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Occurrence of CAE and CLA in Swedish Dairy Goats and Comparison of Serum and Milk as Sampling Material

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Abstract: Caprine arthritis encephalitis (CAE) and caseous lymphadenitis (CLA) are two infectious diseases affecting goat welfare and production throughout the world. There are no current data regarding their prevalence in Sweden, and the aim of this pilot study was therefore to estimate the occurrence in Swedish milk-producing goats, but also to assess the agreement between milk and sera as sample material for diagnosis and to investigate the association between the somatic cell count (SCC) in bulk milk and the occurrence of CAE and CLA. Serum, individual milk, and bulk-tank milk samples were collected from 214 dairy goats in 10 herds. All samples were analysed by ELISA to detect antibodies for CAE and CLA, and 14.6% of the goats were seropositive for CAE, whereas 19.3% of the goats were seropositive for CLA. The agreement between individual milk and serum samples was over 90% for both diseases and individual milk samples can therefore be considered as an alternative material for analysis in a future eradication programme. Based on the limited number of samples, there was also a significant correlation between bulk-milk test results and within-herd seroprevalence for both CAE and CLA. The SCC in bulk-milk samples was measured using a DeLaval cell counter. The medium SCC was 639,000 cells/mL and no association between SCC and CAE or CLA could be found. The results indicate that CAE and CLA are two common diseases in Swedish goat herds, but further studies based on a larger number of herds are needed to draw conclusions about the national prevalence. The results also indicate that milk can be used as a more cost-effective sampling media for diagnosing CAE and CLA compared to serology, which is the standard procedure today. Hopefully, the results can support the establishment of a successful programme to control the diseases, with the ambition to eradicate CAE and CLA in Sweden.

Keywords: small ruminant lentivirus; abscesses; pseudotuberculosis

1. Introduction

Caprine arthritis encephalitis (CAE) and caseous lymphadenitis (CLA) are two chronic, contagious infectious diseases affecting goats. The diseases cause great economic losses due to their association with lower milk production, weight loss and increased mortality, the latter both in adults and in kids [1,2]. The two diseases also have implications on animal welfare and livestock trade, and CLA also has a zoonotic potential [2].

Caprine arthritis-encephalitis virus (CAEV) belongs to the small ruminant lentiviruses (SRLVs) in the *Retroviridae* family, and the disease 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 one year

of age [3]. In Sweden, subclinical diseases are probably the most common [4]. Usually, only a few of the infected goats in a herd display clinical signs, even though many can be infected. Caprine arthritis encephalitis causes a slow infection, and the incubation time can be months to years [5]. 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 [1].

Caseous lymphadenitis is caused by the bacterium *Corynebacterium pseudotuberculosis* and leads to the development of abscesses in regional lymph nodes, in goats predominantly in the head region [3]. The disease is also known as pseudotuberculosis. The entry points of the infection are skin and mucosal membranes, mainly when these have been damaged [3,6]. Bacteria are spread from the infection site to regional internal and external lymph nodes, where abscesses are formed. When abscesses are formed in lymph nodes in the udder, they can result in mastitis [7]. The disease is mainly spread between animals in a herd when subcutaneous abscesses disrupt and the content contaminates the environment. Direct transmission between animals or indirect transmission for example from equipment occurs.

The two diseases are widely spread throughout the world [2,8], but there are no reliable data regarding their prevalence in Sweden. A control and eradication programme was successfully implemented in Norway [9], where most goat herds have been declared free from CAE and CLA. Prior to the control programme, 86% of Norwegian dairy goat herds had CAEV-positive animals and 42% of the animals tested were seropositive [10], and CLA was found in 53% of the herds in northern Norway [11].

Caprine arthritis encephalitis is a notifiable disease in Sweden (SJVFS 2013:23). A voluntary control programme for CAE (SJVFS 2015:17) was launched in Sweden in 1999 and about 12% of the Swedish goats are enrolled [4]. The purpose of the control programme is to detect and eradicate CAE from Swedish goat herds and to prevent introduction into free herds. The programme is based on serological examination of blood samples for antibodies to CAEV with an ELISA test (CAEV/MVV Total Ab ELISA IDEXX). For a more cost-effective programme, dairy herds would benefit from bulk-tank milk (BTM) sampling, which is not included in the Swedish control programme. There is currently no control programme for CLA in Sweden. Norway has, after a successful programme (Healthier goats) [9], declared most goat herds free from CLA and CAE, showing that it is possible to eradicate these diseases. In this programme, surveillance was based on BTM ELISA [12].

