Variations in the CCR5-gene and its effect on CAE, in goat

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Abstract

Caprine Arthritis Encephalitis (CAE) is a disease negatively affecting goats, around the world. A mutation on the CCR5-gene has showed an increase in viral load.

CAE is a lentivirus that affects the monocytes/macrophages. It is a disease similar to HIV in humans and the animal can be a carrier for months to years before symptoms start to show. The symptoms can be severe, arthritis being the most common for adult goats.

This study aimed to find variations in the CCR5-gene that can affect the onset of disease, and to complement earlier studies. It also includes more different breeds, than previously studied. It was also to study the possibility of breeding as a control measure, for the disease.

Blood samples and nose swabs from 127 goats were sequenced and analyzed. Four Swedish breeds were included; Swedish lantras, Göingegoat, Lappgoat and Jämtgoat.

Two mutations were found, one that has previously been shown to possibly have an impact on pro-viral load. There were variations found, in the genotypes, between and within some of the breeds, especially for the breeds Jämtgoat and Göingegoat. There were differences found in genotype- and allele frequencies for the different breeds. This is the second only study on this subject, on Swedish goat breeds.

No significant differences could be found in the correlation between genotype and prevalence of CAE, in the Swedish breeds, though the sample size of sero-positive animals was small.

The genotype- and allele frequencies varied significantly between the breeds, making the possibility for breeding different, for each. Nothing conclusive could be said, in this study, about the possibility of breeding to contain CAE, but the frequencies could be a basis for further studies into the subject. More studies, with more sero-positive animals and increased sample size, need to be conducted.

Keywords: CCR5, CAE, Caprine arthritis encephalitis, Goat, SRLV, Small ruminant lentiviruses

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Abbreviations

SRLV	Small Ruminant Lentiviruses
CAE	Caprine Arthritis Encephalitis
MVV	Maedi/Visna virus
LD	Linkage disequilibrium
HIV	Human immunodeficiency virus
FIV	Feline immunodeficiency virus
BIV	Bovine immunodeficiency virus
HW-equilibrium	Hardy Weinberg equilibrium

1. Introduction

Caprine Arthritis Encephalitis, or CAE, is a disease in goats that can negatively affect a whole farm. Diseased animals may have a negative influence on milk production (Martínez-Navalón *et al.*, 2013 and Tariba *et al.*, 2017). The disease is contagious and the owners have to cull all the infected animals to get rid of the disease.

Cases of CAE are compulsory to report, in Sweden (to the Swedish Board of Agriculture). There are voluntary control programs in place around the world, including Sweden (Gård och Djurhälsan). Farms with small herds or indigenous breeds (allmogegetter in Sweden) may be wary of enrolling into the control program for fear of losing all their animals and genetic material, and because it can be too costly.

CAE is a lentivirus, a sub-family of the retrovirus family. Together with MVV (Maedi-Visna virus) in sheep they form the group SRLV (small ruminant lentiviruses) (Leroux *et al.*, 1995; Blacklaws, 2012). CAE usually affects the brain/nervous system in young animals (encephalitis, though rare) and, most commonly, the joints of adults (arthritis) (SVA, 2021; Patel *et al.*, 2012). Other symptoms include pneumonia, mastitis and cachexia (emaciation).

CAE and MVV strains have been shown to jump species between sheep and goat (Shah *et al.*, 2004, Gjerset *et al.*, 2007). SRLVs are transmitted primarily via colostrum and milk to the young (Blacklaws *et al.*, 2004). It is also transmitted horizontally between individuals. It is thought to be mainly transmitted through aerosols, in those cases, but the exact transmission route is not established. An infected animal can be asymptomatic for months to years after infection, but can still transmit the disease, which makes it more difficult to contain (Blacklaws *et al.*, 2004; Rowe & East, 1997).

There is no effective treatment or vaccine for the disease, with some vaccines exacerbating the clinical symptoms (Patel *et al.*, 2012). Control programs for SRLVs most commonly use the method of first separating the young directly after birth and feeding them treated colostrum/milk and keeping them separate from infected animals (Blacklaws *et al.*, 2004; Reina *et al.*, 2009). All animals will be regularly tested and culled if having a positive result.

Another method is to test and separate animals into different herds and care for them separately, according to generation. The young of the infected animals will

be immediately separated after birth. The infected animals will then be phased out (Reina *et al.,* 2009; Konishi *et al.,* 2011).

