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CASE REPORT

Food/farmed animals

An outbreak of small ruminant lentivirus in a Swedish dairy goat herd

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Abstract

Small ruminant lentivirus causes severe economic losses, and negatively affects animal welfare in goat herds across the world. The aim of this study was to describe the clinical and pathological consequences of the disease in an affected goat herd, as well as to sequence the virus in infected animals. Seventy-six percent of the sampled animals were small ruminant lentivirus seropositive. The most prominent signs of disease were respiratory signs found in clinical examinations and chronic interstitial pneumonia found at postmortem examination. All animals with pneumonia also showed evidence of infection with *Muellerius capillaris*. The cell count in bulk milk was high and most likely related to the high small ruminant lentivirus seroprevalence within the herd. Small ruminant lentivirus was successfully detected in the lungs, bronchial lymph nodes and udder tissues using polymerase chain reaction. The phylogenetic analysis of the obtained sequences revealed the highest similarity with small ruminant lentiviruses in group C, which consists of viruses which have previously been found in Norwegian goats and sheep.

KEYWORDS

caprine arthritis encephalitis virus (CAEV), goats, lungworm, pneumonia, virology

BACKGROUND

Lentivirus is a genus of non-oncogenic viruses belonging to the family *Retroviridae*, and the subfamily *Orthoretrovirinae*. Lentiviruses are characterised by a long incubation time, a high frequency of mutation and an ability to escape the host immune system. Lentiviruses that infect goat and sheep are called small ruminant lentivirus (SRLV) and can be further divided into five groups (A–E) based on their genetic composition. SRLV of group D, however, is suggested to belong to the group A viruses with a divergence in the pol gene.[1] Infection with the virus can cause severe, incurable disease. While the disease in sheep is called maedi-visna (MV) and causes pneumonia and neurological disease in affected animals, the disease in goats is known as caprine arthritis encephalitis (CAE), a disease that occurs in five clinical forms: arthritis, encephalomyelitis, interstitial pneumonia, interstitial mastitis and chronic wasting. Arthritis is the most common clinical sign and is seen mainly in adult goats over 1 year of age.[2] Interstitial pneumonia has only occasionally been reported in SRLV-infected goats. Usually, only a few of the infected goats in a herd display clinical signs, even though many may be infected. Caprine arthritis encephalitis virus (CAEV) causes a slow infection, with the incubation time often spanning months or years.[3] Transmission between animals occurs most commonly via the oral route (mainly through milk and colostrum), but may also occur via inhalation of infected aerosol droplets.[4] CAE causes great economic losses due to its association with lower milk production, weight loss and increased mortality.[4] Moreover, it has major implications for animal welfare and livestock trade. Due to the long incubation time and frequently vague clinical signs, the disease can be very hard to determine, with consequences often being severe for both the animals and the owner.

Historically, MV and CAE and their infective agents have been distinctly separated, although recent studies suggest evidence for inter-species transmission. Phylogenetic studies of SRLV circulating in Norwegian goat and sheep herds have shown that group C was found in mixed herds, while isolates obtained from unmixed sheep flocks clustered in group A.[5] The genotypes of SRLVs circulating in the Swedish sheep and goat population are currently unknown.

SRLVs have been detected in most sheep- and goat-raising countries, including Sweden, where it was first described in the 1970s.[6,7] The prevalence of SRLV in Sweden is not known, though a study from 2018 showed that three out of 10 Swedish goat herds were seropositive for SRLV,[8] while in a more recent study only three out of 20 herds were positive.[9] SRLV infection is a notifiable disease in Sweden (SJVFS 2013:23). A voluntary control programme for SRLV in goats (SJVFS 2015:17) was launched in Sweden in 1999, with about 12% of Swedish goats enrolled.[10] The purpose of the control programme is to detect and eradicate SRLV from Swedish goat herds and to prevent introduction into free herds. The programme is based on serological examination of blood samples for antibodies to SRLV using an ELISA test. Norway has, after a successful programme ('Healthier goats'),[11] declared most dairy goat herds free from SRLV,[12] showing that it is possible to eradicate the disease.

This paper describes a case study carried out in an SRLV-seropositive Swedish goat herd. The aim of the study was to describe the clinical and pathological consequences of the disease, as well as to sequence the virus from infected animals.

