

Subclinical mastitis and clinical arthritis in French Alpine goats serologically positive for caprine arthritis-encephalitis virus

**Bruna Tariba^{1*}, Antun Kostelić¹, Dragica Šalamon¹, Besi Roić³,
Miroslav Benić³, Nikica Prvanović Babić², and Krešimir Salajpal¹**

¹*Department of Animal Science, Faculty of Agricultural, University of Zagreb, Zagreb, Croatia*

²*Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

³*Croatian Veterinary Institute, Zagreb, Croatia*

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ABSTRACT

The prevalence and aetiology of subclinical mastitis in goats seropositive for Caprine Arthritis Encephalitis Virus (CAEV) and the connection with the occurrence of clinical arthritis were investigated on 543 French Alpine goats from intensive production farms in North West Croatia. The aim was to determine if seropositivity to CAEV is connected with susceptibility to subclinical mastitis of bacterial aetiology, and to examine if CAEV is the main reason for the occurrence of clinical arthritis in the investigated sample. All the goats were examined clinically and the presence of arthritis was noted. The blood samples were tested for CAEV antibodies using the immunoenzyme test. Milk samples from each udder half were examined for the bacteriological cause of mastitis. All the collected data were cross-classified in two-way contingency tables. Of the total number of goats, CAEV was serologically confirmed in 50.8%. Subclinical mastitis was confirmed in 52.3% of the goats. Both subclinical mastitis and seropositivity to CAEV were diagnosed in 30% of the total number. Of the total sample 21.9% were seropositive to CAEV and with clinical arthritis. Statistical tests confirmed a positive association for subclinical mastitis occurrence and seropositivity to CAEV. A similar result was obtained for the association between clinical arthritis and seropositivity to CAEV. A weak positive association between subclinical mastitis and clinical arthritis occurrence was also recorded. These results indicate that a developed clinical manifestation of the disease could also influence higher manifestation of subclinical mastitis. Results also suggest that CAEV infection could be one of the main reasons for clinical arthritis occurrence in the investigated herds.

Key words: goats, caprine arthritis encephalitis virus, seropositive, mastitis, arthritis, ELISA, bacteriology

*Corresponding author:

Bruna Tariba, Department of Animal Science, Faculty of Agriculture, University of Zagreb, Svetošimunska 25, 10000 Zagreb, Croatia, Phone: +385 1 2393 992; +385 1 2393 947; E-mail: btariba@agr.hr

Introduction

CAEV is a slow, progressive and incurable disease of goats, spread around the globe (PETERHANS et al., 2004). Due to the virus' ability for cross-infection between goats and sheep, it was classified in the group of small ruminant lentiviruses (SRLV) (LEROUX et al., 1995). Infected goats may stay in a state of unapparent infection for life, and spread the virus or develop various clinical forms, such as: arthritis, synovitis, neurological dysfunctions, indurations of the udder, chronicle interstitial pneumonia, and general wasting (BLACKLAWS et al., 2004). The virus spreads primarily through herds by vertical transmission from dam to kids by colostrum and milk, or through feeding kids with unpasteurized colostrum/milk from CAEV-infected goats (LEITNER et al., 2010). Horizontal transmission is equally important through cohabitation of infected and uninfected animals (ROWE and EAST, 1997). Less investigated routes of transmission are: in utero, contact with the vagina of an infected doe during parturition, via infected blood, with equipment or iatrogenic (ADAMS et al., 1983, ROWE et al., 1991; ROWE et al., 1992; ROWE and EAST, 1997).

French Alpine goats are the main breed for milk production in Croatia. According to the Croatian Agricultural Agency Annual Report (CAA, 2014) there are around 5,500 French Alpine goats in Croatia and 83% of the population is located in the North West of the country. The first herds of the French Alpine breed were imported to Croatia two decades ago. CAEV was first noted and its prevalence was measured by ČAČ et al. (1996), at 7.7% of a sample of 365 animals taken from 29 herds in North West Croatia.

Since CAEV was defined as an immunological disease, causing indurations of target organs and as the udder is one of the main routes of spread of the virus (DESPOINT, 2010), we wanted to determine its prevalence in NW Croatia today. Additionally, we wanted to determine if CAEV seropositivity influenced susceptibility to subclinical mastitis of bacterial aetiology, and to evaluate if clinical arthritis, occurring in herds of French Alpine goats, is connected exclusively to CAEV infections.

Materials and methods

We conducted the research on 543 goats from 6 herds of the French Alpine breed in North West Croatia during 2013. All the animals were kept under intensive milk production conditions with access to an open area. The choice of farms was focused on the largest ones (more than 50 goats) and those operating continuously from the first French Alpine goat imports, enclosed and without animal introduction from other herds, and registered in a breeding program.

