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Abstract: In Switzerland, viruses belonging to two different phylogenetic groups of small ruminant lentiviruses (SRLV) are currently circulating: the caprine arthritis-encephalitis virus (CAEV) and visna/maedi virus (VMV). In the past two decades, a mandatory national control program has led to a very low prevalence of seropositivity, while completely eliminating CAE as a clinical manifestation. However, in order to reduce the high costs and effort associated with this program, adjustments based on the most recent epidemiological knowledge are needed. The purpose of this study was to estimate the seroprevalence of CAEV and VMV using the newest diagnostic tools available, and to identify potential risk factors for infection with these viruses in Switzerland. For the prevalence estimation, a census was carried out including 10,696 farms with a total of 85,454 goats. Blood samples were analysed using a 3-step serological testing algorithm consisting of Chekit ELISA, Western Blot and SU5 ELISA. A risk factor analysis was conducted using logistic regression models built with data obtained from a mail questionnaire, and serological results from the census. The apparent herd-level prevalences were 0.38%, 2.77%, and 3.04% for CAEV, VMV and SRLV, respectively. Animal-level prevalences were 0.06% for CAEV, 0.55% for VMV, and 0.61% for SRLV. No statistically significant risk factors associated with CAEV or VMV infection were identified. However, the proportional high number of CAEV seropositive dwarf goats, in relation to their population size, could indicate that these hobby breeds may slip through some of the official controls. For an infection with SRLV, a medium herd size (7-40 goats) was found to be protective, compared with smaller (OR=1.90, p=0.034) and larger herds (OR=1.95, p=0.038). In conclusion, considering that all CAEV positive animals were culled, these results imply that CAEV is no longer actively spreading and has successfully been controlled in Switzerland. However, given the uncertain pathogenic potential of VMV in goats, future surveillance should also be taking into account the not insignificant number of VMV circulating in the Swiss goat population.

Cover Letter

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Bern, 13 December 2016

Dear Editorial team,

Enclosed, please find the revised manuscript "A census to determine the prevalence and risk factors for caprine arthritis-encephalitis virus and visna/maedi virus in the Swiss goat population" by B. Thomann, L. C. Falzon, G. Bertoni, H.R. Vogt, G. Schüpbach-Regula, and I. Magouras.

We thank for valuable comments, which have substantially contributed to comprehension and clarity of this manuscript.

We respond below to each point brought up during the reviewing process.

On behalf of the authors,

Beat Thomann

*Revision Note

Revision Note:

Dear Reviewer,

Thank you for your valuable comments on the analyses of dwarf goat farms. This helped us to identify a mistake (comparison of farm sizes of dwarf and "non-dwarf goat" farms) that led to a potential misunderstanding of the entire section. We corrected this mistake and also adjusted the methods and results section, which should have improved overall comprehension and clarity.

Reviewer #2: this is much improved although the English is a bit poor in places.

One concern I still have is that the authors are still focusing in on 7 seropositive dwarf goats for which they have done further analysis without presenting initial population data. These results are done as a proportion of positive goats ie not using a population denominator and they then analyse farms with dwarf goats as a proportion of positive farms and report that these farms were smaller than other CAEV positive farms etc but the analysis is not described in the methods and I cannot see these descriptive data or what was tested to develop the final model. These analyses are based on data from 32 farms (line 215) , and contrast to their logistic model where other variables were significant or near significant at p 0.05, it raises the question of how this model was built and which tested e.g. purchase of female goats.

Only the descriptive statistics of the CAEV seropositive goats (former Table 5, now Table 4) is based on the responses (n=34) of the additional questions on individual goats (as described in lines 130-133). All analyses for dwarf goat farms were conducted on a farm level with the same, full dataset that was used for the CAEV risk factor logistic modeling (n=163).

Thanks to the reviewer's comments, we identified a mistake in the manuscript: Dwarf goat farms (n=24, independent of CAEV status) were not smaller than other CAEV positive farms (as stated in the previous version of the manuscript: "Dwarf goat farms were smaller (p<0.001) than other CAEV positive farms." [Revision 1, lines 219-220]), but they were smaller than "non-dwarf goat" farms (n= 139, independent of CAEV status). Thank you for pointing this out! We understand that this mistake has led to confusion on the whole sections of dwarf goat analyses and have corrected and rewritten this paragraph:

"Of the 47 CAEV seropositive goats detected during the census, detailed data on 34 goats located on 32 farms were reported through the questionnaire and available for descriptive statistics (Table 4). Seven of the 34 (20.6%) CAEV positive goats were dwarf goats, whose overall population size (Zwergziegen-IG, 2016) is substantially smaller compared with the population of the main goat breeds in Switzerland, such as Chamois colored or Saanen (SZZV, 2016). Dwarf goat farms (n=24) were smaller (median=8, p<0.001) than "non-dwarf goat" farms (n=139, median=16). Furthermore, fewer (66.7%, p=0.002) dwarf goat owners were professional farmers, compared to owners of other goat breeds (90.0%). Only 37.5% (n=9) of the dwarf goat farms had been tested for SRLV before 2012, compared to 56.8% (n=79, p=0.079) of the farms without dwarf goats." [lines 217-226]

Given that variables such as not tested before 2012 is not significant in the full model and is also 0.08 significance in the model of 32 farms, small farms are more likely to have dwarf goats but these are protective for SRLV positivity (Table 3 supplementary material) what does ia proportional risk model mean? after all these are all positive farms. I still believe there is insufficient logic to present this section of results.