The gene looked at in this study is a variant of the CCR5 gene (C C chemokine receptor, type 5) and has been shown to increase the susceptibility to-, and expression of CAE, in the animal (Colussi *et al.,* 2019). A variant of the gene has also been shown to play a part in resistance to infection of HIV, in humans (Kaslow *et al.,* 2005), and SRLV in sheep (White *et al.,* 2009).

This study is a continuation of previous (student) work in which the resistance for CAE in the CCR5-gene, in goat, was studied (Gunnarsson, 2020).

The aim of this study is to gather more data to validate previous results about the variation in the CCR5 gene and its effect on CAE. Also to include more breeds for examining in-breed and cross-breed variation/spread, of the gene-variant.

Breeding may be an addition to the programs existing today in containing the spread of the virus.

2. Literature review

2.1. Small Ruminant lentiviruses

Small ruminant lentiviruses include the diseases CAE and MVV. These were thought to be separate and species-specific. This has been rebuked and it has been shown that strains of the virus can jump species (Shah *et al.*, 2004; Gjerset *et al.*, 2007; Patel *et al.*, 2012), thus both being reclassified into the group Small ruminant lentiviruses (SRLV).

There are two types of lentiviruses; one affecting both monocytes/macrophages and lymphocytes (as in BIV, HIV, FIV) while the other only affects the monocytes/macrophages (Patel *et al.*, 2012). The SRLV lentiviruses only affect the monocyte/macrophage linage (Blacklaws, 2012; Leroux *et al.*, 2010; Patel *et al.*, 2012). Thus these viruses are not immuno-suppressant, and the animal can still produce antibodies.

Virus replication is induced during monocyte differentiation, as has been shown in sheep in a study by Gendelman et al. (1986). The virus matures together with the monocytes, as they mature into macrophages. The virus shows the most expression at this maturation stage.

Symptoms include pneumonia, arthritis, encephalitis and cachexia (SVA, 2021; Patel *et al.*, 2012). Lesions will be formed in the affected tissue. The lungs and nervous system are mainly affected in MVV and the joints in CAE.

2.1.1. Caprine Arthritis Encephalitis

Caprine arthritis encephalitis (CAE) is a lentivirus, a genus of retroviruses, belonging to the same group as (among others) HIV in human, BIV in cattle and FIV in cats (Patel *et al.*, 2012). CAE mainly infect goats.

The virus does not act as an immunosuppressant like other lentiviruses (Leroux *et al.*, 2010), thus the infected animals still show an immunological response. This is because the CAE virus affects only the macrophage line and not the lymphocytes (see earlier paragraph on SRLV).

Clinical symptoms include arthrithis, encephalitis, mastitis and cachexia (SVA, 2021; Patel *et al.,* 2012). Arthritis is the most common symptom in adult goats while encephalitis is most common in young animals. Lesions form in the affected tissue.

Symptoms can take months to years to surface (SVA, 2021). However asymptomatic animals can still carry and transmit the disease.

2.2. Transmission

The main route of transmission is thought to be through colostrum and milk from infected animals (Blacklaws *et al.,* 2004; Gjerset *et al.,* 2007; Rowe & East, 1997; Shah *et al.,* 2004). The disease can also be transmitted horizontally, between animals, such as in stables were animals are kept near to each other. The exact transmission is not clear but it is thought to be via aerosols, especially from animals with SRLVs affecting the lungs. Other routes of transmission include contact between mother and young directly after birth, milking equipment and via handlers (Blacklaws *et al.,* 2004; Gjerset *et al.,* 2007; Rowe & East, 1997; Shah *et al.,* 2004).

Transmission via semen from affected animals is not thoroughly studied. Though not many studies have been made, one study by Ahmad et al. (2012) has shown evidence for vertical transmission via AI from infected bucks. They used semen infected in vitro and showed that all embryos collected were free from CAE pro-viral DNA and could be used for embryo transfer. So it seems like embryos might not be affected by the virus. However some of the does had pro-viral DNA in uterine smears/swabs, and could therefore potentially be infected from the semen.

Transmission directly from pastures has so far not been shown, though the virus can be transmitted horizontally, as described earlier.

Different strains of SRLVs have been shown to jump species (Shah *et al.,* 2004, Gjerset *et al.,* 2007), mainly between sheep and goat.

2.3. Milk production

Of the studies found, most show no significant impact from CAE on total milk production (Kaba *et al.,* 2012; Leitner *et al.,* 2010 and Nord & Ådnøy, 1997). Two studies by Tariba et al. (2017) and Martínez-Navalón et al. (2013) did however show signs of a negative impact. The study by Kaba et al. (2012), a

cohort study made over a 12-year period, did show a significant difference in percentage of fat and lactose, between sero-positive and sero-negative animals.