The goats had a similar diet and were milked twice a day by machines. Kids were not separated from their dams, so they fed by suckling. Due to these facts we considered that it was possible to investigate how CAEV prevalence increased in separate, enclosed

production units. The sample did not include goats culled due to low production, illness or infertility.

All the goats were clinically examined. The examination included assessment of general condition and gait, as well as inspection of the leg joints. The presence of clinical arthritis (cold swelling and stiffness, with lower mobility) was noted bilaterally. As in BERTONI and BLACKLAWS (1994), animals with a score >7 cm between carpal circumference and metacarpal circumference in at least one joint were considered clinically affected, and animals with a score of <5 cm were considered clinically healthy (asymptomatic).

We collected samples of blood from the jugular vein. Samples of milk were taken from all lactating goats in the first trimester of lactation, during the evening milking, from the foremilk of disinfected teats and after milking out the first flushes of milk. Samples were taken in separate sterile tubes from each udder half. Samples were transported at 4 °C. The infection was diagnosed using the serological immunoenzyme test Chekit CAEV/MVV (Idexx, Switzerland) by the official method for diagnostics of CAEV in Croatia and the method suggested by the World Organisation for Animal Health (OIE, 2007).

Bacteriological tests were performed on the milk samples from each udder half in the referent Laboratory for Mastitis and Raw Milk Quality in the Croatian Veterinary Institute according to standard testing procedures. The data were cross-classified in two-way contingency tables. The associations were tested between CAEV seropositivity and occurrence of subclinical mastitis; CAEV seropositivity and the occurrence of clinical arthritis; and between the occurrence of clinical arthritis and subclinical mastitis, using Chi-square and Fisher's exact test. Contingency was furthermore measured using Pearson's Phi coefficient, also referred to as the mean square contingency coefficient ranging from -1 to +1. All statistical analyses were performed using FREQ procedure of SAS program (SAS, 2004).

Results

CAEV prevalence. The total CAEV prevalence recorded was 50.8% of all the examined French Alpine goats (Fig. 1). CAEV was recorded in all the examined herds.

Clinical arthritis. Out of the entire population clinically examined, clinical arthritis was diagnosed in 31.6% of the animals. Of the total sample number, 21.9% of goats were CAEV infected and with the occurrence of clinical arthritis. A positive association between clinical arthritis and seropositivity to CAEV was confirmed in the examined number of samples, with a Phi coefficient of 0.25 ($P < 0.01$). Clinical arthritis was found in 9.6% of the samples with CAEV seroprevalence not confirmed (Fig. 1).

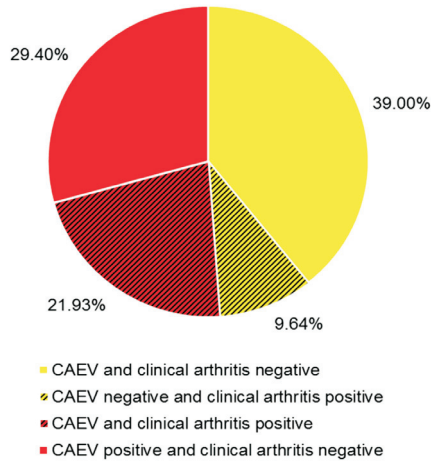


Fig. 1. Prevalence of CAEV and appearance of clinical arthritis

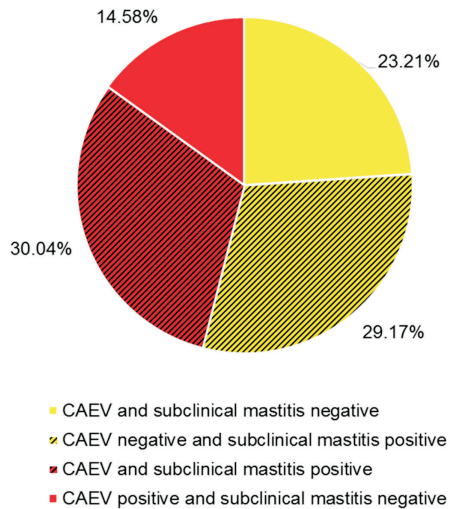


Fig. 2. Subclinical mastitis occurrence in relation to CAEV prevalence

Subclinical mastitis. Subclinical mastitis was diagnosed in 52.3% of the examined goats. Coagulase negative staphylococci (CNS) had the highest prevalence in goat mastitis, and was recorded as a causative agent of subclinical mastitis in 25.6% of the

examined samples. Other bacterial causes appeared in less than 4% and were, in order: *Streptococcus dysgalactiae* (3.5%); *Staphylococcus aureus* (2.8%); *Streptococcus uberis* (1.2%); *Pseudomonas* spp. (0.7%); *E. coli* (0.5%); *Enterobacter* spp., *Pasterella*, *Klebsiella* spp., and *Bacillus* spp. (together <0.5%).