The prevalence of CAE and CLA in Swedish goats is uncertain. The aim of this study was therefore to estimate the occurrence of CAE and CLA in Swedish milk producing goats and to assess the agreement between milk and sera as sample material. Hopefully, the results can support the establishment of a cost-effective programme to control both these diseases, with the ambition to eradicate CAE and CLA in Sweden.

2. Materials and Methods

2.1. Study Design

Serum, individual milk and BTM samples were collected from 214 milking goats in 10 conveniently chosen dairy herds from northern and central Sweden in 2018.

At least 20 lactating goats of different ages in each herd were sampled and palpated for clinical abscesses in external regional lymph nodes (*ln submandibularis*, *ln parotis*, *ln subiliacus* and *ln popliteus*). A sample size of ≥17 goats was estimated to be required to enable detection of infection at the level of ≥15% prevalence within a herd with <100 goats [13]. Number of lactating goats, breed, presence of abscesses, CAE-status if affiliated to the CAE-programme (SJVFS 2015:17), last date for buying live animals and routines for quarantine were recorded. All goats were sampled between July and September which is mid-lactation for most herds, and late lactation for some.

2.2. Sampling Procedure

The goats were sampled adjacent to milking when goats were fed with concentrates. Blood was sampled from the jugular vein and milk was sampled from one udder half. From each herd, a BTM sample was collected.

The blood samples were centrifuged (3000 rpm in 10 min) and serum was aliquoted in new tubes and frozen in −20 °C. The BTM and individual milk samples intended for CAE and CLA analyses were frozen directly in their original test tubes. An aliquot from each BTM was preserved in bronopol for later analyses of the somatic cell count (SCC).

2.3. Analyses of Somatic Cell Count in Bulk Milk

The bronopol-preserved BTM samples were either sent by post or brought to the National Veterinary Institute (SVA) by the sampler for analysis of the SCC with a DeLaval cell counter (DCC, DeLaval International AB, Tumba, Sweden).

2.4. Serological Analyses of Serum and Blood

All serum and milk samples were analysed by ELISA to detect antibodies for CAE and CLA using ELITEST MVV/CAEV (Hyphen BioMed) and ELITEST CLA (Hyphen BioMed, Neuville-sur-Oise, France), respectively. All analyses were performed according to the manufacturer's instructions, with minor adjustments according to a previous study [12]. In brief, for the CAE ELISA, serum was pre-diluted to 1:100, individual milk to 1:50, and BTM to 1:5. For the CLA ELISA, serum was pre-diluted to 1:50, individual milk to 1:20, and BTM to 1:5 before addition to the ELISA plate. Cut-off values were calculated according to the manufacturer's instructions.

2.5. Statistics

ELISA absorbance values from analysis of serum samples and individual milk samples were compared in two-way scatter plots. Pairwise correlation between test results from analysis of serum and milk was estimated by calculating Pearson's correlation coefficient. Dichotomized test results (negative or positive) based on calculated cut-off values were also compared and the proportion of samples where results from analysis of serum and milk agreed was calculated. Testing of serum samples was used as the reference test for calculating the relative sensitivity and specificity of testing of individual milk samples. The number and proportion of test-positive individuals among tested animals were summarised by herd and the correlation to the ELISA bulk-milk result (absorbance) was compared in two-way scatter plots. Data management and analysis were done using Microsoft 365 Excel (Microsoft corporation) and Stata Statistical Software: Release 15. (StataCorp. 2017. College Station, TX, USA: StataCorp LLC.).

3. Results

3.1. Descriptive Statistics

The mean size of the 10 sampled herds was 83.8 lactating goats, ranging from 42 to 165 lactating goats (Table 1). Of the 214 sampled goats, 83 were 1–3 years old and 131 were 4–11 years old. Almost all goats were of Scandinavian landrace, and a few of them cross breeds with Jämtget. All herds were commercial, intensive dairy herds, and all goats were machine milked. One goat was only blood sampled and one was only milk sampled. Therefore, the total number of samples was 213 blood samples and 213 milk samples, and 212 samples with both blood and milk. Six of the herds in the study were affiliated to the Swedish eradication programme for CAE and therefore, previously declared free from disease. Four herds were not affiliated to the programme; hence, their CAE status was unknown. All herds had bought animals in the last three years. None of them had optimal quarantine routines, according to www.sva.se (12 September 2021). The mean SCC was 639,000 cells per mL. See Table 1 for a description of herd data and results.

