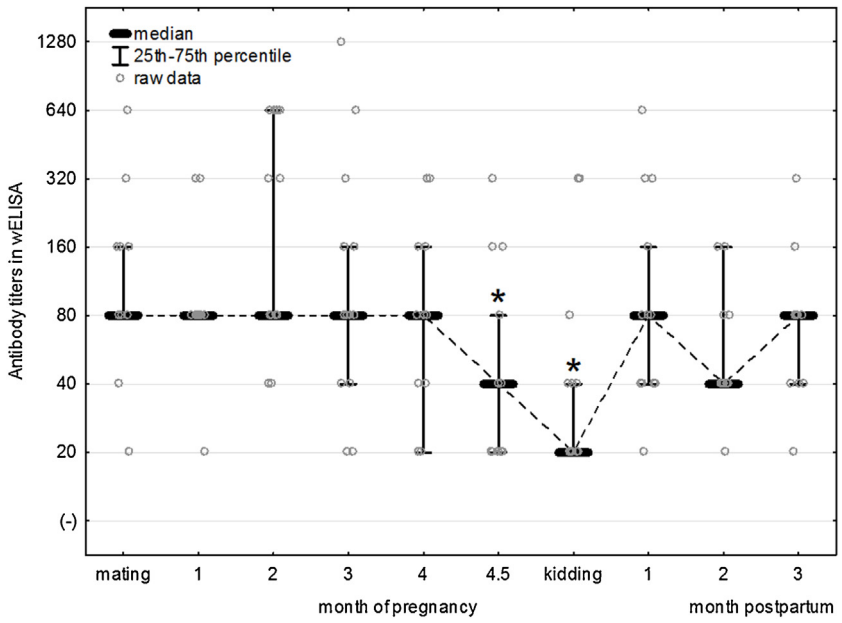

Fig. 1. Antibody titers to small ruminant lentivirus throuhout the study determined using the ID Screen® MVV-CAEV Indirect Screening test (ID.vet Innovative Diagnostics, France) (wELISA). Antibody titers presented on binary logarithm scale after dividing by two. * signifies statistical significance at $\alpha = 0.05$.

Neospora caninum (Kyaw et al., 2005), and in buffalos with *Toxocara vitulorum* infection (Amerasinghe et al., 1994) as well as in sheep with *Trichostrongylus colubriformis* infection (Beasley et al., 2010). We carried out the prospective study to document the change of specific antibody levels in pregnant does chronically infected with SRLV.

2. Materials and methods

2.1. Animals

The study was approved by the 3rd Local Ethical Committee in Warsaw (Approval No. 31/2013, 22 May 2013) and carried out in a dairy goat herd counting roughly 60 dairy does of Polish White Improved (PWI) and Polish Fawn Improved (PFI) breed. A SRLV infection was detected in this herd 20 years ago, first serologically and then by the virus isolation (Kaba et al., 2009). Subsequently, regular serological screening based on a whole-virus ELISA, has been performed twice a year until now and the within-herd seroprevalence ranged from 25% to 75% (Kaba et al., 2011). SRLV in this herd belonged to genotype A, which seems to predominate in Polish goat population (Olech et al., 2012).

Initially, 21 SRLV-seropositive does aged 2 years or more were enrolled in the study at the beginning of a mating season. Eight of them were then excluded due to reproductive failures (5 goats), sudden death (1 goat) and testing negative in at least one of ELISA tests used in the study (2 goats). Finally, 8 PWI and 5 PFI goats, aged from 3 to 7 years with the median of 5 years and interquartile range between 4 and 6 years were included. They had become seropositive to SRLV 1–5 years (median of 2 years) before the onset of the study, however none of them had developed any clinical symptoms of CAE.

2.2. Sampling protocol

All the goats were blood-sampled ten times in all: at mating, at the end of each month of gestation, 2 weeks before kidding, during the week preceding kidding and at the end of each of three months after parturition. Blood was collected to dry tubes and left at room temperature overnight, then centrifuged, and the sera were stored at −20 °C until testing.

2.3. Serological testing

The sera were screened with three commercial ELISA tests – two indirect: ID Screen® MVV-CAEV Indirect Screening test (ID.vet Innovative Diagnostics, France) containing whole-virus antigen (henceforth referred to as wELISA) and IDEXX MVV/CAEV p28 Ab Screening (IDEXX Laboratories, USA) based on recombinant TM and p28 antigen (p28-TM-ELISA) – and one competitive: Small Ruminant Lentivirus Antibody Test Kit, cELISA (VMRD, USA) coated with SU (gp135) antigen (SU-ELISA). They all were based on antigens from caprine isolates of SRLV and have proven highly accurate in diagnosing SRLV infection in goats (Brinkhof and van Maanen, 2007; Herrmann et al., 2003; Nowicka et al., 2014). Manufacturer's cut-offs were used: wELISA – sample-to-positive control ratio percentage (S/P%) of 50%, p28-TM-ELISA – S/P% of 110%, SU-ELISA – percentage of inhibition (%I) of 35%. To determine the antibody titer each serum sample was tested in increasing dilutions: in wELISA and p28-TM-ELISA from 1:20 to 1:2560, whereas in SU-ELISA from 1:1 through 1:256. Then, the reciprocal of the greatest dilution at which a serum yielded the result (either S/P% or %I) greater than the cut-off of the test was considered the end-point antibody titer. For the needs of preparing plots antibody titers were logarithmically transformed using binary logarithm, either directly (SU-ELISA) or after division of the titer by 10 (wELISA and p28-TM-ELISA kits).

2.4. Statistical analysis

Antibody titers were given in figures as median, interquartile range (25th–75th percentile) and raw data. Data were subjected to rank transformation of type RT-1 (Conover and Iman, 1984) and analyzed using repeated-measures analysis of variance. Given that a change in antibody titer should be at least 4-fold to be considered significant (Thrusfield, 2007), titers which differed from the titer at mating no more than 2-fold were assigned the same rank. A Dunnett's post-hoc test was applied to compare antibody titers during pregnancy and three months after parturition with the basal level