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# Influence of Caprine Arthritis Encephalitis Virus infection on milk production of French Alpine goats in Croatia

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*Bruna Tariba*[1]*, *Antun Kostelić*[1], *Besi Roić*[2], *Miroslav Benić*[2], *Dragica Šalamon*[1]

[1]University of Zagreb, Faculty of Agriculture, Department of Animal Science I, Svetošimunska cesta 25, 10000 Zagreb, Croatia
[2]Croatian Veterinary Institute, Savska cesta 25, 10000 Zagreb, Croatia

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**Abstract**

We have investigated the influence of infection with Caprine Arthritis Encephalitis Virus (CAEV), developed clinical arthritis and recorded subclinical mastitis on the length of lactation, milk yield and its composition per lactation. The study was carried out on the sample of 808 goats of French Alpine breed from production farms in Croatia. Samples of blood were taken for serological tests and milk samples for subclinical mastitis detection. Presence of clinical arthritis was noted by inspection and palpation of the joints. Lactation and production data were collected by Croatian Agricultural Agency. The influence of infection status for CAEV and clinical arthritis, separately, were tested on milk yield, length of lactation and milk composition (fat, protein and lactose) using GLM procedure for models including the year of measurement, lactation number of the animal and subclinical mastitis record. CAEV was serologically confirmed in 53.72 % of the total number of goats. From the total sample 23.08 % were infected with CAEV with confirmed subclinical mastitis of bacteriological etiology. In addition, 22.47 % of the total sample had clinical arthritis. Of the total sample 16.95 % were CAEV infected and with diagnosed clinical arthritis. Influence of virus infection was confirmed on all of the analyzed traits, with lower production results. Clinical arthritis had significant influence on decrease in most of analyzed traits, exceptions being total milk, fat amount (%) and lactose content (%) and amount (kg). Diagnosed subclinical mastitis was not found to be significant influence in models of duration of lactation, or fat, protein and lactose content (%).

*Key words*: CAEV, clinical arthritis, milk production, subclinical mastitis

## Introduction

According to Croatian Agricultural Agency data (CAA, 2012) there are 4 imported (Saanen, French Alpine, German Improved Fawn and Boer) and 2 autochthonous breeds of goats reared in Croatia (Croatian white and Croatian spotted goat). The most represented of goat breeds is French Alpine with 7590 animals or 79.39 % of the total number of all milk production goats in Croatia (Mioč et al., 2012). This abundance is a result of growth of goat milk and cheese production industry in last few decades. All of those goats are under production control which is conducted according to the rules of International Committee for Animal Production - ICAR (ICAR, 2003). Controlled milk traits are: lactation length (days), total milk yield (kg/lactation), total milk fat amount (kg), protein and lactose amount (kg), as well as fat, protein and lactose content (%) in milk. Lactation of French Alpine goats lasts in average 269 days with the first period of kids suckling that lasts for about 34 days. Also, they have higher amount and content of milk fat in comparison to other milk breeds present in Croatia (CAA, 2012).

*Corresponding author/Dopisni autor: E-mail: btariba@agr.hr

Caprine arthritis encephalitis virus (CAEV), together with Maedi Visna virus (MVV) and Ovine progressive pneumonia virus (OPPV), belongs to the group of Small ruminant lentiviruses (SRLV), a member of VI. group of *Retroviridae* family, subfamily *Orthoretrovirinae*, genus *Lentivirus* (Petropoulos, 1997). CAEV has the ability to infect lineage monocyte/macrophages, with long incubation period and persistency of the virus despite strong immunological response of the host. Primary way of spreading the virus is via colostrum and milk (Peterhans et al., 2004), but the actual entrance doors and transmission are still unresolved. Herd management and intensive production systems play a major role in virus spreading throughout the herd and around (East et al., 1987; 1993; Greenwood, 1995). Virus dissemination also benefits from the goats' natural behavior (lambs suckling different dames, licking each other's external mucosae, nasal secretions present on muzzles of goats and in feed troughs, leaking of milk, or drinking urine as noted in sires) (Greenwood, 1995; Smith and Sherman, 2009). The risk for infection of healthy goats with infected milk cells during machine milking reflux is also very high. Research conducted on inoculation of the virus directly into the udder demonstrated creation of specific histological lesions after a very short time (Lerondelle et al., 1999). It was concluded that next to digestive system, the udder is one of possible entrance doors of the virus (McDougall et al., 2010). Seroprevalence was increasing rapidly in infected herds for years and that could not be explained only by vertical transmission of the virus (Rimstad et al., 1993). Cohabitation of infected and uninfected animals increased prevalence of the virus significantly (Smith and Sherman, 2009). Infected goats can stay in the state of unapparent infection and spread the virus for life or can develop various clinical forms of illness such are: arthritis, synovitis, neurological dysfunctions, indurations of the udder with hypogalactia, chronicle interstitial pneumonia, cachexia and general wasting (Blacklaws et al., 2004). Udder palpation can show extreme hardness, udder can even be as hard as stone without possibility of external pressure (Hard udder syndrome) in goats with appearance of normal lactation beside that, so should not be misinterpreted as physiological edema. General signs of infection in that case are not present so should not be confused with inflammation (the nearby skin stays relaxed and tissue is not caught with edematous changes, warmth or redness). Milking could appear as totally impossible during parturition (Gregorry et al., 2009). Such state is very important to recognize in order to successfully avoid losses of suckling kids or injuries of such udder by milking machine.