LEARNING POINTS/TAKE-HOME MESSAGES

- Although respiratory disease is not the typical primary clinical manifestation of small ruminant lentivirus in goats, this case study clearly shows that small ruminant lentivirus needs to be taken into consideration when investigating goat herds with respiratory distress.
- Postmortem examinations with virus detection may be necessary for the diagnosis of small ruminant lentivirus as the causative pathogen, as only the serology examination is inconclusive.
- Small ruminant lentivirus can cause significant clinical signs that affect animal welfare.
- Small ruminant lentivirus should be controlled or eradicated for better animal health and welfare.

CASE PRESENTATION

This case study was performed in a dairy goat herd in September 2020 in order to find the reasons for the long history of respiratory signs among the goats. The herd had approximately 60 Scandinavian landrace goats. One third of the goats were 0–2 years old, one third 3–6 years old and one third were 7–10 years old. All goats originated from seven does, bought in 2006 from one dairy farm in the same region, and bucks brought from different herds. The goats were kept in a cold loose housing system with daily access to an outdoor paddock in the wintertime and a pasture in the summertime. Kidding season was February–March, and the kids stayed with the does until 1 month of age, when they were partly weaned overnight. During lactation, the does were milked once daily until December when they were dried. Bucks were brought from other herds, and no quarantine routines were practiced.

To gather information, the owner was interviewed during herd visits, b y email and over the phone. Medical records ($n = 12$), including results from faecal sampling and postmortem examination reports ($n = 4$) from previous herd contacts, were provided by the advisory health organisation Farm & Animal Health. Data were available from 2016 to 2020. According to the owner, the main health issue was respiratory signs, which had been present for a couple of years at that point. The signs were dyspnoea and increased respiratory rates, sometimes with concurrent chronic wasting, but with no discharge and coughing. In some goats, chronic wasting was seen to occur without respiratory distress. There were more severe signs in the summertime. Only adult goats were affected. In some goats, the disease history was short, while in others, clinical signs were worsening over months. There were no problems with neurological diseases, diarrhoea or skin diseases in the herd. The few lameness cases seen were mainly associated with claw lesions. The herd had a couple of clinical mastitis cases each year, but they seldom required medical treatment. Milk production was considered good, but had decreased over the last couple of years, according to the owner's perception. Fertility and neonatal health were good, apart from in the previous year, which had 11 non-pregnant does and one abortion.

In 2018, blood samples from 21 goats were tested for antibodies to SRLV.[8] Fifteen goats were positive (71%). Faecal samples for parasitology were submitted once a year as part of the farm's standard routines. Between 2016 and 2020, the faecal samples frequently showed the presence of *Protostrongylidae* and *Chabertia/Oesophagostomum*, and the sporadic presence of *Nematodirus filicollis* and *Nematodirus spathiger*. Since 2019, all faecal samples were examined for lung worms, and the samples showed the frequent occurrence of *Muellerius* sp. and the sporadic occurrence of *Protostrongylus rufescens*. The herd was treated with anthelmintics following the faecal examination and their veterinarian's prescription. Between 2016 and 2019, four stillborn or weak kids and four adult goats were sent for postmortem examination. One of the kids showed signs of systemic bacterial infection, but no cause of death was determined for the rest of the kids. Among the adult goats, one had purulent metritis with disseminated systemic infection, one was emaciated with no other conclusive findings, and two had pulmonary lesions consistent with infestation of lungworms. No lesions caused by SRLV were suspected, and no further analyses were performed. Despite anthelmintic treatment, the respiratory problems continued.