1

As stated above statistics on dwarf goat farms were not conducted with the 32 CAEV positive farms only, but the same dataset as for the CAEV risk factor analysis (n=163). In the "full model" as the reviewer refers to, "tested before 2012" was checked for an association with CAEV seropositive farm status. In the "additional statistical analyses" the association between "dwarf goat farm" and "tested before 2012" was investigated, but with the same dataset. This methodology was not described in the methods section, and has now been added:

"For the additional statistical analyses on dwarf goat farms, the same data set as for the CAEV risk factor analysis was used. Farm size comparisons are based on differences in medians (Wilcoxon rank-sum test), whereas a chi-square test was used to analyse educational level of the farmers and testing for SRLV before 2012." [lines 158-161]

Thank you a lot for pointing this out!

Former Supplementary Table III (now Table 3) does not indicate that small farms are protective for SRLV positivity. (OR: 1.90, p=0.034).

We believe that by correcting the mistake mentioned above and by adjusting the methods [lines 158-161] and results section [lines 216-226], the additional analyses conducted with dwarf goat farms should now be clear, correct and justified.

tables are muddled and need some reflection –
Table 1 is fine but needs number of herds and animals testd in the title Table 2 and 3, why put these in the main text and not supplementary table 3 which is the model for these data Table 4, need number of herds and case control in title under cases and control this is presumably number, given there are many more controls than cases a % is needed to compare the relative proportions Table 5 - is this the data used ot build the model in 3.2.2 why are other variables not used? is the model appropriate etc

- *The title of Table 1 has been reworded:*
  "Herd-level (n=10,696) and animal-level (n=85,454) seroprevalences (95% CI) for CAEV, VMV and SRLV."

- *Tables 2 and 3 have been moved to the supplementary material (now Supplementary Table I and II). Former Table 3 (now Supp. Table II) shows only descriptive statistics of continuous variables. The data used were from all questionnaire responses (n=341), as described in line 194-196.*

- *Table 4 (now Table 2) was adjusted according to the suggestions, the title has been updated and proportions (%) were added to the table:*
  "Univariable analysis of variables tested for their association with CAEV seropositivity in case (n=32) and control (n=131) herds."

- *Table 5 (now Table 4):*
  This data was not used for any modeling but for characterization purposes only. All logistic regression analyses are based on herd-level data, including the analyses for farms that hold dwarf goats.

supp Table 1 needs number of herds etc, i am not sure this table is needed at all supp Table II again needs numbers time periods etc in title i question if supp III should be in the main text

Thank you for these suggestions. The tables have been adjusted accordingly.

- *Supplementary Table 1 has been removed from the manuscript.*

- *The title of Supplementary Table II (now Supp. Table III) has been reworded and proportions (%) have been added to the table:*
  "Univariable analysis of variables tested for their association with VMV seropositivity in case (n=100) and control (n=131) herds."

- *Supplementary Table III has been moved to the main text (now Table 3).*

A census to determine the prevalence and risk factors for caprine arthritis-encephalitis virus and visna/maedi virus in the Swiss goat population

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1   **Abstract**

2   In Switzerland, viruses belonging to two different phylogenetic groups of small ruminant

3   lentiviruses (SRLV) are currently circulating: the caprine arthritis-encephalitis virus (CAEV)

4   and visna/maedi virus (VMV). In the past two decades, a mandatory national control program

5   has led to a very low prevalence of seropositivity, while completely eliminating CAE as a

6   clinical manifestation. However, in order to reduce the high costs and effort associated with

7   this program, adjustments based on the most recent epidemiological knowledge are needed.

8   The purpose of this study was to estimate the seroprevalence of CAEV and VMV using the

9   newest diagnostic tools available, and to identify potential risk factors for infection with these

10   viruses in Switzerland. For the prevalence estimation, a census was carried out including

11   10,696 farms with a total of 85,454 goats. Blood samples were analysed using a 3-step

12   serological testing algorithm consisting of Chekit ELISA, Western Blot and SU5 ELISA. A

13   risk factor analysis was conducted using logistic regression models built with data obtained

14   from a mail questionnaire, and serological results from the census. The apparent herd-level

15   prevalences were 0.38%, 2.77%, and 3.04% for CAEV, VMV and SRLV, respectively.

16   Animal-level prevalences were 0.06% for CAEV, 0.55% for VMV, and 0.61% for SRLV. No

17   statistically significant risk factors associated with CAEV or VMV infection were identified.

18   However, the proportional high number of CAEV seropositive dwarf goats, in relation to their

19   population size, could indicate that these hobby breeds may slip through some of the official

20   controls. For an infection with SRLV, a medium herd size (7-40 goats) was found to be

21   protective, compared with smaller (OR=1.90, p=0.034) and larger herds (OR=1.95, p=0.038).

22   In conclusion, considering that all CAEV positive animals were culled, these results imply

23   that CAEV is no longer actively spreading and has successfully been controlled in

24   Switzerland. However, given the uncertain pathogenic potential of VMV in goats, future

25　surveillance should also be taking into account the not insignificant number of VMV

26　circulating in the Swiss goat population.

27　**Keywords**

1. Introduction

Caprine Arthritis-Encephalitis (CAE) is a widespread goat disease caused by the CAE-Virus (CAEV), a *Lentivirus* within the family of *Retroviridae*. Another virus of the same genus is the Visna/Maedi Virus (VMV), which is responsible for the Visna-Maedi (VM) disease in sheep. Together, CAEV and VMV form the group of Small Ruminant Lentiviruses (SRLV). Small Ruminant Lentiviruses, depending on the genotype, may cause chronic inflammatory and degenerative lesions in the joints, mammary glands, lungs and brain of goats and sheep (Dawson, 1987; Narayan and Clements, 1989). Of the different SRLV genotypes, B1 is considered as the prototype for CAEV, whereas genotype A1 is the prototype for VMV. While B1 was the principal genotype circulating in Swiss goat herds before the eradication campaign, it seems that, at present, VMV A4 is the dominating SRLV genotype (Cardinaux et al., 2013). From now on, the terms CAEV and VMV will be used to refer to either genotypes B or genotypes A, respectively, to allow for better readability of the manuscript.