In this research, the influence of CAEV infection and the influence of clinical arthritis on lactation (length in days, total milk in kg) as well as on fat, protein and lactose content (%) and total amount (kg) were examined using models correcting for the year of sampling, number of lactation and subclinical mastitis record.

We analyzed clinical arthritis because we wanted to determine dominant causative agent of arthritis since preliminary research showed high presence of clinical arthritis in field conditions (Tariba et al., 2010). In addition, research by Lerondelle et al. (1989) brought out connection between virus caused intramammary lesions and arthritis development.

## Materials and methods

### *Goats*

Research was conducted on 808 goats of French Alpine breed, from 12 herds. Majority of research was concentrated in Međimurje, Varaždin and Istra County because that is the area of the highest Alpine goat farms concentration. Two of the herds were exceptions: one herd in Zagreb County and one in Osijek Baranja County. The samples were taken from all goats in lactation on the examined farms. All animals were kept in condition of intensive milk production, had similar nutrition and were milked twice a day by machine. All were bred by natural mating. Kids were not separated from dames because there was no possibility for replacement nutrition or pasteurization of colostrum and milk in sampled production farms, so they fed by suckling their dames. The sample excluded goats culled due to low production, illness or infertility.

### *Serological tests*

Blood samples were collected from the jugular vein for serological diagnosis of the virus and tested with enzyme-linked immunosorbent assay (ELISA).

Two lines of tests were performed: first, screening test with CAEV/MVV Antibody test kit CHEKIT CAEV/MVV Screening, (IDEXX, Switzerland). After positive reactions, verification tests with higher sensitivity and accuracy (CAEV/MVV Antibody test kit CHEKIT CAV/MVV Verification (IDEXX, Switzerland AG) were performed.

*Arthritis diagnosis*

All goats were clinically examined by assessment of general conditions and gait, as well as inspection and palpation of the extremities joints. Presence of clinical arthritis (cold swelling and stiffness with lower mobility) was noted bilaterally. Clinical arthritis was diagnosed according to the method described by Bertoni et al. (1994).

*Subclinical mastitis tests*

Milk samples were taken from each udder half in separate sterile tubes and investigated for the presence of mastitis using antibiogram according to accredited methods in Laboratory for Mastitis and Raw Milk Quality, Department for Bacteriology and Parasites, Croatian Veterinary Institute, Zagreb, Croatia. Subclinical mastitis was diagnosed in goats with bacteria confirmed in one or in both halves.

*Lactation, milk yield and content*

On all farms with goats registered in CAA selection programs, collection of milk samples and analysis of fat, protein and lactose content, together with measurement of production of each goat are performed once a month. Milk samples were collected using the ICAR method A4 (ICAR, 2003) during three years, and the overall lactation averages per goat were obtained for milk yield, length of lactation, lactose, protein and fat content and amount.

*Models*

The influence of CAEV infection status and clinical arthritis, on lactation milk yield, length of lactation as well as on lactation content and percentage of fat, protein and lactose, during three years of measuring, were tested by analysis of variance using GLM procedure (SAS, 2004) for the following model:

$$y_{ijk} = \mu + D_i + S_s + L_j + Y_k + \varepsilon_{ijk}$$

where: $y_{ijk}$ is the milk production trait (lactation milk yield; length of lactation; fat, protein and lactose content; and fat, protein and lactose amount) of the goat $k$ during lactation $j$; $\mu$ is the milk production trait average; $D_i$ is the diagnosis (of CAEV infection in first set of models, or of clinical arthritis in the other set) with $i=0\text{-}1$; $S_s$ is the diagnosis of subclinical mastitis with $i=0\text{-}1$; $L_j$ is the lactation number of the goat $(j=1\text{-}10)$; $Y_k$ is the year of measurement $(k=1\text{-}3)$; and $\varepsilon_{ijk}$ is the random error. CAEV infection status and clinical arthritis were modelled separately because of the confounding effect. Namely, CAEV is not the only cause of clinical representation of arthritis as shown in research of Tariba et al. (2010).