INVESTIGATIONS

Physical examination performed by the authors

All goats were examined, including recording of general appearance (graded normal, to mildly, moderately or severely affected), body condition scoring (graded from 1–5 using Langston University's body condition score [BCS],[13] where <2 is below normal), palpation of external lymph nodes (graded as normal, mildly, moderately, or severely enlarged) and palpation of the udder (if present). Quarter milk from lactating

does was analysed using a California mastitis test (CMT) and was graded from 1 to 5, where 5 indicated the highest cell count (modified from Schalm et al.[14]). In all female goats over 12 months of age, the respiratory rate was recorded through visual examination of resting goats in the free stall. The number of breaths taken was counted for 15 seconds and multiplied by 4 for the respiratory rate per minute. A respiratory rate of 10–20 breaths per minute was considered normal, 21–30 breaths per minute was considered to be a mild increase of respiratory rate, 31–40 breaths per minute was a moderate increase and over 40 breaths per minute was graded as a severely increased respiratory rate. In bucks and kids, no respiratory rate was measured. Gait examination of all female goats over 12 months of age was performed through visual examination of individual goats when passing from milking parlour to housing. Kids under 12 months were examined visually by moving a group of six animals within the free stall. Gait was graded as normal, lame or with a disturbed moving pattern. Adult bucks were not examined. For goats with an affected general appearance, BCS of 1–2 and/or moderately to severely enlarged lymph nodes, an extended physical examination was performed. This examination included a visual inspection of the mouth mucosa (graded as pale, pink or dark pink), heart, lung and rumen auscultation, cough provocation (negative/positive), palpation of the elbow, carpal, stifle and tarsal joints and the measurement of rectal temperature.

Sampling of blood, milk and faeces, and postmortem examination

Blood for serology was sampled from the jugular vein in all goats over 12 months (49 goats). A bulk tank milk (BTM) sample for measuring somatic cell count (SCC) was collected. Blood and milk samples were brought to the National Veterinary Institute (SVA) for further analysis. The blood samples were centrifugated (3000 rpm for 10 minutes), and serum was aliquoted in new tubes and frozen at −20°C. Faecal samples (7) for parasitology were collected by the goat owner according to the farm's normal procedures (randomly chosen by the owner) and were submitted to Vidilab (Enköping). Four goats with severe respiratory distress (3) and/or lameness (1) were euthanased and transported to SVA for postmortem examination. Postmortem tissue samples from synovial membranes, lungs and mammarian and bronchial lymph nodes were collected and saved for further molecular analysis.

Laboratory analyses of blood, milk, faeces and at postmortem examination

An aliquot from the BTM was analysed with a DeLaval cell counter (DeLaval International). Serum samples were analysed using an indirect ELISA to detect antibodies for CAE using CAEV/MVV Total Ab ELISA (IDEXX) following the manufacturer's instructions. Faecal samples were analysed by a quantitative McMaster technique for the presence of *Trichostrongylidae*. They were also tested using the Baermann funnel technique for the presence of respiratory parasites, such as *Dictyocaulus filaria, Muellerius* sp., *P. rufescens* and *Cystocaulus*.

Reverse transcription polymerase chain reaction, sequencing and phylogenetic analysis

Nucleic acids from the tissue samples of the four autopsied goats were extracted using an IndiMag Pathogen kit (Indical Bioscience) and the extraction robot Maelstrom-9600 (TAN Bead). The elution volume was 100 μL. Before the reverse transcription polymerase chain reaction (RT-PCR) analysis, 5 μL of the nucleic acid extract was treated with 1 μL ezDNase enzyme and 1 μL of ezDNase buffer (Invitrogen) in 5 μL of nuclease-free water at +37°C for 5 minutes 30 seconds. Three separate RT-PCR assays were performed for the detection of the polymerase (pol) and envelope protein (env) genes, as well as the long terminal repeats (LTR) region, respectively, using the previously described primers.[5] The following reaction conditions were used in a total volume of 50 μL: 10 μL of heat-treated nucleic acid extract, 0.5 μM each of forward and reverse primers, 25 μL of 2 × Platinum SuperFi RT-PCR buffer (Invitrogen), 0.5 μL SuperScript IV RT mix (Invitrogen) and 9.5 μL of nuclease-free water. Thermocycling conditions for pol and LTR were 50°C for 10 minutes, 94°C for 2 minutes, followed by 40 cycles of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 1 minute, with a final extension at 72°C for 7 minutes. Thermocycling conditions for env were 50°C for 10 minutes, 98°C for 2 minutes, followed by 40 cycles of 98°C for 10 seconds, 55°C for 10 seconds and 72°C for 1.5 minutes, with a final extension at 72°C for 5 minutes. PCR products were visualised on an agarose gel, and bands with the expected size were considered positive. These were then cut, purified and then submitted for sequencing to Macrogen Europe. Achieved sequences were compared to previously published sequences by using the Basic Local Alignment Search tool for nucleotide sequences (BLASTn; NCBI). Reads were cleaned, forward and reverse reads aligned and a consensus per sample was made using UGENE.[15] A selection of SRLV sequences (pol) was retrieved from the GenBank (NCBI), representing group A–C viruses, to perform phylogenetic analyses. The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes–Cantor model,[16] and evolutionary analyses were conducted in MEGA X.[17]