Table 1. Herd characteristics and test results of the 10 goat herds included in the study to estimate the occurrence of CAE and CLA (Sweden, 2018).

Herd	Herd Size [1]	Samples Size	CAE Status [2]	Presence of Abscesses	SCC [3]	Bulk-Milk CAE ELISA Absorbance	Result [4]	Bulk-Milk CLA ELISA Absorbance	Result [5]
A	42	21	free	no	341	0.204	pos	0.318	pos
B	53	21	unknown	yes	1026	2.282	pos	0.225	neg
C	55	21	free	no	428	0.083	neg	0.254	pos
D	65	21	unknown	no	1025	2.423	pos	0.389	pos
E	67	21	free	no	475	0.167	neg	0.329	pos
F	75	22	unknown	yes	951	0.151	neg	1.527	pos
G	87	20	free	no	189	0.114	neg	0.256	pos
H	89	22	free	no	881	0.241	pos	0.330	pos
I	140	23	free	yes	304	0.108	neg	1.157	pos
J	165	21	unknown	yes	768	0.983	pos	0.228	neg

[1] Number of lactating goats. [2] Based on affiliation to the CAE control programme (see Section 2.1). [3] Somatic Cell Count = number of cells per μL. [4] Positive (pos) or negative (neg) based on cut-off = 0.186. [5] Positive (pos) or negative (neg) based on cut-off = 0.236.

