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Original article

Diagnostic performance of ID Screen® MVV-CAEV Indirect Screening ELISA in identifying small ruminant lentiviruses-infected goats

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Abstract

Diagnostic performance of ID Screen® MVV-CAEV Indirect Screening ELISA in identifying goats infected with small ruminant lentiviruses (SRLV) was evaluated. In total 299 serum samples from the collection of the Laboratory of Veterinary Epidemiology and Economics – 109 truly positive and 190 truly negative – were used. To be enrolled in the study a serum sample had to come from at least 2 year-old goat which had reacted identically in two serological surveys preceding sample collection and was kept in a herd of stable serological status confirmed at least twice during preceding 5 years. Moreover, in seropositive herds at least 20% of goats had to be serologically positive at the moment when the serum sample was collected for the study. The test proved to have high accuracy. Area under curve was 98.8% (95% CI: 97.5%, 100%). Diagnostic performance of the test was almost identical (Youlden's index of 90%, sensitivity >90% and specificity >95%) within a fairly wide range of cut-off values – between 20% and 60%. At manufacturer's cut-off of 50% sensitivity and specificity were 91.7% (95% CI: 85.0%, 95.6%) and 98.9% (95% CI: 96.2%, 99.7%), respectively. For this cut-off positive likelihood ratio was 87 (95% CI: 22, 346) and negative likelihood ratio was 0.08 (95% CI: 0.04, 0.16). In conclusion, the results of this study indicate that ID Screen® MVV-CAEV Indirect Screening ELISA is a highly accurate diagnostic test for SRLV infection.

Key words: small ruminant lentiviruses, caprine arthritis-encephalitis, sensitivity, specificity, ELISA, goat

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Introduction

Small ruminant lentiviruses (SRLV) are a group of closely related genotypes pathogenic for small ruminants. In goats they cause chronic progressive wasting disease called caprine arthritis-encephalitis (CAE). The virus is typically acquired through ingestion of contaminated colostrum and milk, less often via long direct contact between animals. The infection develops slowly and no symptoms are usually apparent for several years. Nonetheless, an infected goat sheds the virus and spreads the infection to other animals in a herd. Hence early identification and elimination of infected animals is crucial for disease control (Patel et al. 2012).

At present, two existing approaches to diagnosing SRLV infection are based on detecting either genetic material of the virus using PCR or antibodies to the virus by means of various serological tests. Even though the infection is lifelong the amount of provirus in blood fluctuates, and occasionally drops to undetectable levels. This renders PCR sensitivity unsatisfying (de Andrés et al. 2005). Serum antibody levels seem to be far more stable and thus serological tests are the mainstay of CAE diagnostics. Historically, agar gel immunodiffusion test (AGID) had been the basic serological method, nowadays substituted by more sensitive immunoenzymatic tests (ELISA) which are now recognized by OIE as the test prescribed for international trade (www.oie.int/manual-of-diagnostic-tests-and-vaccines-for-terrestrial-animals/). Although a competitive ELISA based on monoclonal antibodies to viral envelope antigen has been developed (Herrmann et al. 2003), indirect tests employing either whole virus or recombinant envelope, transmembrane and core proteins as antigens are most commonly used worldwide (de Andrés et al. 2005). A few ELISAs are currently available on the market, one of which is competitive, and the remaining are indirect. Many other have been developed but never commercialized. Diagnostic performance has been investigated and reported for most of them, and their sensitivity (Se) and specificity (Sp) range from 56% to 100% and 95% to 100%, respectively (Archambault et al. 1988, Heckert et al. 1992, Herrmann et al. 2003, Brinkhof and van Maanen 2007).

Recently, a new indirect whole virus ELISA for SRLV infection has emerged on the market. Therefore, the investigation was carried out to evaluate its diagnostic performance in identifying SRLV-infected goats since these data are essential for application of this test in epidemiological studies.