**Results and discussion**

Of the total number of goats examined, 53.72 % had serologically confirmed CAEV infection and 46.28 % were virus free. This prevalence differs substantially from 6.9 % reported by Čač et al. (2000), when illness was reported for the first time in Croatia (sample of 1290, conducted in Međimurje, Varaždin and Istra County). Čač et al. (2000), noted CAEV virus present in 68.08 % of examined herds, but we have found that only one herd of our sample was uninfected (91.7 % infected) (in Međimurje County). In research conducted by Kostelić et al. (2013) all of the examined French Alpine and Saneen herds were positive to CAEV.

Of the total number of animals 22.47 % manifested arthritis on at least one of the joints. Of that number 16.52 % were also CAEV infected, and 5.95 % had clinical arthritis and were virus free. In previous research of bacterial subclinical mastitis on Alpine goats, Tariba et al. (2011) found CNS and *S. aureus* to be predominant. These bacteria can also be suspected as the causative agents of arthritis together with subclinical mastitis (Mallikeswaran and Padmanaban, 1989; Vandenesch et al., 1995). These finding, as well as the percentage of goats showing signs of arthritis, but with no CAEV infection, support our choice of separate models for CAEV infection and clinical arthritis used. Both CAEV and subclinical mastitis on at least one half of the udder were diagnosed concurrently

in 23.08 % of goats, showing the importance of eliminating this possible effect in the models as important. Subclinical mastitis did not influence lactation length, fat, protein or lactose content in CAEV infection models, nor in clinical arthritis development models. Year and lactation were significant in all used models. As the Table 1 shows, we have confirmed significant negative influence of CAEV infection on total lactation milk yield, lactation length, and milk composition. Research in other countries shows similar results, except for the amount and content of lactose (Post et al., 1984; Smith and Cultip, 1988; Kreig and Peterhans, 1990). Measured negative influence of infection could be explained as the consequence of macrophage accumulation in lesions of udder caused by CAEV. High number of macrophages is common in the goat's udder during the dry period but not in lactation. Together with macrophages, uptake of T-lymphocytes by chemotaxis is accented (Desport, 2010). All those cells are accumulating in udder lesions and those accumulations develop into indurations, which finally reduce udder capacity and consequently milk production (Craigo and Montelaro, 2010). Changes in the number and proportions of some milk cells, reduced movement of animals as consequence of developed symptoms of illness and diminished food intake necessarily influence the changes in milk composition. Negative influence of CAEV infection on milk yield and quality inevitably has economic repercussions that in long terms may be higher than the cost of eradication of the virus from the population.

Results of models for clinical arthritis presented in Table 2 confirm negative influence of developed clinical arthritis on the lactation length and protein amount and content as well as fat amount. Although it could be expected that the developed clinical arthritis symptoms would have more severe influence on milk production, it was not so. Namely, the influence of clinical arthritis on total lactation milk yield and fat amount (kg) was not confirmed. This brings out CAEV as the potentially most severe negative influence on milk production. It can be concluded that diagnosis of the virus from blood should be taken seriously, and as pointed out by Kostelić et al. (2013), serious eradication measures should be undertaken in all herds diagnosed with virus. The development of clinical arthritis influences the milk content, but should not be used as possible indicator for culling in case of lenient CAEV eradication program.

Table 1. Influence of the serological status (CAEV infection) on total lactation, total milk production (milked and suckled) and composition of milk

| TRAIT | CAEV | | |
|---|---|---|---|
| | Seropositive LSM±SE | Seronegative LSM±SE | P |
| Length of lactation (days) | 229.14±3.84 | 224.31±3.80 | <0.01 |
| Total milk (kg / lactation) | 540.07±24.01 | 614.88±23.78 | <0.01 |
| Fat (%) | 3.4±0.10 | 3.54±0.10 | <0.01 |
| Fat (kg) | 16.48±0.99 | 19.62±0.98 | <0.01 |
| Protein (%) | 2.79±0.03 | 3.03±0.03 | <0.01 |
| Protein (kg) | 12.83±0.68 | 15.74±0.68 | <0.01 |
| Lactose (%) | 4.04±0.02 | 4.08±0.02 | <0.01 |
| Lactose (kg) | 18.71±0.92 | 20.92±0.91 | <0.01 |

CAEV - Caprine Arthritis Encephalitis diagnosis ($D_i$), as explained in the Models section in Materials and methods;
Seropositive LSM±SE - Least Square Means with Standard Errors obtained for CAEV factor values $D_i = 1$, balanced for the significant effects in the model: year of measurement ($L_l$), number of lactation ($Y_k$);
Seronegative LSM±SE - Least Square Means with Standard Errors obtained for CAEV factor values $D_i = 0$, balanced for the significant effects in the model: year of measurement ($L_l$), number of lactation ($Y_k$);
P - significance value of the tests for the difference of estimated least square means values for $D_i$ (i=0-1) using Tukey-Kramer adjustment for multiple comparisons
Trait - $y_{ijk}$ as defined in the Models section in Materials and methods

Recorded data are of great importance because of the fact that milk fat amount together with total lactation length give frame for formation of the price for the goat milk in EU. Immunological status of the animal is of the utmost importance in the development of CAEV symptoms. Observed goats even with developed symptoms managed to stay in high production, higher than in other countries in which the herd remount is accelerated because of infection (Contreras et al., 2007).