In 30% of the samples, CAEV seroprevalence was concurrent with subclinical mastitis. Additionally, 23.2% of goats were positive for subclinical mastitis but free of the virus. Our analysis confirmed a positive association between subclinical mastitis occurrence and seropositivity to CAEV with a Phi coefficient of 0.25 ($P < 0.01$) (Fig. 2).

Of the examined cases, 18.9% had both clinical arthritis and subclinical mastitis. Of the whole sample, 32% were positive for subclinical mastitis and showed no signs of clinical arthritis. However, a weak positive association between subclinical mastitis and clinical arthritis occurrence was determined, with a Phi coefficient of 0.24 ($P < 0.01$) (Fig. 2).

Discussion

The results obtained in the study showed that the prevalence in the examined goats was even higher than the CAEV prevalence of 6-47% reported in other countries (PETERHANS et al., 2004). Prevalence of CAEV recorded in a previous study by ČAČ et al. (1996) and ČAČ et al. (2000) (in the same area but with different methods of sampling) was substantially lower. Our research showed that the CAEV prevalence had significantly increased over the last two decades. Horizontal transmission of the CAEV virus might lead to cross - species transmission. Although CAEV is typical for goats in intensive production systems, possible horizontal transmission could endanger valuable autochthonous goat breeds (Croatian white and Croatian spotted goat) and sheep flocks, especially in conditions of cohabitation in mixed flocks of goats and sheep which happened in Italian flocks (PISONI et al., 2005).

The high prevalence of clinical arthritis recorded in CAEV seropositive goats was not found in similar research (GREWAL et al., 1986; TORRES-ACOSTA et al., 2003). The result indicates that CAEV could be the main reason for the clinical presentation of arthritis (SMITH and SHERMAN, 2009). The importance of the vertical transmission and the lack of serious eradication measures resulted in the rapid increase in CAEV prevalence during the period with no CAEV control program. Furthermore, the genetics of the animals or the virus strain could explain this result (BERTONI and BLACKLAWS, 2010).

We found the causes of subclinical mastitis to be in line with CONTRERAS et al. (2003), who stated that CNS was the main causative agent of subclinical mastitis, but they differed from those recorded by KOSTELIĆ et al. (2009), who reported *S. aureus* as the most frequent bacterial cause of subclinical mastitis in Croatian French Alpine goats. Improvement of milking technologies and higher udder hygiene are a possible explanation for that.

In 30% of the sample, CAEV seroprevalence was concurrent with subclinical mastitis. This is a higher percentage than that recorded in the research conducted by GREWAL et al.

(1986) and PISONI et al. (2005), who found that in herds with a high prevalence of CAEV only 15% of seropositive goats manifested clinical findings such as arthritis or mastitis. Although CONTRERAS et al. (2003) and LEITNER et al. (2010) did not find a significant association between subclinical mastitis occurrence and seropositivity to CAEV, our analysis confirmed this association. The association obtained in this study could be explained by the larger sample size and the higher prevalence of both subclinical mastitis and CAEV.

To our knowledge, co-occurrence of clinical arthritis and subclinical mastitis has not been investigated in other countries. Although causality cannot be confirmed by the tests that we performed in this study alone, the weak positive association between subclinical mastitis and serologically confirmed arthritis in the examined number of samples suggests that a serious CAEV eradication program might also lead to a decrease in the prevalence of subclinical mastitis, and consequently to an improvement in milk quality. Additionally, our results indicate that the occurrence of clinical arthritis could also be reduced by this program.

The high representation of clinical arthritis in the CAEV seropositive cases implies that further investigation of the virus strain is needed to explain this. Moreover, the presence of clinical arthritis in goats without CAEV reveals the need for further investigation of other possible causative agents.