Nord & Ådnøy, (1997) used data from 1799 goats. They compared an ELISA test for CAE-antibodies with records of milk production.

The study by Leitner et al., (2010) monitored one flock of 248 animals, for three consecutive years. The results did show a significant difference on the milk yield for the first lactation but none on the second to fourth lactation.

Tariba et al. (2012) showed a significant difference in all parameters tested, including total milk yield and total amount of fat, protein and lactose. Blood- and milk samples were collected from 808 goats.

Martínez-Navalón et al. (2013) did a retrospective study, with data on milk production from 22 herds (number of animals not specified).

2.4. Treatment

There is no effective treatment or vaccine for SRLV today (Patel *et al.,* 2012). Attempts to develop vaccines have been made without good results, with some even worsening clinical symptoms.

The main way of controlling the disease is through continuous testing and culling of infected animals, as well as removing the young directly after birth to avoid transmission between mother and young (Reina *et al.,* 2009). The young are then given treated colostrum and milk.

Another method is phasing out the disease by separating the herd into two flocks; infected and non-infected (Konishi *et al.,* 2011; Reina *et al.,* 2009). These animals are then kept separate from each other, and are continuously tested. This requires more work but can be a good alternative for small herds, or if they want to keep genetic material (such as for indigenous breeds with small populations).

2.5. CCR5

The CCR5-gene is located on chromosome 22. The position investigated in this project is in the promoter region (Colussi *et al.,* 2019b). The promoter region is where gene transcription is initiated. The sequence studied is a part of the gene, position 779-1107.

CCR5 is an important co-receptor for macrophage-tropic viruses to be able to enter into the host cell (Colussi *et al.,* 2019)

The expression of the CCR5-gene has been shown to have an impact on the expression of the disease, and to aggravate clinical symptoms (Colussi *et al.*, 2019).

The earlier works by Colussi et al. (2019) and Gunnarson (2020) showed a mutation at loci g1059.T, that Colussi et al. (2019) found could affect the increase of viral load. The mutation displayed a genotype (T'T') that resulted in the increase. The same mutation has been explored in this study.

For HIV, in human, a deletion of the gene showed great resistance to infection (Kaslow *et al.*, 2005). The same has been seen for sheep (White *et al.*, 2009), were pro-viral levels for deleted homozygotes were significantly reduced.

3. Methods and materials

Samples from 127 goats of the Swedish breeds Swedish lantras (25 samples), Göingegoat (17), Lappgoat (7), Jämtgoat (50) and some unknown-/crossbreeds (28), were studied and analyzed. 72 samples were already sequenced before the study started while 55 (60 from the beginning) were sequenced during spring 2021. All samples were collected beforehand. The samples were collected via nose swabs and/or blood samples. They were then sequenced and analyzed for the previously published mutation g1059.T (Colussi *et al*, 2019; Gunnarsson, 2020).

The region of the part of the CCR5 gene sequenced was position 779-1107, in the promoter region of the Caprine reference sequence HQ650162.1 (Colussi *et al.,* 2019b).

The DNA-extraction, for the blood samples, was conducted according to the QIAprep ® Spin Miniprep Kit, Quick start protocol and QIAsymphony® DNA Handbook. The nose swabs had already been sequenced beforehand.

The blood samples were then the put through NanoDrop to measure the concentration and later aliquots were done and a working solution diluted to 4ng/µl.

PCR was then conducted following the BigDye® Direct Cycle Sequencing Kit protocol and sequencing by capillary electrophoresis (BigDye direct sequencing assay).

The sequences were read, as chromatograms, with the program FinchTV Version 1.4.0 (Geospiza 2008). Text files of the sequences were put through the web-program nucleotide BLAST (NCBI 2021), to compare them with each other and spot potential mutations. The results were compared to the chromatograms to confirm them.

Genotype- and allele frequencies were calculated for all the samples collectively and for the respective breeds.

$$Genotypefrequency = \frac{Amount\ of\ the\ genotype}{Total\ number\ of\ individuals}$$

$$Allelefrequency = \frac{Total\ amount\ of\ the\ allele}{Total\ number\ of\ individualsx2}$$

Linkage disequilibrium was calculated to confirm genetic variation in the samples for two found mutations. It was calculated for all samples and the

different breeds with the web-program GenePop (Rousset, 2008). P-values were calculated.

The genotypes, for both the mutations for the different breeds, were tested for Hardy Weinberg equilibrium. With the hypothesis; H_0= the respective breeds are in HW-equilibrium.

