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Serological diagnosis of lentivirus infection in goats raised in Algeria

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Abstract

Introduction: Infection of goats with caprine arthritis encephalitis virus (CAEV) has been detected in variable proportions in many countries all over the world. Here, we investigated the seroprevalence of CAEV in goats raised in Algeria. **Material and Methods:** A serological survey was performed on serum samples from 1,313 goats, including the local breeds (Arabia and Dwarf of Kabylia) and imported European breeds (Alpine and Saanen). Blood samples were taken from goats on 38 farms distributed across four different geographical regions of Algeria. Serum samples were tested for CAEV antibodies using a commercial ELISA. **Results:** A total of 390 serum samples were found to be positive for CAEV, giving an overall seropositivity rate of 29.7% in individual animals and 97.37% (37/38) at the goat farm level. **Conclusion:** These results provide the first large-scale serological evidence for the presence of CAEV infection in both the local and imported breeds of goats raised in Algeria, indicating that the virus infection is widespread.

Keywords: goats, caprine arthritis encephalitis virus, seroprevalence, ELISA, Algeria.

Introduction

Caprine arthritis encephalitis virus (CAEV) is a non-oncogenic retrovirus initially isolated from infected goats in the USA (7, 17). CAEV is genetically and antigenically closely related to the Maedi-Visna virus of sheep (MVV) that was initially isolated from infected sheep in Iceland (24). Both viruses belong to the lentivirus genus of the *Retroviridae* family and *Lentiviridae* subfamily. The nucleotide sequences of the complete genomes of the first isolates of CAEV and MVV were determined (23, 26), and subsequently a variety of complete and partial genome sequences of other isolates from all over the world were reported. Due to farming and livestock production practices and the closeness of the sheep and goat species, these viruses have repeatedly jumped from one species to the other, thereby creating a continuum of virus isolates that were recently grouped under the term “small ruminant lentiviruses” (SRLVs). They are causative agents of lifelong multi-systemic chronic inflammatory

syndrome in affected goats and sheep. The pathogenesis of the goat lentivirus is characterized by fatal leukoencephalomyelitis in kids and chronic progressive arthritis and mastitis in adult goats (6). The main target cells in which this virus replicates productively *in vivo* are those of the monocyte/macrophage lineage (9, 25), with among other tissues bone marrow serving as the main reservoir of infected cells (8). Colostrum is the main route of transmission, although direct animal contact and sexual activity may also be incriminated. A variety of cells in the reproductive tracts of both male and female goats were shown to be permissive to the goat lentivirus *in cellulo* (3, 14). Eradication programmes which aim to prevent virus spread and progressively eliminate lentivirus infection from flocks include pasteurisation of colostrum and milk as well as segregation and culling of seropositive animals (27, 19). The virus persists in infected animals despite generation of virus-specific immune responses, and delayed seroconversion of latently infected goats can take many years (21).

Clinical manifestations of infection are frequently insidious; goats may develop arthritis several years after infection (15).

Previously, the agar gel immunodiffusion (AGID) test was used as the regular serological method for detection of virus infection. However, the reproducibility and the sensitivity of this assay are questionable and antigen preparation is expensive and time consuming. ELISA methods were developed based on the specific detection of antibodies against purified Gag proteins of the goat lentivirus (8, 12) and more recently against recombinant Env glycoproteins (8, 11). ELISA was shown to be more sensitive than the AGID tests and validation of the ELISA for use in goats was reported with 100% sensitivity and 96.4% specificity (11). Thus, the use of ELISA is preferable in extensive serological surveys for lentivirus infection in raised goats.

To our knowledge, there is only a single AGID-based study of the seroprevalence of lentivirus infection in goats which has been conducted in Algerian herds (1). In 1994, the authors used Maedi-Visna antigens in AGID to highlight the lack of lentivirus infection in endogenous goat herds before the coming of imported goats. Since then, goat farming has undergone numerous changes including rises both in goat numbers in herds and the numbers of herds, adoption of different breeding practices and altered orientation of production. In this study, we used a reliable commercial ELISA to evaluate the prevalence of lentivirus in goat herds located in several regions of Algeria.