Statistics

Fisher´s exact test, $p < 0.05$, was performed to find associations between age and BCS, age and respiratory rate, and goats chosen for extended physical examination and seropositivity for SRLV.

DIFFERENTIAL DIAGNOSIS

Although the herd had a known history of being positive for SRLV, lungworm was considered to be the main cause of the problems. Even after the first postmortem examinations, SRLV was not suspected, as the lungs were so heavily infested with *Muellerius* sp.

TABLE 1 Postmortem examination and PCR results for four goats with SRLV symptoms.

Clinical history			Postmortem diagnosis				SRLV positive (RT-PCR)			
Goat	Age (years)	Clinical signs	SRLV-associated pneumonia	Lungworms	SRLV-associated mastitis	SRLV-associated synovitis	Lungs	Udder	Synovial membrane	Bronchial lymph node
1	7	Dyspnoea	X	X			pol, env	n.d.	n.d.	n.d.
2	5	Dyspnoea	X	X			pol, env	n.d.	n.d.	n.d.
3	10	Lameness		X	X	X	pol	Neg	Neg	Neg
4	4	Dyspnoea	X	X			pol, env	pol	Neg	pol, env

Abbreviations: Env, envelope protein gene; Neg, negative; n.d., not done (no access of tissue); PCR, polymerase chain reaction; pol, polymerase gene; SRLV, small ruminant lentivirus.

TREATMENT

The goats were dewormed with ivermectin and treated with NSAIDs and antibiotics, with little to no effect, according to the owner.

OUTCOME AND FOLLOW-UP

Physical examinations performed by the authors

Three goats had a mildly affected general appearance. Three goats had a few mildly enlarged lymph nodes. Four goats had a BCS below 2. The mean BCS in the herd was 2.6. There was no significant association between age and BCS. No goats had hard udders indicative of SRLV mastitis. The mean CMT in the herd was 1.96. The median respiratory rate was 24 per minute (range: 18–80). There was no association between age and respiratory rate. Two goats were lame. One of them was culled and submitted for postmortem examination. There were no neurological signs in the herd. Among the goats where a physical clinical examination was performed ($n = 9$): all had normal-coloured mouth mucosa; three goats had a heart rate greater than 90 (reference range: 70–90); no goats had heart murmurs or arrhythmias; one goat had dyspnoea, with increased respiratory rate and increased respiratory sounds on both sides at auscultation; one goat had mildly decreased rumen frequency; three goats had a rectal temperature greater than 39.5°C (reference range: 38.5°C–39.5°C); all goats had a negative cough provoking test; and one goat was lame with a swollen and sore hip joint. There was no significant association between the goats chosen for extended physical examination and seropositivity for SRLV.

Laboratory analyses of blood, milk and faeces

Thirty-seven of the 49 goats (76%) tested positive for antibodies to SRLV. The BTMSCC was 1,011,000 cells/mL. Faecal samples from seven goats (age: 2–10 years) showed the presence of *Trichostrongylidae*, *Chabertia/Oesophagostomum, Muellerius* sp. and *P. rufescens*.

Postmortem examinations

Four goats had postmortem examinations in 2020 (see Table 1 for more details). Two of them, 7 and 5 years old, respectively, had signs of dyspnoea. The postmortem examination revealed both chronic interstitial pneumonia, consistent with SRLV infection, and granulomatous pneumonia, caused by an infestation of lungworms. The elder goat was known to be seropositive for SRLV, because it was tested in 2018. Samples from lung tissue were tested for *Mycoplasma* spp. genetic material using PCR, and cultured aerobically for bacteria from both goats with negative results. The third goat, 10 years old, was euthanased due to progressive lameness. It had chronic arthritis, with lymphoplasmacytic synovitis in the right knee joint, and lymphoplasmacytic mastitis, both findings considered to be consistent with SRLV infection. It also had lung lesions, which were consistent with lungworm-associated pneumonia. The fourth goat, 4 years old, was similar to the first two goats. It had a clinical history of dyspnoea and showed pathological lesions consistent with both SRLV infection and infestation of lungworms. PCR analyses for SRLV were performed from synovial membranes, lung tissue, udder and bronchial lymph nodes; these are described below.