A Chi2-test was used to compare the correlations for the two mutations, in the whole sample set and between- and within the breeds. It was also used to calculate the correlation between genotype and instance of disease, for ten goats with confirmed CAE (sero-positive), from four farms that had at least one positive case of CAE. The Chi2 function in Excel and the Chi-square calculator from the web site Social Science Statistics was used for calculation.

4. Results

Two different mutations were found (see appendix 2), one of which was the g1059.T mutation mentioned earlier, that has been shown to have a correlation with increased pro-viral loads (Colussi *et al.*, 2019). This will further be named as "Mutation 1". The other mutation found will further be named as "Mutation 2". Table 1 shows the genotype frequencies for mutation 1 and 2 respectively. Table 2 shows the allele frequencies.

Table 1. Genotype frequencies for the two found mutations in the CCR5-gene. Separated for the different breeds.

Genotype/Breed	All samples	Swedish lantras	Göingegoat	Lappgoat	Jämtgoat	Unknown/Crossbreeds
Mutation 1						
T'T'	0.82	0.80	0.23	0.71	1.00	0.89
T'C'	0.16	0.20	0.59	0.29	0.00	0.11
C'C'	0.2	0.00	0.18	0.00	0.00	0.00
Mutation 2						
A'A	0.40	0.64	0.06	0.71	0.30	0.50
A'C'	0.42	0.36	0.53	0.29	0.42	0.43
C'C'	0.18	0.00	0.41	0.00	0.28	0.07

Table 2. Allele frequencies for the two mutations. Separated for the whole population and the different breeds.

Allele/Breed	All samples	Swedish lantras	Göingegoat	Lappgoat	Jämtgoat	Unknown /Crossbreeds
Mutation 1						
T’	0.90	0.90	0.53	0.86	1.00	0.95
C’	0.10	0.10	0.47	0.14	0.00	0.05
Mutation 2						
A’	0.61	0.82	0.32	0.86	0.51	0.71
C’	0.39	0.18	0.68	0.14	0.49	0.29

Jämtgoat was found to only exhibit the T’T’ genotype, for the first mutation, with an allele frequency of 100%. The genotypes were much more evenly spread for the second mutation, the same with the allele frequency (51% for A’ and 49% for C’).

Göingegoat had the highest frequency for the C’C’ genotype, of the first mutation. It was also the same for the second mutation. There were also many heterozygotes for both of the mutations.

Swedish lantras (and the Unknown group) had a high frequency of the T’T’ genotype.

Lappgoat had genotype frequencies of 71% for T’T and 29% for T’C for the first mutation and the same for A’A and A’C respectively, for the second mutation. If combined with the results from Gunnarsson (2020) the frequencies were 61% for T’T’, 39% for T’C, 61 % for A’A’ and 39% for A’C’ (from a total of 18 individuals).

The allele frequencies in Lappgoat were 86% for T’, 14% for C’ (of the first mutation), and 86% for A’ and 14% for C’ (of the second mutation). Combined with Gunnarsson (2020) they were 81% for T’, 19% for C’ for the first mutation and 81 % for A’ and 19 % for C’, for the second mutation.

4.1. Linkage disequilibrium

The result of the LD test showed that there was a significant correlation between the two mutations for all the breeds, except for Jämtgoat that could not be calculated. This since it only displayed one genotype for mutation 1. Contingency tables for LD, for the different breeds, and p-values can be seen in appendix 4

The T'T genotype for mutation 1 was often paired with the A'A genotype of mutation 2. The same could be seen for the T'C' genotype together with the A'C' genotype, for mutation 1 and mutation 2 respectively.

4.2. Chi2-test

The test showed that the correlation between the two mutations were significant for all the samples combined as well as for within the breeds of Göingegoat, and the Unknown/Crossbreed (see table 3).

The correlation between genotype and disease (CAE) was not significant, with a p-value of 0.97 for mutation 2 and 0.53 for the first mutation. The T'C' & C'C' genotypes were put together in the calculation, since there were so few with the C'C' genotype.

Observed and expected values, for the tests, can be found in appendix 3.

Table 3. p-values for correlation between genotype and CAE, and for correlation between the genotypes within the breeds.

	CAE	All samples	Swedish lantras	Göinge goat	Lapp goat	Jämt goat	Unknown /Crossbreed
p-value	Mut1=0.53 Mut2=0.97	0.0000021	1.9	0.048	1.33	0.49	0.002

A CHI2-test was also done to see if there were significant differences between genotypes for mutation 1 between the breeds. The tests showed significant differences between Swedish lantras-Göingegoat, Swedish lantras-Jämtgoat, Göingegoat-Lappgoat, Göingegoat-Jämtgoat and Jämtgoat-Lappgoat. No significant difference was found between Swedish lantras and Lappgoat. See p-values in table 4.