Material and Methods

Study area. The present study was carried out from May 2013 to December 2015 in northern and central regions of Algeria where the great majority of goats are raised. Three regions are located in the north-east, one in the west central, two in the north central, and one in the south central territory of Algeria (Fig. 1).

Flocks and blood sampling. Blood sampling was performed on 1,313 randomly selected goats of the most dominant breeds raised in Algeria: Saanen ($n = 220$, 16.76%), Alpine ($n = 81$, 6.17%) (imported breeds) Arabia ($n = 943$, 71.82%), and Dwarf of Kabylia ($n = 69$, 5.26%) (local breeds). Goats were sampled from 38 flocks scattered all over the study area. The majority of the 27 farms were from the north-east, five farms were from the north central, three farms were from the west central, and the three last farms were from south central regions of the country. It is important to underline that with the exception of the single Tizi Ouzou-region farm (practicing strict intensive breeding), all the other farms practiced an uncontrolled trade in animals with a regular introduction of new animals on almost all farms.

Blood samples were taken from the jugular vein of each goat into clot activator vacuum tubes. Sera were separated from clots after centrifugation at 5,000 g for 5 min and then transferred on ice to the laboratory of the National Veterinary School of Algiers, and stored then at -20°C until examined.

The farms were divided into three categories depending on the type of farming they practiced: i – the extensive breeding method ($n = 9$) where goats were raised in a free-stall system with pasture feeding and frequent mingling of herds, especially with sheep; ii – the intensive breeding method ($n = 8$) where goats were in high density in small spaces in closed-buildings housing for a high production system, and iii – the mixed breeding method (semi intensive) ($n = 21$) in which the goats were raised alternately outdoors, with regular contact with other animals and herds, and indoors, most often housed in covered buildings. It is important to note that, apart from a single breeding operation in Tizi Ouzou which separated newborn kids from their mothers, all of the breeders elected to have the kids suckle until weaning.

Screening test. The collected serum samples were analysed to assess their content of CAEV antibodies using an indirect screening ELISA (Maedi-Visna/CAEV) (IDvet, Grabels, France). The test against the viral envelope glycoprotein gp28 was performed according to the manufacturer's instructions.

The sensitivity and specificity of this ELISA (100% and 97.8%, respectively; information provided by the manufacturer) were used to convert the theoretical seroprevalence to the real seroprevalence using the formula reported by Rogan and Gladen (22). The OD value was determined using the following formula: $S/P (\%) = (\text{OD sample} - \text{OD negative control}) / (\text{OD positive control} - \text{OD negative control}) \times 100$.

A value greater than or equal to 40% is considered a positive result, whereas if it is less than 30%, it is considered negative. Values between 30% and 40% are considered doubtful. A herd was considered positive when at least one animal in the herd was tested positive.

This test was chosen as it is routinely used in serosurveys of CAEV infection worldwide, and because of its high sensitivity and specificity.

Seroprevalence calculation. Individual seroprevalence was determined by evaluating the proportion of seropositive animals out of the total of examined samples in a herd. The herd seroprevalence was determined by calculating the proportion of herds with at least one seropositive goat out of all examined herds.

Statistical analysis. The data obtained were analysed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). The risk factors for the seroprevalence of goat lentivirus were assessed using SPSS version 15.0 (IBM SPSS, Armonk, USA). Pearson's chi-squared test was used to compare the serological status of CAEV infection and to evaluate

the differences between the three types of breeding on the goat farms. P values less than 0.05 were considered statistically significant. Pearson's chi-squared test was also applied to check differences between outcome variables in a univariate analysis. Probability of less than 0.05 was then considered statistically significant (18).

Results

Serum samples were tested for the presence of lentivirus antibodies. Analysis of the data at the individual goat level included the following variables: study area (districts), goat breed, type of breeding, and gender. The samples were collected from a total of 956 males and 357 females located in seven districts: Jijel (n = 156), Bejaia (n = 366), Tizi Ouzou (n = 253), Bouira (n = 150), Algiers (n = 36), Ain Defla (n = 49), and Djelfa (n = 303).