## Conclusions

The research brings out the conclusion that prevalence of CAEV in Croatia has increased unacceptably since the first report of the virus in the country. Obtained results confirmed significant negative influence of CAEV infection on total lactation and milk composition. Clinical arthritis had similar influence, but did not affect total lactation milk yield. Special attention should be given to plans of CAEV eradication because serious measures should include culling of all infected animals and that could mean great financial loss for the state as well as for the farmers. Since the research confirmed that there are herds in Croatia which are virus free, those should be used as a base for formation of new virus free herds.

## *Utjecaj infekcije virusom AEK na proizvodnju mlijeka francuske alpske koze u Hrvatskoj*

**Sažetak**

Istraživan je utjecaj infekcije virusom artritis encefalitisa koza (AEK), kliničkog artritisa i subkliničkog mastitisa na trajanje laktacije, ukupno mlijeko i sastav mlijeka. Istraživanje je provedeno na uzorku od 808 koza pasmine francuska alpska koza s proizvodnih mliječnih farmi u Hrvatskoj. Uzeti su uzorci krvi za serološka testiranja i uzorci mlijeka za detekciju subkliničkog mastitisa. Prisutnost kliničkog artritisa dijagnosticirana je pregledom i palpacijom zglobova. Podaci o laktacijama i proizvodnji preuzeti su od Hrvatske poljoprivredne agencije (HPA). Zasebno su testirani utjecaji AEK infekcije i kliničkog artritisa na ukupno mlijeko, trajanje laktacije i sastav mlijeka (mast, protein, laktoza) pomoću GLM procedure za modele koji su uključivali godinu mjerenja, broj laktacije i subklinički mastitis. Infekcija AEK virusom potvrđena je u 53,72 % koza od ukupnog broja koza. Od ukupnog uzorka 23,08 % inficirano je s AEK i imalo subklinički mastitis bakterijske etiologije. Nadalje 22,47 % od ukupnog broja imalo je klinički artritis. Od ukupnog broja 16,95 % bilo je AEK pozitivno i imalo klinički artritis. Utjecaj

Table 2. Influence of developed clinical arthritis on lactation milk production and its composition

| TRAIT | No arthritis LSM±SE | Clinical arthritis LSM±SE | P |
|---|---|---|---|
| Total lactation (days) | 238.49±3.57 | 228.25±4.72 | <0.01 |
| Total milk (kg/lact.) | 581.85±22.28 | 549.01±29.48 | N.S. |
| Fat (%) | 3.71±0.08 | 3.55±0.11 | N.S. |
| Fat (kg) | 18.60±0.93 | 16.52±1.23 | <0.05 |
| Protein (%) | 2.94±0.04 | 2.83±0.03 | <0.01 |
| Protein (kg) | 14.62±0.65 | 12.99±0.86 | <0.05 |
| Lactose (%) | 4.06±0.01 | 4.06±0.02 | N.S. |
| Lactose (kg) | 20.07±0.87 | 18.72±1.44 | N.S. |

No arthritis LSM±SE - Least Square Means with Standard Errors obtained for clinical arthritis diagnosis factor values $D_i = 1$, balanced for the significant effects in the model: year of measurement ($L_j$), number of lactation ($Y_k$);
Clinical arthritis LSM±SE - Least Square Means with Standard Errors obtained for clinical arthritis diagnosis factor values $D_i = 0$, balanced for the significant effects in the model: year of measurement ($L_j$), number of lactation ($Y_k$);
P - significance value of the tests for the difference of estimated least square means values for $D_i$ (i=0-1) using Tukey-Kramer adjustment for multiple comparisons
Trait - $y_{ijk}$ as defined in the Models section in Materials and methods
N.S. - not significant.

infekcije virusom AEK potvrđen je za sva analizirana svojstva u smislu smanjenja proizvodnih rezultata. Klinički artritis je pokazao značajan utjecaj na smanjenje većine proizvodnih vrijednosti osim na ukupno mlijeko, udio (%) masti laktoze i ukupnu laktozu (kg). Subklinički mastitis nije značajno utjecao na trajanje laktacije, kao ni udio (%) i udio masti, proteina i laktoze.

*Ključne riječi*: AEK virus, klinički artritis, proizvodnja mlijeka, subklinički mastitis

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