3.2. Test Results for Individual Animals

The ELISA test results from the analysis of serum and milk from individual goats are shown in Figures 1 and 2. The cut-off values calculated for the results from the CAE ELISA varied between plates and were 0.1565, 0.1920, and 0.2041 for analysis of serum, and 0.1855, 0.2484, and 0.2854 for analysis of milk. For the CLA ELISA, the cut-off values also varied between plates and were 0.2222, 0.2488, 0.4183, and 0.4735 for analysis of serum, and 0.2363, 0.2774, and 0.2819 for analysis of milk. The number of animals seropositive in the CAE ELISA was 31 (14.6%), and 28 (13.2%) goats were positive in milk. In the CLA ELISA, 41 animals (19.3%) were positive in serum and 36 (17.0%) in milk. The ELISA absorbance values from the analysis of serum samples versus individual milk samples are presented in Figure 1 (CAE) and Figure 2 (CLA). The correlation between absorbance values based on the analysis of serum and milk and seroprevalence was 0.976 ($p < 0.001$) for CAE and 0.829 ($p < 0.001$) for CLA. The number of test-positive animals based on the results from the analysis of serum and milk is presented in Table 2. The relative sensitivity and specificity of testing milk was 90.3% (95% confidence interval: 74.2–98.0) and 100.0% (95% confidence interval: 98.0–100.0) for CAE, and 87.8% (95% confidence interval: 73.8–95.9) and 93.0% (95% confidence interval: 88.1–96.3) for CLA.
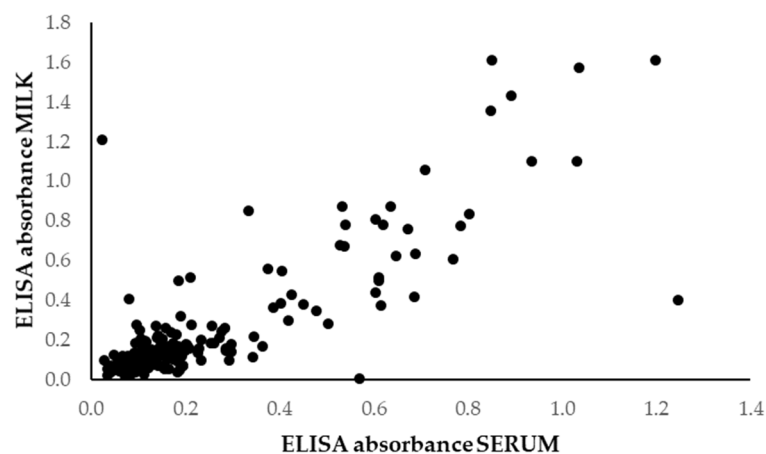
Figure 1. CAE-ELISA absorbance values in serum (*x*-axis) vs. milk (*y*-axis) in 212 lactating goats included in the study to estimate the occurrence of CAE and CLA (Sweden, 2018).
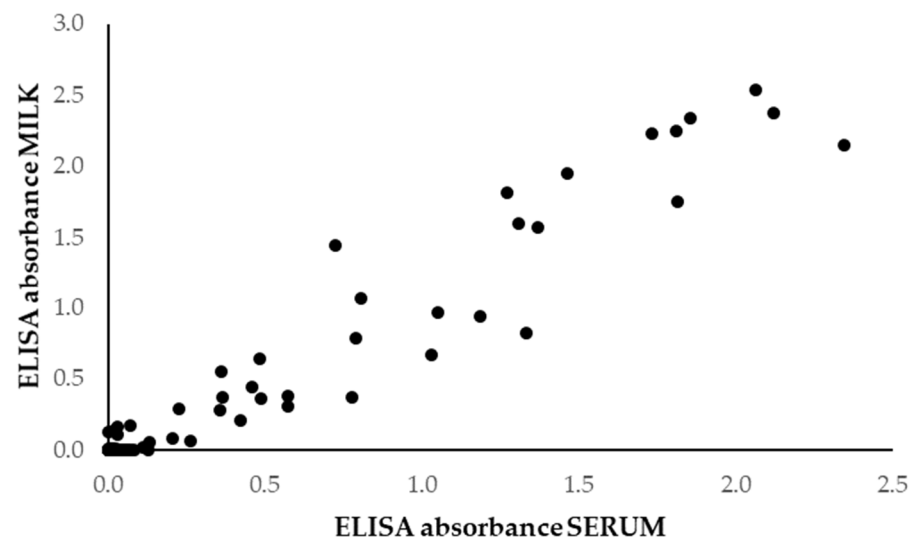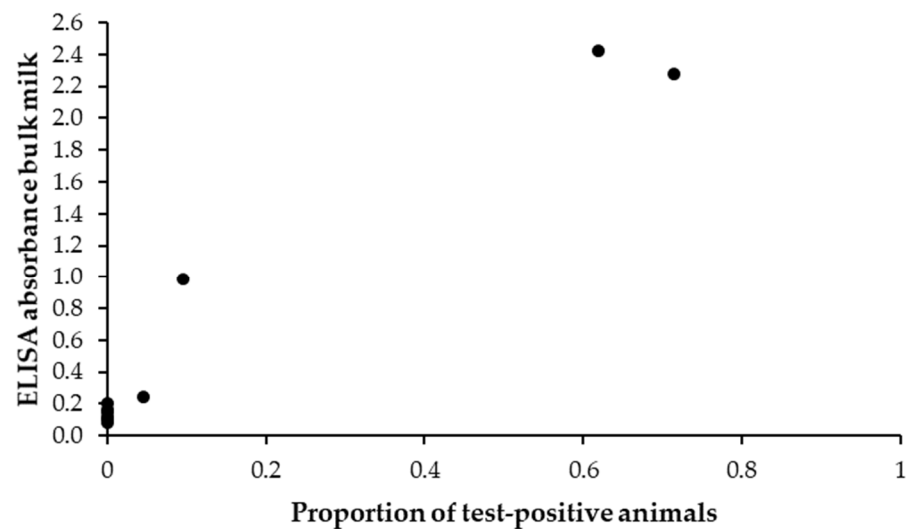
Figure 2. CLA-ELISA absorbance values in serum (*x*-axis) vs. milk (*y*-axis) in 212 lactating goats included in the study to estimate the occurrence of CAE and CLA (Sweden, 2018).