Seroprevalence of goat lentivirus in Algerian goat herds. ELISA detected specific antibodies against the goat lentivirus antigens in 390 animals, giving an overall (raw) individual seroprevalence of 29.70% among goats raised on farms in Algeria. At herd level, among the 38 farms from which the blood samples were collected only one (1/38, 2.63%) in the Tizi Ouzou area in the north-east region was found to be free from the goat lentivirus as no animal (Saanen breed) was found to be seropositive. All other farms (37/38, 97.37%) were found to contain seropositive goats for CAEV as shown in Table 1.

Table 1. Global prevalence of CAEV in Algeria

	Total	Positive	Prevalence (%)
Animals tested	1,313	390	29.7
Herds tested	38	37	97.37

Regional variation of goat lentivirus infection.

Lentivirus seropositive goats were found to be spread across all studied regions. For the north-east, north central, west central, and south central regions, the seroprevalence rates were found to reach 29.68% (230/775), 36.56% (68/186), 46.94% (23/49), and 22.77% (69/303), respectively as reported in Table 2. These results have high statistical significance with respect to the geographical variation in the prevalence of CAEV infection (Pearson's chi-squared test $P = 10^{-11}$).

From the results in Table 2, the CAEV seroprevalence in Algerian goats varies significantly ($P < 0.005$) between different geographical areas, with the highest recorded in west central Algeria (46.94%)

and the lowest in the south central part (22.77%). This might be explained by augmented industrialised farming practices in the west central region compared to the south central region which are associated with higher risks of virus transmission.

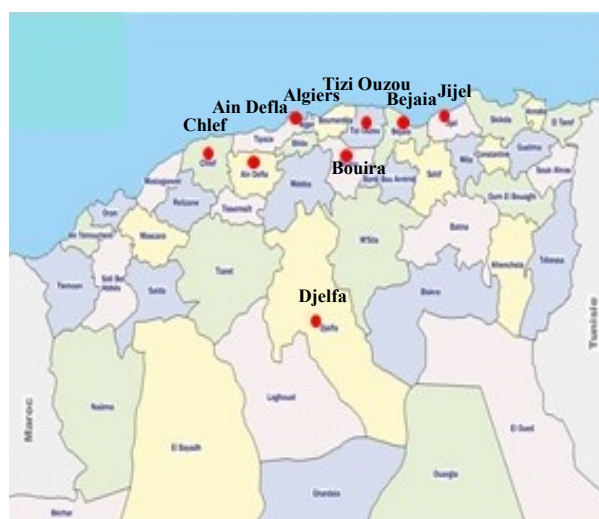


Fig. 1. Geographic localisation of tested herds in the northern part of Algeria

Variation of CAEV seroprevalence according to goat breeds. The data reported in Table 3 summarizing the total CAEV-seropositive animals and prevalence by breed (Saanen, Alpine, Arabia and Dwarf of Kabylia) show diversity. The lowest seropositive proportion, of 10.0% (22/220), was in Saanen breed goats, the highest, of 46.91% (38/81), was in Alpine, followed by the Dwarf of Kabylia breed with 36.23% (25/69), and an intermediate rate of 32.34% (305/943), was observed in the Arabia breed. The differences are statistically significant ($P < 0.05$).

Effects of breeding methods. Our survey reveals a high global lentivirus seropositivity rate among goat farms in Algeria (97.37%; 37/38). To address variance in seropositivity across the different types of breeding method, the prevalence of lentivirus infection was analysed, and we noted that goats from semi-intensive breeding farms were significantly more infected by goat lentivirus (46.67%, 49/105) than goats raised in extensive (29.98%, 307/1024) or intensive systems (18.48%, 34/184). These differences were found to be statistically significant ($P < 0.05$) (Table 4).

A significant statistical difference (Pearson's chi-squared test < 0.05) in seropositive rates between the extensive, intensive, and semi-intensive breeding methods was noticed (29.98%, 18.48%, and 46.67%, respectively).