Table 4. p-values for mutation 1, between the different breeds

	Swedish lantras-Göingegoat	Swedish lantras-Jämtgoat	Göingegoat-Lappgoat	Göingegoat-Jämtgoat	Jämtgoat-Lappgoat	Swedish lantras-Lappgoat
p-value	0.00028	0.0067	0.0276	0.00001	0.0032	0.63

The test for HW equilibrium showed that all breeds were in equilibrium, for both of the mutations. Degree of freedom was one. All values were below the threshold X^2-value of 3.84, meaning that the test was significant for $p<0.05$. See table 5.

Table 5.X^2-valuesfor test of HW-equilibrium.

	Swedish lantras	Göingegoat	Lappgoat	Jämtgoat
Mutation 1	0.31	0.20	0.21	0.00
Mutation 2	1.20	0.75	0.21	1.27

5. Discussion

5.1. Milk production

The loss of milk production from infected animals showed mixed results, in previous studies, however even if no direct losses on an individual level could be measured, there would still be a loss on the total production since the infected animals would be removed from the herd and culled.

5.2. Treatment

No treatments or vaccines are available today so alternative methods are crucial in containing CAE. It is both a production- and animal welfare issue. The control programs used today result in huge losses since many animals in affected herds need to be culled. There is also more work with separating the young and healthy from sick animals. Especially if the "phasing out" method mentioned earlier is used, since then several separate groups need to be looked after. More care is also needed so that the disease does not spread via handlers and equipment, in this case. Therefore breeding would be a good complement to existing methods, if it is viable. This will be discussed more later on in the text.

5.3. CCR5 and CAE

As in previous studies two mutations on the CCR5 gene were found (Colussi *et al.,* 2019 & Gunnarsson, 2020). There were significant differences between the mutations within the breeds Göingegoat and the Unknown/Crossbreeds, and within all the samples, as shown in the result section (table 3). There were some significant differences for the first mutation between the breeds, except for between Swedish lantras-Lappgoat. Many of the animals, who were heterozygous, were as such on both mutation loci. This indicates that these mutations are correlated with each other. The mutations seem to follow each other, but what

effect the second mutation has on CAE is not known. Since the effect of the second mutation is unknown the focus has been on mutation 1, in this study, as it has proven to be of interest from previous studies.

The C'C' combination of the first mutation seemed the most prevalent in Göingegoat, compared to all the other breeds.

Ten of the sampled animals were confirmed carriers of CAE (sero-positive). The chi2-test showed no significant difference between genotype and prevalence of disease for both of the mutations. Only the herds that had at least one case of CAE were included in the calculations, since it was not possible to know if animals from the other herds had been subjected to the virus or not. Therefore they would not be a fair representation.

In other studies the prevalence of the first mutation (T'T') had an impact on pro-viral levels (Colussi *et al.,* 2019). In their study they showed that the T'T' genotype increased the pro-viral load while it was the opposite for the C'C' genotype. However Gunnarsson (2020) could not find any significant correlation between the mutation and prevalence of disease for any of the mutations, as was the case in this study. Gunnarsson (2020) studied two breeds with a total of 96 samples. In this study there were four breeds studied with a total of 127 samples, broadening the study material, as was the aim of this study.

The correlation between the first mutation and CAE could not be proven in the study presented here. Since in this study only ten of the animals were confirmed sero-positive more samples need to be studied to reach any conclusive results.

All Jämtgoat included in this study were homozygous for the first mutation (T'T'). If previous results hold that this mutation could increase the viral load, then this breed might be more susceptible to CAE, assuming that we included a representative sample from the entire population. Similarly Göingegoat might be more resistant to the disease since most of them were either homozygous for mutation 1 (C'C') or heterozygous (T'C'). More studies need to be conducted on this to reach any conclusions.

The breeds were chosen based on the fact that mostly Swedish lantras had been studied previously (and some Lappgoats) (Gunnarsson (2020). Gunnarsson had samples from 85 Swedish lantras and 11 Lappgoats. In this study samples from 25 Swedish lantras, 17 Göingegoats, 7 Lappgoats and 50 Jämtgoats were analyzed. The indigenous breeds (primarily Göingegoat and Lappgoat) have small populations, thus fewer samples were possible to collect. Samples from Jämtgoat were easier to collect since more were available. That is why there were more samples of Jämtgoat, and fewer from the other breeds.