In Switzerland, a voluntary eradication program for CAE in goats was launched in 1984, when seroprevalence in the Swiss goat population was around 60-80% (Peterhans et al., 2004). In 1998, eradication of CAE became mandatory, and in the same year the last confirmed clinical CAE case in Switzerland was reported. From 2006 until 2011, the Swiss Federal Food Safety and Veterinary Office (FSVO) reported prevalences of 0.77% to 10.28% (FSVO 2006 – 2011), based on an annual sampling of 25% of all goat farms. However, previous diagnostic tests could not distinguish between CAEV and VMV (Zanoni, 1998), and estimates published before 2012 therefore refer to infections with all SRLV, including goats seropositive for VMV. Following the introduction of an additional diagnostic test which permitted to distinguish between the two genotypes, in 2012 the Swiss federal authorities

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54   decided to conduct a census and restrict the eradication campaign to the CAEV genotypes

55   only.

56   Despite previous achievements and the ongoing eradication program, seroconversions,

57   mainly related to VMV, continue to occur in Switzerland. To reduce further transmission of

58   these viruses, it is important to understand which factors are associated with CAEV and VMV

59   infections in goats. The main routes of transmission of SRLV are either vertically, through

60   ingestion of infected colostrum or milk, or horizontally through inhalation of respiratory

61   secretions, though intra-uterine infection can also occur (Blacklaws et al., 2004). In addition,

62   natural cross species transmission in mixed flocks from goat to sheep, and *vice versa*, has also

63   been described (Germain et al., 2008; Leroux et al., 1997; Pisoni et al., 2005; Shah et al.,

64   2004). Potential risk factors described in the literature include close contact to SRLV

65   seropositive sheep, the import of goats from abroad, the presence of seropositive bucks in a

66   herd, as well as large herds (Brülisauer et al., 2005; Kaba et al., 2013). As mentioned

67   previously however, diagnostic tools available at the time when these studies were conducted

68   could not distinguish between CAEV and VMV infections, and their results often refer to

69   SRLV in general. Given the advent of new diagnostic tools, a risk factor analysis for the

70   separate genotypes could shed light on specific differences between the viruses.

71   The objectives of this study were to use the census data based on the new diagnostic test

72   to (i) provide for the first time SRLV prevalence estimates in the Swiss goat population,

73   discriminating between CAEV and VMV, and (ii) determine risk factors associated with

74   CAEV and VMV infection in goats. Results from this study will permit the veterinary

75   authorities to make evidence based decisions on future surveillance and control of this

76   disease, thus optimizing resource allocation.

77

2. Materials and methods

2.1 Prevalence estimation

2.1.1 Census and laboratory analysis

As part of the national CAE control program, a census of all goat herds registered in Switzerland was conducted and coordinated by the FSVO. Goat herds were visited and sampled by veterinarians between October 2011 and August 2012. The sampling unit was the single animal and blood samples (5-10ml whole blood) were taken from all goats that were six months of age or older. The diagnostic procedure consisted of different serological methods forming a 3-step serial testing algorithm (Figure 1). All blood samples were initially screened using Chekit CAEV/VMV Total Antibody Screening ELISA Test (Idexx Switzerland AG, Liebefeld, Switzerland) by one of the ten accredited regional laboratories. Positive and indeterminate sera were then sent to the National Reference Laboratory at the Institute of Veterinary Virology, University of Bern, where the same ELISA test was repeated. If the ELISA positive result was confirmed, a Western Blot (WB) was performed, and WB positive sera were then analysed using a panel of surface subunit (SU) 5 peptides derived from different SRLV genotypes (Bertoni et al., 2014; Mordasini et al., 2006). This SU5 ELISA analysis permitted to distinguish between CAEV and VMV positive sera; undifferentiated sera were classified as "CAEV positive by default".

2.1.2 Statistical analysis

The estimation of herd- and animal-level prevalences was based on the census data and the confirmed seropositive results from the National Reference Laboratory. A herd was considered CAEV or VMV seropositive if at least one goat had a corresponding SU5 ELISA positive test result. All apparent prevalences (AP) were then converted into true prevalence (TP) estimates by means of the Rogan and Gladen estimator (1978). In order to visualize seropositive cases, a map with the spatial distribution of positive farms was designed using

103   ArcGIS 10 (ESRI, 2010). Case farms were located based on their postal code. Information

104   about the density of small ruminants at the municipality level (FSVO) was mapped as a

105   background layer.

106   *2.2 Risk factor analysis*

107   *2.2.1 Study design and study population*

108   A case-control study was conducted to determine risk factors associated with a CAEV and

109   VMV positive farm status. For the cases, all farms that tested positive in the census were

110   selected and three groups of cases were defined: (i) CAEV, (ii) VMV and (iii) SRLV. CAEV

111   cases were defined as farms that tested CAEV seropositive in the SU5 ELISA. All farms that

112   tested VMV positive, but without CAEV and "CAEV positive by default" results, were

113   treated as VMV cases. Finally, SRLV cases were all those farms that had at least either one

114   CAEV or VMV but had no "CAEV positive by default" test result. Controls were selected

115   randomly from farms that tested negative in the census and had no history of SRLV

116   seropositivity in the previous five years (2006 until 2011). Since only 41 farms were tested

117   positive for CAEV and 284 farms positive for VMV, all these farms were included in our

118   study. The number of control farms required was estimated using a logistic regression power

119   analysis conducted in PASS12 (Hintze, 2013), to detect an odds ratio (OR) for risk factors

120   greater than 2, with a power of 80% ($\alpha = 5\%$). Based on these calculations, a minimum of 120

121   controls was necessary.