Table 2. Individual prevalence of CAEV by region

Region	District	Total Samples	Positive Samples	Rate (%)	Prevalence by region (%)	P value
North-east	Jijel	156	48	30.77	29.68	0.0004
	Bejaia	366	142	38.8		
	Tizi Ouzou	253	40	15.81		
North central	Bouira	150	66	44.0	36.56	
	Algiers	36	2	5.56		
West central	Ain Defla	49	23	46.94	46.94	
South central	Djelfa	303	69	22.77	22.77	

Table 3. Distribution of CAEV-positive status by goat breed

Breed	Total tested	Seropositive	Prevalence %	P value
Arabia	943	305	32.34	0.000021
Saanen	220	22	10.0	
Dwarf of Kabylia	69	25	36.23	
Alpine	81	38	46.91	

Table 4. Proportion of seropositive status by breeding method in Algerian goat herds

Breeding method	Positive	Total	Prevalence	P value
Extensive	307	1,024	29.98	0.0001
Intensive	34	184	18.48	
Semi-intensive	49	105	46.67	

Discussion

CAEV-associated pathogenesis was widely described in diseased goats in the United States in 1974 (5) and a few years later the virus was first isolated from an arthritic goat (7) and encephalitic kid (17). Since then, the presence of CAEV has been described in goats raised on all continents (27). Several serological surveys reported varying incidences in different countries ranging from less than 1% in Switzerland (29) to higher than 80% in Italy (10).

The detection of CAEV antibodies in goats is considered a diagnosis of infection. However, this indicates that the animal has a history of contact with the virus but does not distinguish animals that have abortive infection or which have cleared their infection from animals undergoing productive virus replication. In addition, animals expressing viral antigens weakly will not induce a strong immune response, and some slow low virus replication in animals remains under the limits of ELISA detection and specimens from such animals are thereby considered negative (4).

The seroprevalence of CAEV in Algeria remains poorly evaluated. Indeed, apart from a single study

more than two decades ago (1), there is no other report on the CAEV situation in Algeria. In addition, the existing tools at the time when the first study was conducted were not as accurate as the actual ELISA either in sensitivity or specificity. Moreover, that study was made earlier than the changes in goat management and breeding practices in Algeria and the import of great numbers of goats from Europe. Therefore, the data reported in the present study can be considered a pioneer study in the epidemiology of CAEV infection in goats raised in Algeria.

During the last two decades, there have been significant changes in goat breeding in Algeria due to the rapidly growing demand for goat meat, dairy products, and dairy by-products for the fast-growing population, and also due to the proliferation of food processing industries. To address demand, there have been many imports of goats from different European countries, and among the countries are some that are known to have high seroprevalence of CAEV (13).

The import of live animals is one of the major risk factors for SRLV transmission. Several examples illustrate these facts, especially concerning MVV which was introduced to Iceland after importation of

a herd of 20 Karakul sheep in 1933 from Halle in Germany; it would later be proved that two rams infected with MVV were the source of the spread of the disease in two geographically separated districts (24).

However, because of its mode of transmission (colostrum contamination), CAEV seems to be spread more by the movement of dairy goats. Algeria resorts every year to the massive import of goats from European countries, and this movement could be the origin of the wide dissemination of CAEV in Algerian farms, especially if we consider that all herds in the present study contained imported goats.

This is the first large-scale CAEV serological survey in goats raised in Algeria using ELISA diagnosis. According to the results presented here, CAEV is widespread on goat farms in Algeria as we found 29.70% of goats to be seropositive and 97.37% of all farms testing positive for CAEV infection.

It has been reported by Rimstad *et al.* (21) that the sensitivity of an ELISA based on p28 was similar to and as reliable as the Western blot based on whole virus antigens, and suggested that it offers simplicity both methodological and technical. ELISA based on p28 only was found to be easily adapted for large-scale serological screening and the estimation of the population prevalence rate. The Western blot remains the confirmatory gold standard method.