The breeds should be well represented since they are from several different herds, from across the country. Jämtgoat-samples were collected both from herds with a focus on milk production and those with a focus on preserving the breed. Farms with Göingegoat and Lappgoat are mainly focused on breed-preservation.

With Swedish lantras it is hard to know if the incidence of disease is because of genetic factors or because so many of those herds have been actively working with prevention programs. They have conducted continuous testing, separating the young at birth and culled infected animals.

The allele frequency of C', of the first mutation, for Swedish lantras was 10% in this study compared to the study of Gunnarsson (2020) with a frequency of 14%. For Lappgoat the frequency, for the same allele, was 14% and 23% respectively, though there are only seven samples in this study. The differences in allele frequencies were significant in both studies. Lappgoat showed a higher frequency of the C' allele than Swedish lantras did.

It might be viable to breed on the first mutation in the future but the connection between the CCR5-gene and the previously studied casein gene, CSN1S1, (Gunnarsson, 2020) is not known. It is also not known if other genes play a role in expression of/susceptibility to CAE.

More studies have to be done before these results can be used for breeding, as there is no conclusive correlation between these mutations and CAE, in Swedish goats, yet.

The basis for breeding on the mutation is different for the breeds. It should be easier on Göingegoat since they have the most variation and the most of the T'C' and C'C' genotype, for the first mutation. In Jämtgoat, however, it might be impossible since none of the individuals in this study showed any other variation than the T'T' genotype. There will have to be more samples taken to seek variation in genotype for this breed. If one is found then it might be possible to breed on it, but not as it looks now.

Right now there is not enough data to support the link between the mutation on the CCR5 gene and expression of CAE in Swedish goats. Only a few samples (10) could be used to analyze this link. Other, unknown, genes might also factor into the onset and expression of the disease.

The deletion of the gene had showed increased resistance both for HIV in human and for sheep (Kaslow *et al.,* 2005 & White *et al.,* 2009). The gene is co-receptor that enables macrophage-tropic viruses to enter the host cells (Colussi *et al.,* 2019). It might be this that makes the CCR5 gene important when it comes to expression of the CAE virus, as it is a macrophage-tropic virus. Have not found anything on if the deletion of this gene would affect other parts of the immune system.

Animals with CAE can still produce antibodies as this disease does not affect the T-cells, and are not immuno-suppressant (Leroux *et al.,* 2010). So even if they produce antibodies they can still be sick/infected and develop symptoms. As well as transmit the disease.

If breeding becomes a viable option in containing the disease it could be a helpful tool, together with existing control programs. If the disease could be

further contained it would decrease production losses connected to it. It would also increase animal welfare, since it is a disease with severe symptoms that leads to suffering and the animal ultimately being culled. Containing it would also decrease the risk of losing breeds/genetic materials from being forced to cull sick animals. All this would also improve the economic aspect of the production, with healthier and more productive animals.

6. Conclusion

There were no significant correlations found between genotype and prevalence of disease, but only ten animals were sero-positive. More studies need to be conducted, and more animals tested for CAE, to increase the sample size and the validity. More animals of all Swedish breeds need to be sampled, especially for the indigenous breeds.

There were significant differences in genotype within and between some of the breeds for the mutation.

There were big differences in genotype- and allele frequencies. Breeding would therefore be easier on some breeds, since they show more variation in genotype.

This study show genotype- and allele frequencies for the Swedish goat breeds and can be used as a basis for further studies on the correlation between them and instance of CAE.

More needs to be studied before the possibility of breeding as a way of stopping the disease.

In the future, if breeding becomes a viable method, this might be used to increase the chance of containing the disease and therefore positively affect production and animal welfare.

Right now there is not enough data to support the link between the mutation on the CCR5 gene and expression of CAE in Swedish goats. Other, unknown, genes might also factor into the onset and expression of the disease.

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Appendix 1

List of genotypes for all individuals, used in GenePop for LD. 0101=A'A', 0102=A'C', 0202=C'C' for mutation 2. 0303=T'T', 0304=T'C', 0404=C'C' for mutation 1.