Molecular diagnostics and phylogenetic analysis

RT-PCR detected SRLV pol and LTR in the lungs of all four goats, whereas env was detected in lung tissues of three of the goats. Synovial membrane, udder and bronchial lymph node from at least one of the goats were also RT-PCR positive. Sequencing confirmed SRLV pol in two goats, env in one goat and LTR in two goats. Phylogenetic analysis revealed that the SRLV isolate (GenBank accession number OQ858252) in the studied herd clustered with other group C viruses (Figure 1), previously detected in Norwegian sheep and goat herds.

DISCUSSION

Approximately 76% of the tested animals were seropositive for SRLV, indicative of a high within-herd prevalence. The most prominent signs of disease that might relate to infection with SRLV were respiratory signs and chronic interstitial pneumonia. Single findings of arthritis and mastitis could also be related to infection. All animals with chronic interstitial pneumonia showed evidence that, together with results from the analysis of faeces, indicated the presence of the lungworm *Muellerius capillaris*. This was considered to contribute to the respiratory disease and illustrates the importance of considering both diseases when examining a goat with respiratory disease. The cell count in bulk milk was high and most likely related to the high SRLV seroprevalence within the herd. Moreover, SRLV was successfully detected in lung,

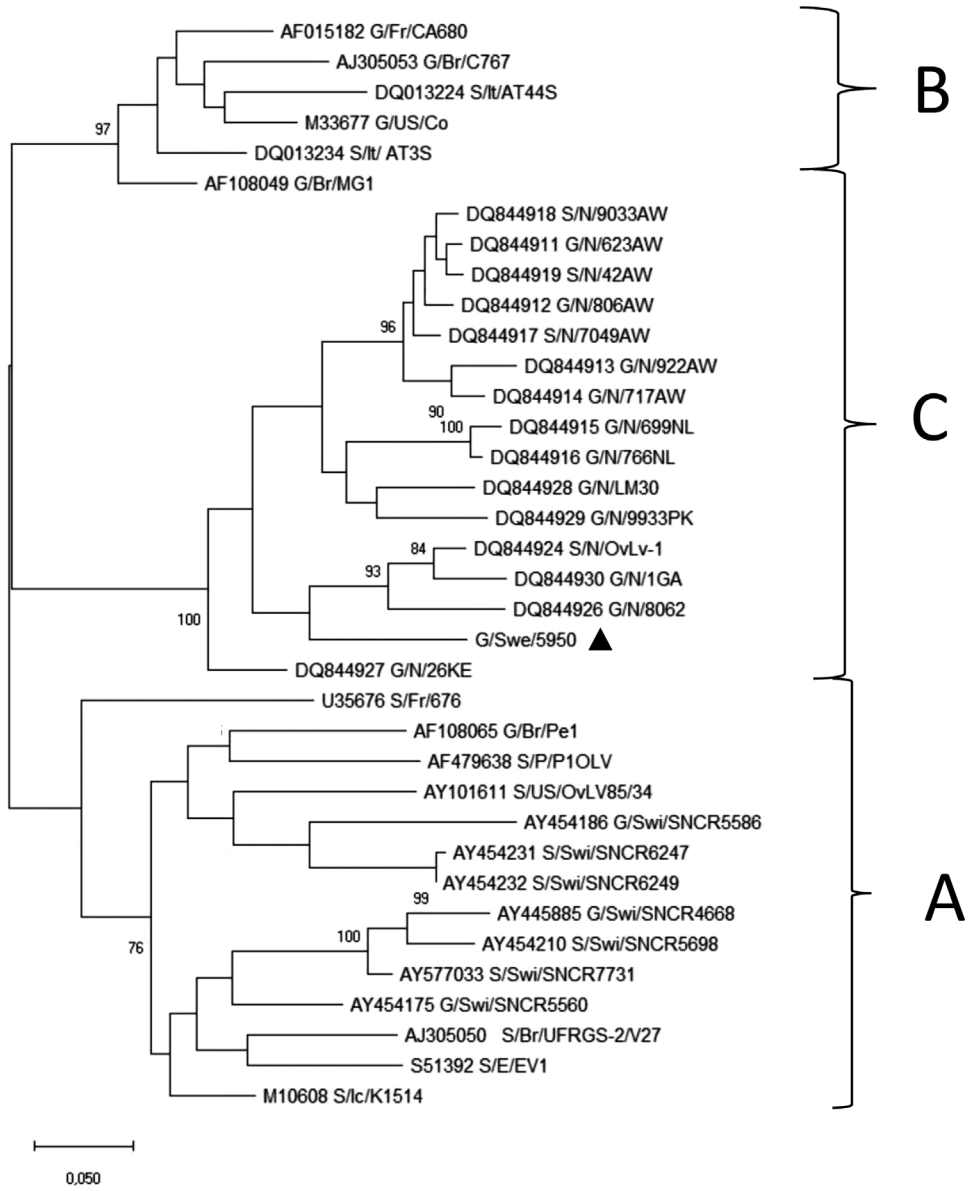

FIGURE 1 Phylogenetic relationship between the small ruminant lentivirus (SRLV) identified in the herd of this study compared to a selection of previously sequenced SRLVs of group A–C, based on partial polymerase gene sequences (405 nucleotides). The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes–Cantor model.[16] The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Bootstrap values below 75% are omitted. The analysis involved 36 nucleotide sequences. There was a total of 405 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.[17] Sequences are identified by GenBank accession number, if it was from a sheep (S) or goat (G), country code (Fr = France, Br = Brazil, It = Italy, US = United States of America, N = Norway, Swe = Sweden, P = Portugal, Swi = Switzerland, E = England, Ic = Iceland) and isolate name. The triangle indicates the isolate from this study (GenBank accession number OQ858252).

bronchial lymph node, synovial membrane and udder tissues. The phylogenetic analysis of the obtained sequences revealed the highest similarity with SRLVs from group C, which consists of viruses previously found in Norwegian goats and sheep.[5]