In terms of livestock, the present study reports a herd infection rate of 97.37% (37/38), which is higher than that reported for most of the industrialised countries. The high seroprevalence rate reported in our study may have several explanations: firstly that the sensitive commercial ELISA in our study may have detected lower antibody concentrations; possibly that local breeds are more sensitive to CAEV resulting in increased virus diffusion following contact with imported European infected goats; or finally that the uncontrolled exchange of breeding adult males between farms during the breeding season exacerbated the infection spread. Studies have shown that semen can contain the virus (3, 29), but its role in viral transmission remains controversial (4). However, low and highly virus-contaminated sperm used for artificial insemination infects recipient goats, and CAEV-infected males can transmit the virus to females by other routes. Breeding males appear to be a significant route of infection within flocks and herds, and their loan seems to be such a route between herds. Another hypothesis can be advanced to explain the high rate of infection at the herd level. It is well known that the contact between animals from herds raised extensively and those raised semi-intensively during free-grazing periods increases the transmission risks of CAEV from one herd to another.

The overall individual seroprevalence reported in this study (29.70%) was higher than the rates reported in Switzerland and Southern Mexico (less than 1%) (28) but was significantly lower than that reported in Passirian goats from Northern Italy (81.5%) (10). Very

few studies have been undertaken in Africa, except for a few surveys that reported the different status of SRLV in Nigeria, Mozambique, Morocco, and Sudan (2, 16, 20). The individual seroprevalence of goats raised in Algeria obtained in the present work falls between the relatively high CAEV infection rates reported in developed countries where farms are highly industrialized and do not conduct long-term eradication programmes, and the low seroprevalence recorded in countries where goat farm management is of the traditional type or in countries which have conducted serious long-term eradication programmes, such as Switzerland. The fast-growing industrialisation and attendant modification of goat farm management in Algeria could be one of the factors of this increased rate of CAEV-seropositivity in goats. The seroprevalence rates reported in different studied regions demonstrate the wide dissemination of goat lentivirus among Algerian goat farms across the country. The highest seropositive rate (46.94%) was in the west central region of Algeria, exceeding those found in the north central (36.56%), north-east (29.68%), and the south central regions (22.77%). The high proportion of seropositive goats observed in the west central region might result from the low number of sampled animals ($n = 49$). This might not reflect the true incidence of CAEV in this region. Although, it has been suggested that the size of the herd does not influence the risk of infection by CAEV (30), others reported a strong inverse correlation and a direct impact of the herd size on the incidence of this disease within a goat breeding centre.

A tiny difference in seropositivity rates was observed between females (30.25%) and males (29.5%) with no statistical significance. One can expect a higher rate of infection in females than males since they are kept longer in the traditional type of breeding operation, while the males are used as lambs for meat.

The sensitivity of the different breeds of goats was found to be statistically significant ($P = 2 \times 10^{-5}$) in the present work. Indeed, the highest rate of CAEV seropositive goats (46.91%) was in the imported Alpine goats, and for comparison the Dwarf of Kabylie, Arabia, and Saanen goats emerged with the seropositivity rates of 36.23%, 32.34%, and 10.0%, respectively. In 1994, Achour *et al.* (1) reported that the breeds of goats raised in Algeria were free from lentivirus infection. Granted that the assay used then is known to have been less sensitive than the ELISA used in our study, however, our data provide evidence of a dramatic increase in seropositivity during the last three decades.

It is well known that the breeding practice is one of the risk factors for SRLV infection. Goat breeds in an extensive and semi-intensive system are significantly more infected (29.98% and 46.67%, respectively) than those managed in intensive conditions (18.48%) ($P < 0.05$).

In conclusion, the present study provides useful information about the current CAEV seroprevalence and herd management practices of goats in Algeria. Data from this investigation will help the setup of a programme of measures for restriction of virus diffusion and for eradication. The high lentivirus seroprevalence in the great majority of goats raised in Algeria indicates the need for adequate disease management. Caprine arthritis encephalitis will be brought under control by establishing measures to prevent new infections such as culling infected goats in the herds. Moreover, evidence of the species barrier crossing from goat to sheep calls for more sustained monitoring of livestock practices and movement of animals both nationally and internationally.

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Animal Rights Statement: The authors certify that animals were handled in accordance with local Ethical Committee laws and regulations concerning animal welfare rules.

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