122   *2.2.2 Questionnaire*

123   Epidemiological data from each case and control farm were collected through a standardized

124   mail questionnaire. As the questionnaire was distributed to farmers in December 2013 none of

125   the farms that tested positive in the 2012 CAE census were still under animal movement

126   restrictions at the time the data were collected. The questionnaire consisted of 33 questions

127   grouped into the following 5 categories: (i) farm type, breeds and number of animals; (ii)

128 farming know-how and education; (iii) contact with goats from other farms through trade,

129 breeding, shows or common pasture; (iv) contact with own or foreign sheep through common

130 pasture; (v) farm status for CAEV infection in the previous five years. Farmers with CAEV

131 positive goats were also asked to provide specific information on each CAEV positive goat.

132 These additional seven questions, which aimed to gather information on the age, sex, breed,

133 origin, place of purchase and contact with sheep, were used for descriptive purposes only. The

134 questionnaire was pre-tested on goat owners to ensure clarity of the questions. To reduce bias

135 due to possible changes in management practices or increased disease awareness following

136 the census results, and to account for the time lag between when the census and the survey

137 were conducted, questions on animal movement or contact were phrased as "mean over the

138 past two years", "on average", "normally" or "temporarily". The questionnaire was

139 distributed in German, French or Italian language and is available from the corresponding

140 author upon request.

141   *2.2.3 Statistical analyses*

142   The questionnaire data were transferred into an Excel spreadsheet (Microsoft Excel

143 2010) and analysed using STATA 13 (StataCorp., 2013). Predictor variables were checked for

144 missing values and possible errors in data transcription, and continuous variables were also

145 checked for linearity and restructured if needed.

146   To identify possible risk factors, the three case groups, (i) CAEV, (ii) VMV, and (iii)

147 SRLV, were defined as binary outcomes (positive or negative) in the model-building process.

148 For each of the three independent models, the corresponding data set was used and

149 univariable associations between the putative risk factors as independent variables and the

150 serological status were tested using logistic regression. Univariable ORs and *p* values were

151 calculated and ranked. Independent variables that were significantly associated with outcomes

152 at a liberal *p* value of <0.20 were assessed for co-linearity (Pearson correlation coefficient

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153 >0.8) and defined as candidate risk factors. The multivariable logistic regression models were

154 built using a stepwise backward elimination procedure, initially including all candidate risk

155 factor as independent variables. Based on likelihood-ratio tests, candidate risk factors that

156 were significant ($p<0.05$) were retained in the final model and were tested for two-way

157 interactions ($p<0.05$). The goodness-of-fit of the final models were tested using the Hosmer-

158 Lemeshow test. For the additional statistical analyses on dwarf goat farms, the same data set

159 as for the CAEV risk factor analysis was used. Farm size comparisons are based on

160 differences in medians (Wilcoxon rank-sum test), whereas a chi-square test was used to

161 analyse educational level of the farmers and testing for SRLV before 2012.

3. Results

3.1 Seroprevalence and spatial distribution

Within the framework of the census, 85,454 individual goats located on 10,696 different farms were tested. A total of 729 animals from 452 farms were classified as WB positive, of which 47 (6.4%) animals were further classified as CAEV infected and 473 (64.9%) as VMV infected by means of the SU5 ELISA. The remaining 209 (28.7%) WB positive sera were classified as "CAEV positive by default" as they could not be further described using this panel of SU5 peptides; these were therefore excluded from further prevalence estimation. However, for the purposes of the national eradication program, these animals were considered as potentially CAEV infected and were therefore culled.

The 47 CAEV positive goats were located on 41 farms. Of these farms, 36 (87.8%) had 1 positive goat each, 4 farms had 2 positive goats, and 1 farm had 3 positive goats; the median within-herd prevalence was 5.9% (min=0.5%, max=66.7%). The 473 VMV infected goats were located on 296 farms. The majority of these farms (78.7%) had only one VMV seropositive goat. Twelve farms (4%) had both CAEV and VMV positive goats, resulting in a total of 325 SRLV positive farms. Consequently, the AP at a herd level were 0.38%, 2.77%, and 3.04% for CAEV, VMV and SRLV, respectively, while the TP estimates were 0.40%, 32.21% and 32.27%. The animal-level AP were 0.06%, 0.55% and 0.61%, while TP estimates were 0.06%, 6.40% and 6.46% for CAEV, VMV and SRLV, respectively. All prevalence estimates are listed in Table 1.

The 41 CAEV positive farms were evenly distributed over the whole territory of Switzerland and no regional patterns could be observed (Figure 2). Similarly, the VMV positive farms were detected all over Switzerland; however, regional patterns in accordance with the regional differences in animal density and topography, were noticeable for these farms.

187   *3.2 Risk factor analysis*

188   *3.2.1 Study population and response rate*

189   Based on the diagnostic test results, questionnaires were administered to all 452 farms

190   that tested positive with the WB, including the 127 "CAEV positive by default" farms.

191   However, the latter were only considered for descriptive purposes and later excluded from the

192   risk factor analysis. Overall, 752 questionnaires (including 300 for control farms) were sent

193   out, of which 13 could not be delivered. Of the 739 delivered questionnaires, 396 were

194   completed and returned, resulting in an overall response rate of 54%. Of these, 55 farms had

195   to be omitted either because the farm identification was missing (n=37) or because they no

196   longer kept goats (n=18). The response rate for CAEV positive farms was 78% (32 of 41).