Materials and Methods

Serum samples and reference procedure

In total 299 serum samples from the collection of the Laboratory of Veterinary Epidemiology and Economics – 109 truly positive and 190 truly negative – were used. To be enrolled in the study a serum sample had to come from a goat which: 1) was at least 2 year-old; 2) had reacted identically in two serological surveys preceding sample collection; 3) was kept in a herd of stable serological status confirmed at least twice during preceding 5 years. Moreover, in seropositive herds at least 20% of goats had to be serologically positive at the moment when the serum sample was collected for the study. All the samples were tested with one of two other ELISAs – Chekit CAEV/MVV monophasic (Dr. Bommeli AG, Bern, Switzerland) and ELISA MAEDI VISNA/CAEV (Institut Pourquier, Montpellier, France). According to Brinkhof and van Maanen (2007) both tests had Se of 94% and Sp of 97%. Serum samples enrolled came from herds for which prevalence rate was either approaching 0% (in the case of healthy individuals; for the needs of calculations within-herd prevalence of 1% was assumed) or exceeding 20% (in the case of diseased individuals). Parallel testing performed in a population in which prevalence was 1% yielded 100% probability that a sample was truly negative. On the other hand, serial testing performed in a population in which prevalence was at least 20% yielded 99.6% probability that a sample was truly positive.

ELISA and testing protocol

ID Screen® MVV-CAEV Indirect Screening ELISA (IDvet Innovative Diagnostics) is a whole-virus indirect test. The test was performed according to the manufacturer's manual (VISNAS ver. 0312 GB). Briefly, 10 µl of control negative serum (wells A1-A2), control positive serum wells (B1-B2), and tested sera were mixed with 190 µl of dilution buffer and incubated at 21°C for 45 min. Then, the plate was washed three times, filled with 100 µl of properly diluted anti-ruminant IgG-HRP conjugate and incubated at 21°C for 30 min. Finally, after last washing the plate was filled with 100 µl of the 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution and after 15-min. incubation the reaction was ceased with 100 µl of the sulfuric acid-based stop solution. The plate was read at 450 nm wavelength in the BioTek microplate reader. The results were considered valid only if optical density of a positive control serum

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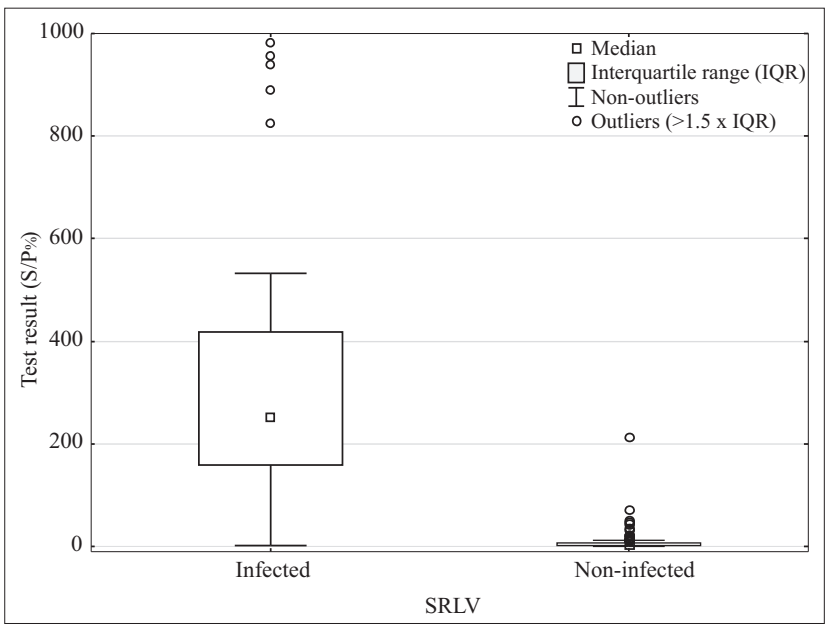

Fig. 1. Box plot of the ELISA results by disease status.

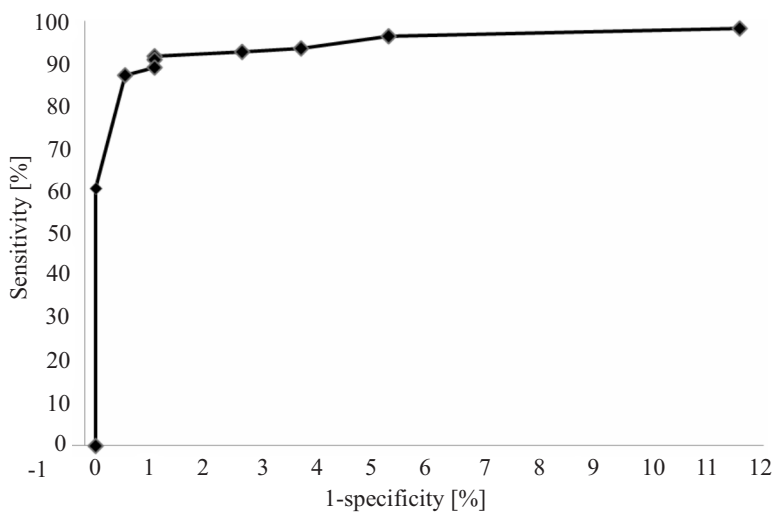

Fig. 2. Receiver operating characteristic (ROC) curve of the ELISA for detection of SRLV-infected goats.