The importance of vertical transmission and the lack of serious eradication measures resulted in a rapid increase in CAEV prevalence during the period with no CAEV control program (TARIBA et al., 2010). Horizontal transmission of the CAEV virus might lead to cross-species transmission. The recorded high prevalence of CAEV on Croatian French Alpine dairy goat farms and the further risks it represents for the small ruminant production, are the main reasons why a CAEV control program should be created urgently in Croatia. Although the production of French Alpine goats in Croatia has developed rapidly over the last two decades, replacement feeding or pasteurization of the colostrum and the milk for kids, as well as the eradication of CAEV through separation of kids from the infected dams will be expensive and labour intensive. Therefore, creation and implementation of a CAEV control program is a great challenge for Croatian dairy goat production.

Conclusion

The prevalence of CAEV has increased enormously over the last two decades, and is resulting in the appearance of subclinical mastitis. In total, we can conclude that serious and appropriate CAEV eradication and control measures should be developed and applied to reduce the prevalence of CAEV disease and possibly also improve the milk quality production.

References

- ADAMS, D. S., P. KLEVJER-ANDERSON, J. L. CARLSON, T. C. MCGUIRE, J. R. GORHAM (1983): Transmission and control of caprine arthritis-encephalitis virus. *Am. J. Vet. Res.* 44, 1670-1675.

- BERTONI, G., B. BLACKLAWS (2010): Small Ruminant Lentiviruses and Cross-Species Transmission. (M. Desport, Ed.). Caister Academic Press. Norfolk UK.
- BERTONI, G., M.-L. ZAHNO, R. ZANONI, H. R., VOGT, E. PETERHANS, G. RUFF, W. P., CHEEVERS, P. SONIGO, G. PANCINO (1994): Antibody reactivity to the immunodominant epitopes of the caprine arthritis-encephalitis virus gp38 transmembrane protein associates with the development of arthritis. *J. Virol.* 68, 7139-7147.
- BLACKLAWS, B. A., E. BERRIATUA, S. TORSTEINSDOTTIR, N. J. WATT, D. DE ANDRES, D. KLEIN, G. D. HARKISS (2004): Transmission of small ruminant lentiviruses. *Vet. Microbiol.* 10, 199-208.
- CROATIAN AGRICULTURAL AGENCY ANNUAL REPORT (2014): Breeding of sheep, goats and small animals, (Z. Barač, Ed.). CCA, Križevci 2014.
- CONTRERAS, A., C. LUENGO, A., SANCHEZ, J. C. CORRALES (2003): The role of intramammary pathogens in dairy goats. *Livestock Prod. Sci.* 79, 273-283.
- ČAČ, Ž., M. LOJKIĆ, L. JEMERŠIĆ (1996): Caprine arthritis-encephalitis syndrome - The first serological approval in Croatia. In: Proceedings of 1st Croatian Congress of Microbiology with International Participation (V. Delić, Ed.). Zagreb, Croatian Microbiological Society. p. 10.
- ČAČ, Ž., M. LOJKIĆ, B. ROIĆ, L. JEMERŠIĆ (2000): Serološka dijagnostika bolesti artritis encefalitis koza. *Prax. Vet.* 48, 167-172.
- DESPOINT, M. (2010): Lentiviruses and Macrophages. Caister Academic Press. Norfolk UK.
- GREWAL, A. S., R. W. BURTON, J. E. SMITH, E. M. BATTY, P. E. GREENWOOD, R. NORTH (1986): Caprine retrovirus infection in New South Wales: Virus isolations, clinical and histopathological findings and prevalence of antibody. *Austr. Vet. J.* 63, 245-248.
- KOSTELIĆ, A., M. CERGOLJ, V. RUPIĆ, B. TARIBA, M. BENIĆ, V. GANTNER, I. ŠTOKOVIĆ (2009): Prevalence and aetiology of subclinical mastitis in goats. *It. J. Anim. Sci.* 8, 134-136.
- LEITNER, G., O. KRIFUCKS, L. WEISBLIT, Y. LOVI, S. BERNSTEIN, V. MERIN (2010): The effect of caprine arthritis-encephalitis virus infection on production in goats. *Vet. J.* 183, 328-333.
- LEROUX, C., G. CORDIER, I. MERCIER, J. CHASTAND, M. LYON, G. QUÉRAT, T. GREENLAND, R. VIGNE, J. F. MORNEX (1995): Ovine aortic smooth muscle cells allow the replication of visna-maedi virus *in vitro*. *Arch. Virol.* 140, 1-11.
- OIE (2007): World Organisation for Animal Health; Terrestrial Animal Health Code. Chapter 2.4.4, Caprine arthritis encephalitis. 16th ed. Office International des Epizooties, Paris.
- PETERHANS, E., T. GREENLAND, J. BADIOLA, G. HARKISS, G. BERTONI, B. AMORENA, M. ELIASZEWICZ, R. A. JUSTE, R. KRASSNIG, J. P. LAFONT, P. LENIHAN, G. PETURSSON, G. PRITCHARD, J. THORLEY, C. VITU, J. P. MORNEX, M. PEPIN (2004): Routes of transmission and consequences of small ruminant lentiviruses (SRLVs) infection and eradication schemes. *Vet. Res.* 35, 257-274.
- PISONI, G., A. QUASSO, P. MORONI (2005): Phylogenetic analysis of small-ruminant lentivirus subtype B1 in mixed flocks: Evidence for natural transmission from goats to sheep. *Virol.* 339, 147-152.