197   Finally, a full data set was available for 341 farms. Descriptive statistics of the categorical and

198   continuous variables of these farms are presented in Supplementary Table I and

199   Supplementary Table II, respectively.

200   *3.2.2 Logistic regression*

201   Based on the case definition, the risk factor analysis was conducted on 32 CAEV, 100

202   VMV and 125 SRLV case farms. A total of 131 controls were used for the risk factor

203   analysis. The univariable analysis identified "purchase of female goats" (p=0.093, OR=2.17)

204   as the only predictor variable marginally associated (p<0.10) with a positive CAEV status,

205   while the association between dwarf goat ownership and CAEV positive status was just above

206   the threshold value (p=0.208, OR=1.88). Thus, no multivariable model for CAEV could be

207   built. Results of the univariable analysis for other variables considered relevant are presented

208   in Table 2. The final multivariable model for VMV did not identify any significant risk

209   factors either. Nevertheless, four variables were marginally significant in the univariable

210   analysis: "herd size" (small vs. medium herds, OR=1.82; large vs. medium herds, OR=1.89)

211   and "Appenzell breed" (OR=3.21) as candidate risk factors, while "know-how" (OR=0.53)

212 and "breeding done with own buck" (OR=0.62) as putative protective factors for VMV in

213 goats (Supplementary Table III). For an infection with SRLV, the final model included the

214 following two variables: "Appenzell breed" (OR=4.10) and "herd size" (small *vs*. medium

215 herds, OR=1.90; large *vs* medium herds, OR=1.95) (Table 3).

216 *3.3 Additional statistical analyses*

217 Of the 47 CAEV seropositive goats detected during the census, detailed data on 34 goats

218 located on 32 farms were reported through the questionnaire and available for descriptive

219 statistics (Table 4). Seven of the 34 (20.6%) CAEV positive goats were dwarf goats, whose

220 overall population size (Zwergziegen-IG, 2016) is substantially smaller compared with the

221 population of the main goat breeds in Switzerland, such as Chamois colored or Saanen

222 (SZZV, 2016). Dwarf goat farms (n=24) were smaller (median=8, *p*<0.001) than "non-dwarf

223 goat" farms (n=139, median=16). Furthermore, fewer (66.7%, *p*=0.002) dwarf goat owners

224 were professional farmers, compared to owners of other goat breeds (90.0%). Only 37.5%

225 (n=9) of the dwarf goat farms had been tested for SRLV before 2012, compared to 56.8%

226 (n=79, *p*=0.079) of the farms without dwarf goats.

4. Discussion

This is the first study in Switzerland where, by means of newly applied diagnostic tools, SRLV infected goats could be further classified as CAEV (genotypes B) and VMV (genotypes A) infected. This, in turn, allowed for genotype specific prevalence estimations and risk factor analyses. In addition, the prevalence estimations published in this study are based on a census, compared to earlier Swiss publications.

Only 47 CAEV positive goats (TP animal-level 0.06%), located on 41 farms (TP herd-level 0.40%) were detected, indicating that the eradication campaign is progressing successfully. A comparison with previous publications should be done with caution as, due to the lack of discriminatory tests for the different genotypes until recently, as they refer to a global SRLV seroprevalence. Furthermore, different studies reported either AP or TP, and should therefore not be compared directly. The herd-level AP for SRLV described in this study (3.04%) differs from previous estimates for Switzerland (0.77% - 10.28%, FSVO 2006 - 2011); it is higher compared to that reported for 2006 and 2007, but lower than that reported between 2008 and 2011. However, the estimates in 2008 and 2009 could have increased due to the ongoing bluetongue vaccination campaign (FSVO, 2009, 2010), which might have generated false positive results in the Screening ELISA Test (Valas et al., 2011). For the years 2010 and 2011, the reported estimates are considered as not representative as either the sample sizes were too small or the samples were not taken randomly (FSVO, 2011, 2012). The large difference between SRLV AP and TP estimates in this study is driven by the poor sensitivity of the Screening ELISA for VMV genotypes, which comprise the vast majority (91%) of SRLV seropositive goats detected during the census, and resulted a combined overall sensitivity of 8.6% (Cardinaux et al., 2013). Since the SRLV positive results could not be subdivided into CAEV or VMV prior to the 2012 census, it remains unclear how much of the previous SRLV estimates were due to VMV cases. VMV seropositive goats are currently

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252 excluded from mandatory culling as VMV infection in goats is still considered as non-

253 pathogenic (despite recent histological evidence by Deubelbeiss et al., (2014) that VMV could

254 cause mastitis) and VMV prevalence may thus rise in the coming years. Considering the fact

255 that all goats that tested CAEV positive were culled, it might be assumed that VMV

256 prevalence (TP herd-level: 32.21%, TP animal-level: 6.40%) currently equals SRLV

257 prevalence.

258 A considerable number of WB positive animals escaping SU5 ELISA classification

259 (28.7%) were reported by the National Reference Laboratory as "CAEV positive by default"

260 and therefore culled. This decision was taken to reduce the risk that infected animals with

261 false negative SU5 ELISA results may jeopardize the success of the eradication campaign.

262 Previous work has shown that the sensitivity of the SU5 ELISA test is excellent with well

263 characterized viruses but may fail with variant strains, leading to possible false negative cases

264 (Bertoni et al., 2014).

265 During the 2012 census, numerous farms were tested for SRLV for the first time, and

266 several of these were hobby farms that were not previously registered. It is plausible that

267 dwarf goats are held on such farms, with potentially less professional management practices.

268 Even though, before the 2012 census, the dwarf goat farms were not tested significantly less

269 frequently compared with other farms (p=0.079), a trend is identifiable. This might be a

270 possible explanation for the proportionally high number of CAEV seropositive dwarf goats

271 detected.