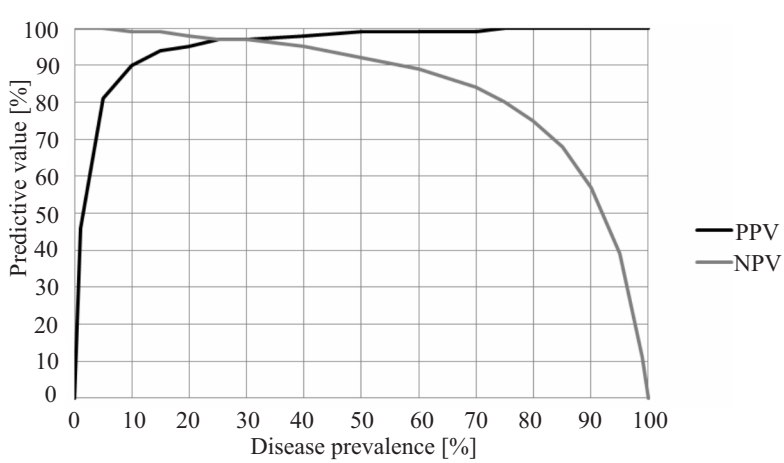

Fig. 3. Positive (PPV) and negative (NPV) predictive value of the test result by SRLV-infection prevalence in population.

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Table 1. Accuracy of the ELISA at different cut-off values.

Cut-off	Number of goats		Se	95% CI for Se		Sp	95% CI for Sp		*J*	95% CI for *J*	
	diseased	healthy									
10%	2	168	98.2%	93.6%,	99.5%	88.4%	83.1%,	92.2%	86.6%	81.4%,	91.8%
20%	2	12	96.3%	90.9%,	98.6%	94.7%	90.6%,	97.1%	91.1%	86.3%,	95.8%
30%	3	3	93.6%	87.3%,	96.9%	96.3%	92.6%,	98.2%	89.9%	84.6%,	95.2%
40%	1	2	92.7%	86.2%,	96.2%	97.4%	94.0%,	98.9%	90.0%	84.6%,	95.4%
50%	1	3	91.7%	85.0%,	95.6%	98.9%	96.2%,	99.7%	90.7%	85.3%,	96.1%
60%	1	0	90.8%	83.9%,	94.9%	98.9%	96.2%,	99.7%	89.8%	84.2%,	95.4%
70%	2	0	89.0%	81.7%,	93.6%	98.9%	96.2%,	99.7%	87.9%	81.9%,	94.0%
80%	2	1	87.2%	79.6%,	92.2%	99.5%	97.1%,	99.9%	86.6%	80.3%,	93.0%
220%	29	1	60.6%	51.2%,	69.2%	100.0%	98.0%,	100.0%	60.6%	51.4%,	69.7%
990%	66	0	0.0%	0.0%,	3.4%	100.0%	98.0%,	100.0%	60.6%	51.4%,	69.7%

(OD_{PC}) was higher than 0.350 and OD_{PC} was more than three times higher than optical density of a negative control serum (OD_{NC}).

Then optical density of a serum sample (OD_{sample}) was recalculated into percentage of OD_{PC} ($S/P_{\%}$) adjusted by OD_{NC} with the formula:

$$S/P_{\%} = (OD_{sample} - OD_{NC}) / (OD_{PC} - OD_{NC}) \times 100\%$$

Statistical analysis

Distribution of continuous test results in SRLV-infected and uninfected goats was described using median (Me), interquartile range (IQR) and positional coefficient of variability (CV_Q), and displayed on a box-and-whisker plot. Tukey's rule was used to identify outliers (Tukey, 1977).