CCR5 Goat LD
Loc1, Loc2
POP
001, 0101 0303
002, 0102 0304
003, 0102 0304
004, 0101 0303
005, 0102 0303
006, 0101 0303
007, 0102 0304
008, 0101 0303
009, 0101 0303
010, 0101 0303
011, 0102 0304
012, 0202 0304
013, 0101 0303
014, 0102 0303
015, 0102 0303
016, 0101 0303
017, 0101 0303
018, 0102 0303
019, 0102 0303
020, 0101 0303
021, 0101 0303
023, 0102 0304
024, 0102 0304
026, 0102 0303
027, 0101 0303
028, 0202 0303
029, 0101 0303
030, 0102 0303

031, 0101 0303
033, 0101 0303
034, 0101 0303
035, 0101 0303
036, 0101 0303
037, 0101 0303
038, 0101 0303
040, 0101 0303
041, 0102 0303
042, 0101 0303
044, 0202 0304
045, 0102 0303
046, 0102 0303
047, 0102 0303
048, 0101 0303
049, 0101 0303
050, 0101 0303
051, 0102 0303
052, 0101 0303
053, 0101 0303
054, 0102 0303
055, 0101 0303
056, 0101 0303
057, 0102 0303
058, 0102 0303
059, 0102 0303
060, 0101 0303
061, 0102 0304
062, 0101 0303
063, 0102 0304
064, 0101 0303
065, 0101 0303
066, 0101 0303
067, 0101 0303
068, 0102 0304
069, 0102 0303
070, 0102 0304
071, 0202 0404
072, 0202 0404
073, 0102 0304
074, 0102 0304

075, 0202 0304
076, 0102 0304
077, 0102 0303
078, 0202 0304
079, 0202 0404
080, 0102 0303
081, 0202 0304
082, 0101 0303
083, 0102 0304
084, 0202 0304
085, 0102 0303
086, 0202 0303
087, 0202 0303
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091, 0102 0303
092, 0202 0303
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114, 0102 0303
115, 0102 0303

116, 0202 0303
117, 0101 0303
118, 0101 0303
119, 0202 0303
120, 0102 0303
121, 0102 0303
122, 0102 0303
123, 0202 0303
124, 0202 0303
125, 0101 0303
126, 0101 0303
127, 0101 0303
128, 0101 0303
129, 0202 0303
130, 0102 0303
131, 0101 0303
132, 0101 0303

Appendix 2

Sequence for a homozygote on both mutation loci. Red is mutation 2(C'C'), gold is mutation 1(T'T').

Homozygote
GCTCAGTCCCTCAGTTGTGTCAGATTTCTTGC AAACCCATGGACTGTATGCAGCCCACCAGGC TCCTCCATCCATTTTTCTAGGCAAGAATACTT AAGTGGTTTGCCATTTCCTCCTCCAGGCGAT CTTTCCATCCCAGGGATCACACACACATCTC CTGTGTCAGCAAGTAGGTTCTTCACCACTGA GCCAGCTGGGAAGCCCAGGTTTAGGGGGAT AACAGGGTTAATGTGAGAGGTTCCCTCCACT TTAAAGTCAGTTTCAGCTGGCTTCCACAAAT ACCAAGTGGGTCAGAATCTCTCACCCTCTGA GCCTCCCCATTGATAAGGGTCATAGCTGTTT CCTG

Sequence for a heterozygote on both mutation loci. Red is mutation 2(A'C'), gold is mutation 1(T'C').

Heterozygote
CAGTCCCTCAGTTGTGTCAGATTTCTTGCAAACC CATGGACTGTATGCAGCCCACCAGGCTCCTCCA TCCATTTTTCTAGGCAAGAATACTTAAGTGGTTT GCCATTTCCTCCTCCAGGCGATCTTTCCATCCCA GGGATCAMACACACATCTCCTGTGTCAGCAAGT AGGTTCTTCACCACTGAGCCAGCTGGGAAGCCC AGGTTTAGGGGGATAACAGGGTTAATGTGAGAG GTTCCCTCCACTTTAAAGTCAGTTTCAGCTGGCT TCCACAAAYACCAAGTGGGTCAGAATCTCTCAC

Appendix 3

Observed and expected values for Chi2-test of mutation 1 and mutation 2, respectively, in correlation with prevalence of CAE.

Observed value	T'T'	T'C'	C'C'	**Total**
Disease	9	1	0	10
No disease	22	5	0	27
Total	31	6	0	37
Expected value	T'T'	T'C'	C'C'	**Total**
Disease	8.38	1.62	0	10
No disease	22.62	4.38	0	27
Total	31	6	0	37

Observed value	A'A'	A'C'	C'C'	**Total**
Disease	6	3	1	10
No disease	16	10	1	27
Total	22	13	2	37
Expected value	A'A'	A'C'	C'C'	**Total**
Disease	5.94	3.51	0.54	10
No disease	16.06	9.49	1.46	27
Total	22	13	2	37

Observed and expected values for chi2-test for correlation between the genotypes of the two mutations in the whole population and within the breeds.