In a similar study from a dairy goat herd in Korea, the main clinical sign was arthritis,[18] which is considered as the most common clinical manifestation of SRLV in goats. In a case report from the United States, the author describes a concurrent infection with SRLV and lungworm.[19] In yet another study, more than half of the autopsied goats from a Polish SRLV type A-positive dairy goat herd had histological lesions of chronic interstitial pneumonia.[20] Contrary to our case, these goats exhibited no gross lesions, and the main clinical sign in the herd was arthritis.

Further research within this area could possibly explain a connection between viruses in group C and respiratory signs. The study also concludes that the effect of SRLV in Swedish goat herds can be severely detrimental and that an effective control programme is therefore much needed. Although lungworm infestation probably worsened the clinical signs, we still believe SRLV was the main underlying cause of respiratory signs in the herd. Our experience is that lungworm is a common finding in Swedish goat herds, often without any clinical signs of disease. Still, it is important to implement good parasite-mitigation strategies in all goat herds, and perhaps even more in herds with known SRLV infection. The interplay between lungworms and SRLV needs to be further explored before we can give any final recommendations. Nevertheless, this case report stresses the importance

of good biosecurity, where this herd serves as an example on how a disease can progress if no actions are undertaken. This herd started with goats with an unknown SRLV status and continued to buy bucks without proper quarantine routines.

AUTHOR CONTRIBUTIONS

Ylva Persson, Emelie Hedlund Salenstedt and Jonas Johansson Wensman conceived and designed the project. Emelie Hedlund Salenstedt and Ellen Andersson acquired the data. Ylva Persson, Emelie Hedlund Salenstedt, Ellen Andersson and Jonas Johansson Wensman analysed and interpreted the data and wrote the paper.

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CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest.

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ETHICS STATEMENT

The animal study protocol was approved by the Swedish Ethical Committee on Animal Research (Uppsala djurförsöksetiska nämnd); approval number C 148/13 and 5.8.18-15533/2018.

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