Relationship between sensitivity (Se) / specificity (Sp) and cut-off value were presented using receiver operating characteristic (ROC) curve, for which area under curve (AUC) was computed. Confidence interval of 95% (95% CI) was computed for Se and Sp at each value of the cut-off using Wilson's score method (Altman et al. 2000). The most optimal cut-off value was chosen on the basis of Youlden's index (*J*) (Thrusfield 2005). For this cut-off value predictive value for positive (PPV) and negative (NPV) result were presented for the whole range of possible prevalence rates. Moreover, likelihood ratios of the positive (LR+) and negative (LR-) result along with logarithmic method 95% CI were provided (Thrusfield 2005). Calculations were performed in EpiTools (Sergeant 2014) and WinEpiscope while plots were prepared in Excel (Microsoft Office 2007) and Statistica 10 (StatSoft Inc.).

Results

Median $S/P_{\%}$ (IQR) for infected and uninfected goats were 252.1% (263.8%) and 3.2% (4.2%), respectively. In both groups $S/P_{\%}$ were equally dispersed (CV_Q of 53% and 66%, respectively) (Fig. 1).

The ELISA proved to have high accuracy. AUC was 98.8% (95% CI: 97.5%, 100%) (Fig. 2). Diagnostic performance of the test was almost identical (*J* of 90%, Se >90% and Sp >95%) within a fairly wide range of cut-off values – between 20% and 60% (Table 1). Manufacturer's cut-off of 50% ensured both PPV and NPV of at least 80% for the range of prevalence rates between 5% and 75%, whereas both PPV and NPV of at least 90% for the range of prevalence rates between 10% and 60%, with simultaneous PPV and NPV of 95% for prevalence of 25-30% (Fig. 3). For manufacturer's cut-off value LR+ was 87 (95% CI: 22, 346) and LR- was 0.08 (95% CI: 0.04, 0.16).

Discussion

Reliability of any study regarding test accuracy depends mostly on correct determination of true health status of studied animals. This, in turn, depends on quality of gold standard used. Procedures applied so far in investigations regarding accuracy of ELISAs for SRLV infection have mostly consisted in comparison with another imperfect test such as agar gel immunodiffusion (Castro et al. 1999), radioimmunoprecipitation (Vander Schalie et al. 1994, Herrmann et al. 2003), western blotting (Clavijo and Thorsen 1995) or another ELISA (Simard et al. 2001). Rarely, has it been based on comparison with

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combination of two or three of the aforementioned tests (Hecker et al. 1992, Rimstad et al. 1994, Kwang et al. 1995). Given that no test can be considered 100% sensitive and specific more complex procedure was employed in our study to declare a goat healthy or diseased. To meet eligibility criteria a goat not only had to react identically in two consecutive serological surveys carried out with one of two widely recognized ELISAs but it also had to come from a herd of a certain well-evidenced serological status. Such a protocol raised probability of a right classification of study goats to nearly 100%.

Both Se and Sp of the test are comparable with other ELISAs for SRLV infection. The test proves to be a useful diagnostic tool ensuring high validity of both positive and negative results. Its result strongly affects the probability of the disease in a goat – positive result increases the pre-test probability roughly 90-fold while negative result decreases it more than 10-fold. Moreover, changing the cut-off within the range between 10% and 80% ensures Se of 98% or Sp of over 99% depending on current user's needs.

Main limitation to the study is the fact that SRLV genotype responsible for infection in study goats was not determined. Given that accuracy of ELISAs can be affected by genotype of the infecting virus (Carroza et al. 2009, de Andrés et al. 2013), it is possible that diagnostic performance may differ between goat populations. However, Polish goats seem to be infected mainly with genotypes A and B (Olech et al. unpublished data), which also predominate in other European countries (Kuhar et al. 2013, Rachid et al. 2013). This fact allows for cautious generalization of obtained results to other European goat populations.

Even though the number of samples from known-infected and known-uninfected animals was much lower than required by OIE standards (300 and 1000 animals, respectively) (Jacobson 1996) it is comparable with vast majority of other studies (de Andrés et al. 2005), of which none have met OIE criterion. Furthermore, a number of enrolled animals allowed for fairly precise estimation of parameters as indicated by quite narrow 95% confidence intervals.

In conclusion, the results of this study imply that ID Screen® MVV-CAEV Indirect Screening ELISA is a highly accurate diagnostic test for SRLV infection in goats.