Observed value (whole pop.)	T'T	T'C'	C'C'	Total
A'A'	51	0	0	51
A'C'	38	15	0	53
C'C'	14	6	3	23
Total	103	21	3	127
Expected value	T'T	T'C'	C'C'	Total
A'A'	41.36	8.44	1.21	51
A'C'	42.98	8.76	1.25	53
C'C'	18.66	3.8	0.54	23
Total	103	21	3	127

Observed value (Swedish lantras)	T'T	T'C'	C'C'	Total
A'A'	16	0	0	16
A'C'	4	5	0	9
C'C'	0	0	0	0
Total	20	5	0	25
Expected value	T'T	T'C'	C'C'	Total
A'A'	12.8	3.2	0	16
A'C'	7.2	1.8	0	9
C'C'	0	0	0	0
Total	20	5	0	25

Observed value (Göingegoat)	T’T	T’C’	C’C’	Total
A’A’	1	0	0	1
A’C’	3	6	0	9
C’C’	0	4	3	7
Total	4	10	3	17
Expected value	T’T	T’C’	C’C’	Total
A’A’	0.23	0.59	0.18	1
A’C’	2.12	5.29	1.59	9
C’C’	1.65	4.12	1.23	7
Total	4	10	3	17

Observed value (Lappgoat)	T’T	T’C’	C’C’	Total
A’A’	5	0	0	5
A’C’	0	2	0	2
C’C’	0	0	0	0
Total	5	2	0	7
Expected value	T’T	T’C’	C’C’	Total
A’A’	3.57	1.43	0	5
A’C’	1.43	0.57	0	2
C’C’	0	0	0	0
Total	5	2	0	7
Observed value (Jämtgoat)	T’T	T’C’	C’C’	Total
A’A’	15	0	0	15
A’C’	19	1	0	20
C’C’	13	0	0	13
Total	47	1	0	48
Expected value	T’T	T’C’	C’C’	Total
A’A’	14.69	0.31	0	15
A’C’	19.58	0.42	0	20
C’C’	12.73	0.27	0	13
Total	47	1	0	48

Observed value (Unknown)	T’T	T’C’	C’C’	Total
A’A’	15	0	0	15
A’C’	11	1	0	12
C’C’	1	2	0	3
Total	27	3	0	30
Expected value	T’T	T’C’	C’C’	Total
A’A’	13.5	1.5	0	15
A’C’	10.8	1.2	0	12
C’C’	2.7	0.3	0	3
Total	27	3	0	30

Appendix 4

Contingency table for genotypic disequilibrium, for all samples.

Mutation 2/Mutation 1	T'T'	T'C'	C'C'	Total
A'A'	51	0	0	51
A'C'	38	15	0	53
C'C'	14	6	3	23
Total	103	21	3	**127**

Contingency table for genotypic disequilibrium,, for Swedish lantras

Mutation 2/Mutation 1	T'T'	T'C'	C'C'	Total
A'A'	16	0	-	16
A'C'	4	5	-	9
C'C'	-	-	-	-
Total	20	5	-	**25**

Contingency table for genotypic disequilibrium,, for Göingegoat

Mutation 2/Mutation 1	T'T'	T'C'	C'C'	Total
A'A'	1	0	0	1
A'C'	3	6	0	9
C'C'	0	4	3	7
Total	4	10	3	**17**

Contingency table for genotypic disequilibrium,, for Lappgoat

Mutation 2/Mutation 1	T'T'	T'C'	C'C'	Total
A'A'	5	0	-	5
A'C'	0	2	-	2
C'C'	-	-	-	-
Total	5	2	-	**7**

Contingency table for genotypic disequilibrium,, for Jämtgoat

Mutation 2/Mutation 1	T'T'	T'C'	C'C'	Total
A'A'	15	0	-	15
A'C'	21	0	-	21
C'C'	14	0	-	14
Total	50	0	-	**50**

Contingency table for genotypic disequilibrium,, for Unknown/Crossbreeds

Mutation 2/Mutation 1	T'T'	T'C'	C'C'	Total
A'A'	15	0	-	15
A'C'	10	1	-	11
C'C'	0	2	-	2
Total	25	3	-	**28**

p-values for LD

Breed	All samples	Swedish lantras	Göinge goat	Lappgoat	Jämt goat	Unknown /Crossbreed
p-value	0.00	0.002	0.029	0.046	-	0.009