Table 2. CAE and CLA ELISA test results in serum and milk from 212 lactating goats included in the study to estimate the occurrence of CAE and CLA (Sweden, 2018).
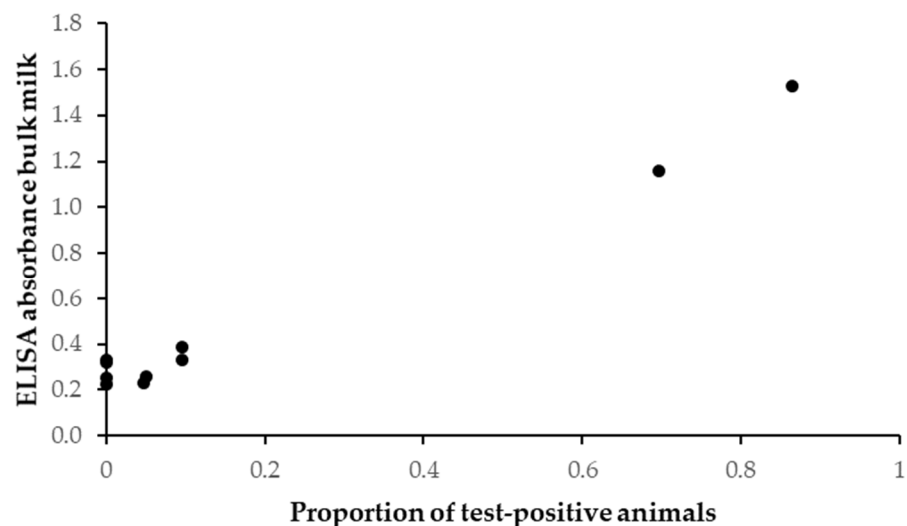
		Milk				
Disease	**Serum**	**Neg**		**Pos**		**Total**
		n	%	*n*	%	
CAE	Neg	181	100.0	0	0.0	181
	Pos	3	9.7	28	90.3	31
	Total	184	86.8	28	13.2	212
CLA	Neg	159	93.0	12	7.0	171
	Pos	5	12.2	36	87.8	41
	Total	164	77.4	48	22.6	212

3.3. Bulk-Milk Test Results

The number of test-positive herds in the bulk-milk CAE ELISA at the calculated cut-off (absorbance ≥ 0.186) was 5. In the CLA ELISA, the number of test-positive herds at the calculated cut-off (absorbance ≥ 0.236) was 8. Bulk-milk ELISA absorbance values of herds relative to their within-herd seroprevalence are presented in Figure 3 (CAE) and Figure 4 (CLA). Seroprevalence of the herd was used as a reference test (absence of seropositive animals corresponding to a negative herd test and presence of seropositive animals corresponding to a positive herd test) and compared to bulk-milk absorbance values to explore the potential diagnostic sensitivity and specificity of the bulk-milk ELISAs. For CAE, all four herds with seropositive animals tested positive in bulk milk, whereas five out of six seronegative herds tested negative. The herd seroprevalence of the bulk-milk-negative herd was 4.6%, corresponding to one individual goat. The serum sample of this goat was re-analysed in another ELISA (IDEXX CAEV/MVV Total Ab Test, Hoofddorp, The Netherlands), which resulted in a negative result. For CLA, five out of six seropositive herds (83.3%) tested positive in bulk milk, whereas just one out of four seronegative herds tested negative in bulk milk. The herd seroprevalence of the seropositive but bulk milk negative herd was 4.8%, whereas the other sero- and bulk-milk-positive herds had seroprevalences ranging from 5.0% to 86.4%.

Figure 3. Absorbance value of bulk milk analysed in CAE ELISA compared to proportion of test-positive goats (serum) in the herd in 10 herds and 213 lactating goats included in the study to estimate the occurrence of CAE and CLA (Sweden, 2018).

Figure 4. Absorbance value of bulk milk analysed in CLA ELISA compared to proportion of test-positive goats (serum) in the herd in 10 herds and 213 lactating goats included in the study to estimate the occurrence of CAE and CLA (Sweden, 2018).

3.4. Association between CAE/CLA and Herd Variables

Clinical abscesses were found in four of the ten herds (40%), and two of these had seropositive goats and tested positive for CLA in the bulk-milk ELISA (Herds F and I; Table 1). No statistically significant association between CLA positivity in bulk milk and clinical abscesses was found ($p = 0.133$). Positive bulk milk and seropositive goats were found in three of the four herds with previously unknown status for CAE, whereas five out of six CAE-free herds were seronegative and bulk-milk-negative. One CAE-free herd was bulk-milk-positive with a single seropositive goat, later declared negative in a second ELISA. No statistically significant association between CAE status according to the Swedish CAE-programme and bulk-milk CAE ELISA results was found ($p = 0.524$). Moreover, no association between CAE nor CLA and SCC in bulk milk was found.

4. Discussion

This is the first scientific report of CAE and CLA in Swedish goats. The presence of antibodies for CAE and CLA was investigated in 10 dairy goat herds, using individual

serum and milk samples, and bulk milk. The results indicate that both CAE and CLA are common among Swedish dairy goats and that milk is a good diagnostic media for detecting antibodies for CAE and CLA.