272 Since only few publications distinguish between CAEV and VMV, knowledge on the

273 transmission routes for each genotype is limited. Vertical transmission through colostrum and

274 milk appears to be the main infection route for CAEV-infected goats. The situation in sheep

275 appears to be more complex, with horizontal transmission of SRLV playing a more important

276 role (Alvarez et al., 2005; Bertoni et al., 2014; Peterhans et al., 2004). This indicates that

277 alternative control measures might be necessary when aiming to control SRLV in sheep

278 populations. In CAEV and VMV co-infected goats, there is evidence that VMV is more

279 efficiently transmitted to the suckling kids, suggesting a rapid spread of VMV in goat

280 populations (Pisoni et al., 2010). Further research is needed to understand how efficiently

281 CAEV is transmitted horizontally, both within goat herds, as well as between goats and sheep.

282 A low horizontal transmission rate of CAEV could explain the presence of only a single

283 seropositive goat in each CAEV case farm, as well as the even and almost random distribution

284 of CAEV cases over the country. Even if some CAEV positive goats have escaped detection

285 due to imperfect diagnostic tests, we expect a very slow spread of the virus in the future

286 (unpublished data). In contrast, the regional patterns observed with VMV cases are suggestive

287 of a more efficient horizontal transmission of these viruses.

288   No significant risk factors for an infection with CAEV could be determined in this

289 study, which differs from other publications (Brülisauer et al., 2005; Kaba et al., 2013),

290 though the univariable regressions identified an association between CAEV positive status

291 and "purchase of female goats", similar to previous findings by Brülisauer et al. (2005).

292 However, as mentioned before, past studies refer to risk factors for an infection with SRLV

293 and not only with CAEV. Additionally, a major limitation in our risk factor analysis was the

294 small number of CAEV case farms (n=32) included (despite the high response rate).

295 Ironically, this fact can be seen as a negative "side effect" of the success of the eradication

296 campaign over the last two decades, which has led to a low prevalence. Furthermore, the

297 retrospective study design might have introduced bias due to potential improved disease

298 awareness and consequent changes in management practices following the census results.

299   Although no risk factors for an infection with VMV were determined, the univariable

300 analysis identified seven variables below the threshold point (p<0.20). This difference in

301 number of putative risk factors, compared to the CAEV analysis, may be attributed to the

14

302 higher number of VMV case farms (n=100) included in the analysis. SRLV risk factors

303 obtained from the present case-control study, similar to the SRLV prevalence, were strongly

304 influenced by the large proportion of VMV case farms. The observed higher odds (OR=1.90)

305 of SRLV seropositivity in small herds, compared to medium sized herds, could be explained

306 by less professional management practices often observed on such smaller farms. On the other

307 hand, large herds could be at a higher odds (OR=1.95) of infection due to greater animal

308 movements which may favor disease transmission (Kaba et al., 2013). Goats from the

309 Appenzell breed were found to be a significant risk factor for SRLV (OR=4.10), and this

310 tendency was also observed with VMV positive status. However, only a small number of

311 participating farms (n=12) held goats from the Appenzell breed.

Conclusion

312

313   While the disappearance of clinical cases of CAEV-induced arthritis in Switzerland

314   suggested that the eradication campaign had succeeded in eliminating virulent CAEV strains

315   from the goat population, this study, based on a detailed epidemiological analysis of a

316   serological survey involving the entire goat population, further confirms and expands this

317   observation. Therefore we believe that classical CAEV genotypes are no longer circulating

318   between goats in Switzerland, permitting us to conclude that these viruses may be considered

319   as eradicated. In contrast, it seems that VMV genotypes may be actively transmitted in goat

320   herds, pointing to the importance of regularly monitoring these infections, particularly since

321   their pathogenic potential is still uncertain. In this respect, we are currently developing

322   epidemiological models that will permit to perform this monitoring in a targeted and

323   economically efficient way.

324

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413

Figure 1

3-step serological testing algorithm used to determine SRLV status with corresponding test sensitivities/specificities (%) for CAEV and VMV.

[1]CAEV: 97.9/98.1; VMV: 43.0/100
[2]CAEV: 98.0/95.0; VMV: 35.0/100
[3]CAEV: 99.0/99.0; VMV: 57.0/50.0

Figure 2

Spatial distribution of CAEV (n=41) and VMV (n=284) seropositive farms.

Table1

1 **Table 1**
2 Herd-level (n=10,696) and animal-level (n=85,454) seroprevalences (95% CI) for CAEV,
3 VMV and SRLV.

	CAEV (genotypes B)	VMV (genotypes A)	SRLV (genotypes A and B)
Herd-level			
Number of seropositive herds	41	296	325[a]
Apparent seroprevalence	0.38%	2.77%	3.04%
True seroprevalence	0.40%	32.21%	32.27%
	(0.34-0.46)	(31.92-32.50)	(31.97-32.57)
Animal-level			
Number of seropositive animals	47	473	520
Apparent seroprevalence	0.06%	0.55%	0.61%
True animal seroprevalence	0.06%	6.40%	6.46%
	(0.06-0.07)	(6.35-6.44)	(6.41-6.51)

4 [a] 12 farms with both CAEV and VMV positive animals.

Table2

1 **Table 2**

2 Univariable analysis of variables tested for their association with CAEV seropositivity in case (n=32) and control (n=131) herds.