- ROWE, J. D., N. E. EAST, M. C. THURMOND, C. E. FRANTI (1991): Risk factors associated with caprine arthritis-encephalitis virus infection in goats on California dairies. *Am. J. Vet. Res.* 52, 510-514.
- ROWE, J. D., N. E. EAST, C. E. FRANTI, M. C. THURMOND, N. C. PEDERSEN, G. H. THEILEN (1992): Risk factors associated with the incidence of seroconversion to caprine arthritis-encephalitis virus in goats on California dairies. *Am. J. Vet. Res.* 53, 2396-2403.
- ROWE, J. D., N. E. EAST (1997): Risk factors for transmission and methods for control of caprine arthritis-encephalitis virus infection. *Vet. Clin. North Am. Food Anim. Pract.* 13, 35-53.
- SAS (2004): SAS/STAT Software, Release 9.1.3.
- SMITH, M. C., D. M. SHERMAN (2009): *The goat medicine*, 2nd ed. Ames, IA, Wiley-Blackwell, USA.
- TARIBA, B., A. KOSTELIĆ, B. ROIĆ, M. BENIĆ, D. ŠALAMON (2010): Effect of caprine arthritis-encephalitis virus on frequency of subclinical mastitis of French alpine goats in Croatia. In: *Proceedings of 39th Croatian Dairy Symposium with international participation.* (Samardžija D., V. Volarić, Eds.) Zagreb, Hrvatska mljekarska udruga, pp. 81-82.
- TORRES-ACOSTA, J. F. J., E. J. GUTIERREZ-RUIZA, V. BUTLERB, A. SCHMIDTB, J. EVANSB, J. BABINGTONB, K. BEARMANB, T. FORDHAMB, T. BROWNLIEB, S. SCHROERC, E. CÁMARA-GA, J. LIGHTSEYB (2003): Serological survey of caprine arthritis-encephalitis virus in 83 goat herds of Yucatan, Mexico. *Small Rum. Res.* 49, 207-211.

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SAŽETAK

Istražena je povezanost prevalencije i etiologije supkliničkog mastitisa s pojavnošću kliničkog artritisa u 543 francuske alpske koze serološki pozitivne na virus artritisa-encefalitisa (AEK) iz proizvodnih farmi sa sjeverozapada Hrvatske. Cilj je bio odrediti je li serološka pozitivnost na AEK povezana s pojavnošću supkliničkog mastitisa bakterijske etiologije i istražiti je li AEK glavni uzrok pojavnosti kliničkog artritisa u pretraženih koza. Sve su koze bile pregledane na pojavu kliničkog artritisa. Uzorci krvi iz jugularne vene bili su testirani na AEK imunoenzimnim testom. Uzorci mlijeka, uzeti iz svake polovine vimena zasebno, pretraženi su na prisutnost bakterijskih uzročnika mastitisa. Svi prikupljeni podatci unakrsno su klasificirani u kontingencijskim tablicama. Od ukupnog broja koza, AEK je serološki potvrđen u 50,8%. Supklinički mastitis je potvrđen u 52,3% koza. Istodobna pojava supkliničkog mastitisa i AEK zabilježena je u 30% od ukupnog broja, dok je 21,9% koza s kliničkim artritisom bilo serološki pozitivna na AEK. Statističke su analize potvrdile pozitivnu povezanost pojave supkliničkog mastitisa i AEK seropozitivnosti. Slični su rezultati dobiveni i za povezanost pojavnosti kliničkog artritisa i AEK seropozitivnosti. Također je zabilježena slaba pozitivna povezanost između supkliničkog mastitisa i kliničkog artritisa. Rezultati upućuju na to da bi AEK mogao utjecati na učestalost supkliničkog mastitisa. Rezultati također nalažu dodatno istraživanje AEK infekcije kao glavnog uzroka pojave kliničkog artritisa u istraženim stadima.

Ključne riječi: koze, artritis-encefalitis, serološki nalaz, mastitis, artritis, ELISA, bakteriologija