Seropositivity for CAE was found in 14.6% of the individual goats (based on serum samples) and in 3 out of 10 herds, but in 75% of the herds not affiliated to the control programme. This is higher than previously estimated figures of a herd prevalence of 15% in non-affiliated herds [14] but lower than the previous prevalence study in Sweden where all herds were infected [15] and earlier reported prevalence from Norway before the eradication campaign, where 86% of the herds and 42% of the goats had antibodies for CAE [3]. Almost 90% of the bulk-milk samples within the Norwegian eradication campaign were positive for antibodies for CAE [12]. Historically, Sweden was, together with Norway, Canada, France, Switzerland, and USA, considered one of the countries with highest CAE prevalence due to an intensive goat dairy production. Previously, all these countries had a seroprevalence of over 65%, but thanks to control programmes, most of the countries have been able to decrease the CAE burden substantially [3].

In this study, there was a high correlation between serum and individual milk samples for detection of antibodies for CAE. Only three goats were misclassified and tested negative for antibodies using individual milk samples while being positive based on testing of serum. Moreover, all herds with seropositive goats but one tested positive when using bulk milk, and the bulk-milk-negative herd had a herd seroprevalence of 4.6%, corresponding to one tested goat. The serum sample from this goat was re-analysed in another ELISA with a negative result. Moreover, there was a clear difference in the absorbance values between negative herds and herds with seropositive animals in the bulk-milk ELISA, even in a herd where only 9.5% of the goats tested positive. The number of herds included is, however, limited, and a more comprehensive study is needed. Nevertheless, bulk-milk testing to identify CAE-positive herds followed by testing of individual milk to identify CAE-positive goats, could be a cost-efficient method to use in the ongoing voluntary CAE control programme.

Almost one-fifth (19.3%) of the goats included in this study, from 6 out of 10 herds, were seropositive for CLA, which is in line with studies from Norway, where 53% of the herds were seropositive for CLA [11]. This was before Norway started their eradication campaign in 2002 [12]. Four of the ten herds in this study had goats with clinical abscesses, but there was no association between CLA seropositive bulk milk and findings of abscesses. Not all goats per herd were clinically examined, so there could have been herds where abscesses remained undetected. There could also have been other reasons than CLA for clinical abscesses.

For CLA, the correlation between antibody-positive serum and individual milk samples was acceptable, although not as good as compared to CAE; 12.2% of the goats that tested positive in serum were negative in milk. Bulk-milk analysis identified 83.3% of the seropositive herds, whereas only one of four seronegative herds was correctly classified. Two herds with a high proportion of seropositive goats (69.6% and 86.4%, respectively) had clearly higher absorbance values than seronegative herds or herds with a low proportion of seropositive goats (5.0–9.5%). Taken together, there might be a need to further evaluate cut-off values in a larger set of serum and milk samples, and, if needed, adjust them, before the test scheme (bulk milk followed by individual milk samples) is implemented on a larger scale, such as in a control programme.

The results from the SCC analyses were inconclusive and no clear association between SCC and CAE-antibodies could be found. This was not expected since there is a well-established relationship between a high SCC and CAE infection [16]. The two herds with the highest CAE prevalence also had the highest SCC, but for the rest of the herds, no such relation could be seen. The limited number of herds is possibly one explanation for our discrepant result. Nevertheless, SCC as an indicator of milk quality is important to measure when studying CAE and CLA. In the Norwegian eradication campaign, the SCC decreased and milk yield increased in herds where CAE and CLA was eradicated [12,17].

There is a lack of reliable and up-to-date data on the number of dairy goat herds in Sweden, but in the most recent data from the Swedish Board of Agriculture [18], the number of farms that milked their goats was 230, which is less than 10% of the total goat population in Sweden. Furthermore, at least half of the farms are small scale and use the milk mainly for household purposes. The number of commercial dairy farms can therefore be estimated to be 115 with a total of 3340 goats [18]. Therefore, a limitation of our study is that it only covers a minor proportion of the dairy goat herds in Sweden. Further studies, including a larger randomly selected sample size, are therefore needed to estimate the seroprevalence of CAE and CLA in the Swedish goat population.

5. Conclusions

Antibodies for CAE and CLA were detected in the studied goat herds, indicating that both diseases are commonly present in Swedish dairy goat herds. Agreements between individual milk and serum samples were good for both diseases and individual milk samples can therefore be considered as an alternative material for analysis in a future eradication programme. For a CAE control programme, bulk-milk samples to identify positive herds followed by individual milk samples to identify positive animals could be a cost-efficient, non-invasive, yet reliable sampling scheme.

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Informed Consent Statement: Not applicable.

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Conflicts of Interest: The authors declare no conflict of interest.

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