Acknowledgements

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References

Altman D, Macnin D, Bryant T, Garder M (2000) Statistics with confidence intervals and Statistical Guidelines, 2 nd ed BMJ Books p 45-48.

de Andrés D, Klein D, Watt NJ, Berriatua E, Torsteinsdottir S, Blacklaws BA, Harkiss GD (**2005**) Diagnostic tests for small ruminant lentiviruses. Vet Microbiol 107: 49-62.

de Andrés X, Ramtrez H, Bertolotti L, San Román B, Glaria I, Crespo H, Jáuregui P, Minguijón E, Juste R, Leginagoikoa I, Pérez M, Luján L, Badiola JJ, Polledo L, Garcta-Marín JF, Riezu JI, Borrás-Cuesta F, de Andrés D, Rosati S, Reina R, Amorena B (**2013**) An insight into a combination of ELISA strategies to diagnose small ruminant lentivirus infections. Vet Immunol Immunopathol 152: 277-288

Archambault D, East N, Perk K, Dahlberg JE (**1988**) Development of an enzyme-linked immunosorbent assay for caprine arthritis-encephalitis virus. J Clin Microbiol 26: 971-975.

Brinkhof J, van Maanen C (**2007**) Evaluation of five enzyme-linked immunosorbent assays and an agar gel immunodiffusion test for detection of antibodies to small ruminant lentiviruses. Clin Vaccine Immunol 14: 1210-1214.

Carrozza ML, Mazzei M, Lacerenza D, Del Chiaro L, Giammarioli M, Marini C, Rutili D, Rosati S, Tolari F (**2009**) Seroconversion against SU5 derived synthetic peptides in sheep experimentally infected with different SRLV genotypes. Vet Microbiol 137: 369-374.

Castro RS, Leite RC, Resende M, Gouveia AM (**1999**) A labelled avidin-biotin ELISA to detect antibodies to caprine arthritis-encephalitis virus in goats' sera. Vet Res Commun 23: 515-522.

Clavijo A, Thorsen J (**1995**) Serologic diagnosis of caprine arthritis-encephalitis by ELISA with two recombinant proteins in a parallel testing format. J Immunoassay 16: 419-436.

Jacobson RH (**1996**) Validation of serological assays for diagnosis of infectious diseases. In: Manual of Standards for Diagnostic Tests and Vaccines, OIE, France, pp 8-15.

Heckert RA, McNab WB, Richardson SM, Briscoe MR (**1992**) Evaluation of an enzyme-linked immunosorbent assay for the detection of antibodies to caprine arthritis-encephalitis virus in goat serum. Can J Vet Res 56: 237-241.

Herrmann LM, Cheevers WP, McGuire TC, Adams DS, Hutton MM, Gavin WG, Knowles DP (**2003**) Competitive-inhibition enzyme-linked immunosorbent assay for detection of serum antibodies to caprine arthritis-encephalitis virus: diagnostic tool for successful eradication. Clin Diagn Lab Immunol 10: 267-271.

Kuhar U, Barlič-Maganja D, Zadnik T, Grom J (**2013**) Molecular and genetic characteristics of small ruminant lentiviruses in Slovenia. Acta Vet Hung 61: 135-146.

Kwang J, Keen J, Cutlip RC, Kim HS, de la Concha-Bermejillo A (**1995**) Serological diagnosis of caprine lentivirus infection by recombinant immunoassays. Small Ruminant Res 16: 171-177.

Patel JR, Heldens JG, Bakonyi T, Rusvai M (**2012**) Important mammalian veterinary viral immunodiseases and their control. Vaccine 30: 1767-1781.

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Rachid A, Croisé B, Russo P, Vignoni M, Lacerenza D, Rosati S, Kuzmak J, Valas S (**2013**) Diverse host-virus interactions following caprine arthritis-encephalitis virus infection in sheep and goats. J Gen Virol 94: 634-642.

Rimstad E, East N, DeRock E, Higgins J, Pedersen NC (**1994**) Detection of antibodies to caprine arthritis-encephalitis virus using recombinant gag proteins. Arch Virol 134: 345-356.

Simard C, Kibenge MT, Singh P, Dixon P (**2001**) Simple and rapid method for production of whole-virus antigen for serodiagnosis of caprine arthritis-encephalitis virus by enzyme-linked immunosorbent assay. Clin Diagn Lab Immunol 8: 352-356.

Tukey JW (**1977**) Exploratory data analysis. Ed Addison-Wesley, pp 87-113.

Thrusfield M **(2005)** Veterinary epidemiology. 3rd ed., Blackwell Publishing Ltd., Oxford, pp 305-330.

Vander Schalie J, Bradway DS, Besser TE, Evermann JF (**1994**) Evaluation of a kinetic enzyme-linked immunosorbent assay for detection of caprine arthritis-encephalitis virus-specific antibodies. J Vet Diagn Invest 6: 30-33.

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