Variable	Description	Cases		Controls		*p* value	Odds Ratio	Odds Ratio (95% CI)
		n	%	n	%			
Animal show	Goats taken to breed shows or goat markets at least every other year	10	31.3	41	31.3	0.996	1.00	0.43-2.30
Breeding	Breeding of animals done on farm	27	84.4	116	88.5	0.520	0.70	0.23-2.09
Breed Appenzell	Appenzell breed present in herd	2	6.3	3	2.3	0.264	2.84	0.45-17.78
Breed dwarf goat	Dwarf goat breed present in herd	7	21.9	17	13.0	0.208	1.88	0.70-5.01
Contact alpine	Common alpine pasture with foreign goats/sheep	10	31.3	37	28.2	0.737	1.15	0.50-2.67
Contact sheep	Keep goats and sheep together with direct contact	7	21.9	21	16.0	0.434	1.47	0.56-3.83
Herd size						0.642		
	Medium herd (7-40 goats)	15	46.9	71	54.2	(ref)	1.00	-
	Small herd (1-6 goats)	8	25.0	33	25.2	0.777	1.15	0.44-2.97
	Large herd (>40 goats)	9	28.1	27	20.6	0.341	1.58	0.62-4.03
Know-how	Know-how based on agricultural education and/or membership in a goat association	27	84.4	114	87.0	0.695	0.81	0.27-2.38
Own buck	Breeding done with own buck(s)	22	68.8	87	66.4	0.801	1.11	0.48-2.55
Purchase	Purchase of at least one goat per year	15	46.9	59	45.0	0.852	1.08	0.50-2.34
Purchase female	Purchase of at least one female goat per year	9	28.1	20	15.3	0.093	2.17	0.88-5.37
Tested before 2012	Farm tested for SRLV before the 2012 census (2006-2011)	16	50.0	72	55.0	0.614	0.82	0.38-1.78
Wildlife						0.403		
	No observed contact with wildlife	24	75.0	98	74.8	(ref)	1.00	-
	Observed contact with ibex	3	9.4	5	3.8	0.241	2.45	0.55-10.97
	Observed contact with chamois and other Swiss wildlife	5	15.6	28	21.4	0.556	0.73	0.25-2.09

3

Table3

1 **Table 3**

2 Final multivariable logistic regression model of risk factors associated with small ruminant

3 lentiviruses (SRLV) infection in goats.

Variable	Description	se	*p* value	Odds Ratio	Odds Ratio (95% CI)
Breed Appenzell	Appenzell breed present in herd	2.83	0.041	4.10	1.06-15.84
Herd size			0.039		
	Medium herd (7-40 goats)		(ref)	1.00	-
	Small herd (1-6 goats)	0.57	0.034	1.90	1.05-3.43
	Large herd (>40 goats)	0.63	0.038	1.95	1.04-3.66

4

Table4

1 **Table 4**
2 Characterization of individual goats (n=34) that tested CAEV seropositive during the 2012
3 census.

Variable	Categories	n	%
Sex	Female	28	82
	Male	6	18
Origin	Own breeding	20	59
	Bought in	14	41
Place of purchase (n=14)	Private	14	100
	Market	0	0
Contact with sheep (n=33)	Yes	8	24
	No	20	61
	Not known	5	15
Breed	Dwarf goat	7	20
	Mixed breed	7	20
	Saanen	6	18
	Chamois colored	4	12
	Grisons striped	3	9
	Peacock goat	3	9
	Buren	2	6
	Valais Blackneck	1	3
	Nera Verzasca	1	3

4

1 **Supplementary Table I**

2 Descriptive statistics of categorical variables in a study to determine risk factors for SRLV

3 seropositivity (n=341).

Variable	Description	Categories	n	%
Alpine pasture	Goats taken on alpine pasture in the summer	Yes	134	39
		No	207	61
Animal show	Goats taken to breed shows or goat markets at least every other year	Yes	112	33
		No	229	67
Breeding	Breeding of animals done on farm	Yes	290	85
		No	51	15
Contact alpine	Common alpine pasture with foreign goats/sheep	Yes	106	31
		No	235	69
Contact sheep	Keep goats and sheep together with direct contact	Yes	64	19
		No	277	81
Contact wildlife	Observed contact (<5meters) with wildlife animals	Yes	89	26
		No	252	74
Farm type	Goat farm production type	Milk	131	38
		Other	210	62
Know-how	Know-how based on agriculture education and/or goat association membership	Yes	281	82
		No	60	18
Purchase	Purchase of at least one goat per year	Yes	142	42
		No	199	58
Tested before 2012	Farm tested for SRLV before the 2012 census (2006-2011)	Yes	191	56
		No	150	44

4

1   **Supplementary Table II**
2   Characterization of continuous variables in a study to determine risk factors for SRLV
3   seropositivity (n=341).

Variable	Mean	Median	Min	Max
Number of goats per farm				
Overall (n=341)*	32.2	14	1	300
CAEV (n=32)	34.5	11	3	165
VMV (n=100)	36.7	12.5	2	300
SRLV (n=125)	35.4	12	2	300
Controls (n=131)	30.0	13	1	270
Number of bucks (n=341)	1.9	1	0	26
Number of purchased goats / year (n=142)†	2.7	1.5	0	60
Number of sheep (n=80)††	32.6	11.5	2	600

4   * including CAE positive by default tested farms
5   † only for farms purchasing goats
6   †† only for those farms where sheep were also present on the farm

1 **Supplementary Table III**

2 Univariable analysis of variables tested for their association with VMV seropositivity in case (n=100) and control (n=131) herds.

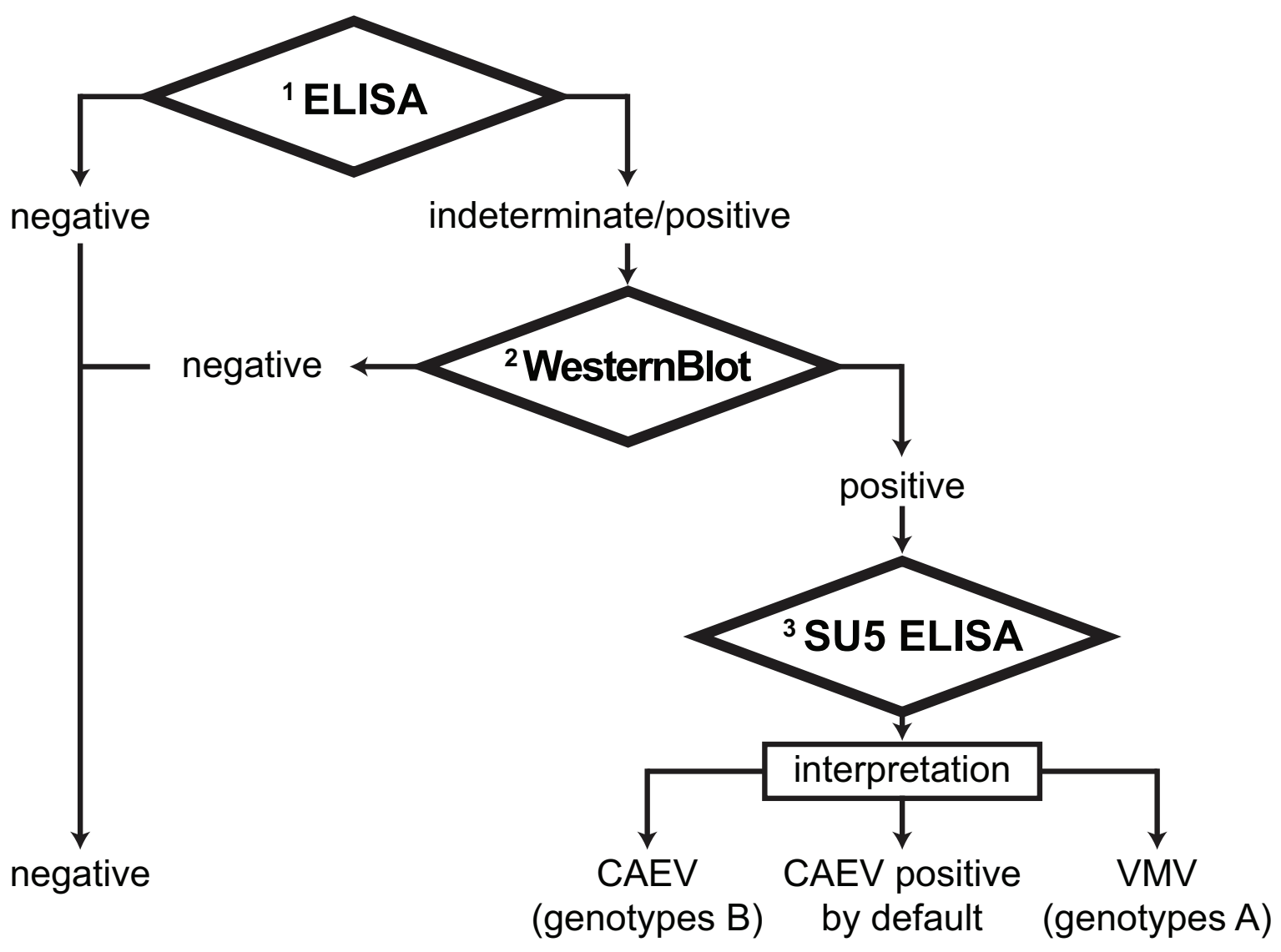| Variable	Description	Cases		Controls		*p* value	Odds Ratio	Odds Ratio (95% CI)
		n	%	n	%			
Animal show	Goats taken to breed shows or goat markets at least every other year	30	30.0	41	31.3	0.832	0.94	0.53-1.66
Breeding	Breeding of animals done on farm	82	82.0	116	88.5	0.162	0.59	0.28-1.24
Breed Appenzell	Appenzell breed present in herd	7	7.0	3	2.3	0.097	3.21	0.81-12.75
Breed dwarf goat	Dwarf goat breed present in herd	18	18.0	17	13.0	0.293	1.47	0.72-3.03
Contact alpine	Common alpine pasture with foreign goats/sheep	34	34.0	37	28.2	0.348	1.31	0.75-2.30
Contact sheep	Keep goats and sheep together with direct contact	21	21.0	21	16.0	0.333	1.39	0.71-2.72
Herd size						0.071		
	Medium herd (7-40 goats)	39	39.0	71	54.2	(ref)	1.00	-
	Small herd (1-6 goats)	33	33.0	33	25.2	0.059	1.82	0.98-3.39
	Large herd (>40 goats)	28	28.0	27	20.6	0.058	1.89	0.98-3.64
Know-how	Know-how based on agricultural education and/or membership in a goat association	78	78.0	114	87.0	0.072	0.53	0.26-1.06
Own buck	Breeding done with own buck(s)	55	55.0	87	66.4	0.078	0.62	0.36-1.06
Purchase	Purchase of at least one goat per year	36	36.0	59	45.0	0.167	0.69	0.40-1.17
Purchase female	Purchase of at least one female goat per year	18	18.0	20	15.3	0.579	1.22	0.61-2.45
Tested before 2012	Farm tested for SRLV before the 2012 census (2006-2011)	63	63.0	72	55.0	0.219	1.40	0.82-2.38
Wildlife						0.169		
	No observed contact with wildlife	70	70.0	98	74.8	(ref)	1.00	-
	Observed contact with ibex	10	10.0	5	3.8	0.071	2.80	0.92-8.55
	Observed contact with chamois and other Swiss wildlife	20	20.0	28	21.4	1.000	1.00	0.52-1.92

3

Figure1

Figure2
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SRLV positive herds

▲ CAEV positive (41), including dual infected (CAEV & VMV) herds (12)

○ VMV positive (284)

Goats and sheep per km² (community-level)

< 10
11 - 50
51 - 100
> 100

0   25   50   100 kilometers