

Development and Field Testing of a Real-Time PCR Assay for Caprine Arthritis-Encephalitis-Virus (CAEV)

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Abstract: Caprine arthritis/encephalitis (CAE) of goats and occasionally sheep are persistent virus infections caused by a lentivirus (CAEV). This viral infection results in arthritis in adult animals and encephalitis in kids. Prognosis for the encephalitic form is normally poor, with substantial economic loss for the farm. In this context an early/fast laboratory diagnosis for CAEV infection could be useful for effective prophylactic action. In this work we performed a quantitative real time PCR designed on the CAEV *env* gene to detect/quantify in goat/sheep samples, viral RNA or proviral DNA forms of CAEV. This procedure was validated in 15 sheep, experimentally infected with CAEV or with a highly correlated lentivirus (visna maedi, MVV); in addition, a total of 37 clinical goat specimens recruited in CAEV positive herds were analyzed and compared using serological analysis (Elisa and AGID). All samples infected with MVV resulted negative. In sheep experimentally infected with CAEV, proviral DNA was detectable 15 days post infection, whereas the serological methods revealed an indicative positivity after 40-60 days. This method showed a sensitivity of 10² *env* fragments/PCR with a linear dynamic range of quantitation from 10³ to 10⁷ *env* fragments/PCR; the R2 correlation coefficient was 0.98. All subjects with a clinical diagnosis for Caprine Arthritis-Encephalitis (CAE) resulted CAEV DNA positive.

Keywords: Caprine arthritis-encephalitis, CAEV, lentivirus, real time PCR, agar gel immunodiffusion (AGID), enzyme-linked immunosorbent assay (ELISA).

INTRODUCTION

Among the group of small ruminant lentiviruses (SRLV), Caprine arthritis-encephalitis virus (CAEV) is able to infect goats and occasionally sheep and other related ruminants [1, 2]. The major transmission route of this virus is through the ingestion of virus-infected milk from adult goats by kids via *colostrum*, by iatrogenic transmission or by lateral transmission through prolonged close contact, with infected animals. All breeds and ages of goats are susceptible to infection, and once established, it persists throughout the animal's life. CAEV infection of goats in newborn kids may lead to encephalitis, interstitial pneumonia and in adult animals to chronic arthritis and interstitial mastitis, and all may contribute to impaired milk production [3]. Infection with CAEV has been identified worldwide [4-8]. CAEV has a tropism for monocytes and macrophages; the infection of

host cells involved in the immune system carries the virus throughout the body with multi organ involvement and progression to the disease can take months to years, causing chronic inflammatory diseases. CAEV is closely related to the maedi-visna virus (MVV), but it is a genetically distinct lentivirus. As nucleotide sequence information has increased, it has been shown that MVV and CAEV should not be regarded as sheep-specific and goat-specific pathogens respectively, but rather that they should be more correctly referred to as SRLV [1, 9, 10]. In the diagnostic field, serological tests are routinely applied to detect SRLV infections. Although third-generation ELISAs, like those used in this study, are highly sensitive and specific [11], seroconversion can, however, be relatively slow and variable and there is no simple relationship between the moment of conversion and the initial infection. To be able to prove the availability of actual viral particles in blood and tissues, a PCR test may be used, as in the present study. Theoretically, SRLV can be present in the clinical samples (blood, organs, semen) in three forms: namely (i) incorporated proviral DNA (in macrophages), (ii) as virions (complete virus particles with their ssRNA core and protein coat released by budding from the plasma membrane) and (iii) as free virus (released

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by cell lysis). In practice, CAEV proviral-DNA and viral mRNA could be detected by PCR base methods. The current most widely used test for caprine arthritis-encephalitis (CAE) is agar gel immunodiffusion (AGID) recommended by the World Animal Health Organization (OIE,2009). However, this seems to underestimate infection incidence especially in animals with delayed sera conversion [12]. This can impede early or precise serological diagnosis in animals, which could be an efficient infection source. By direct diagnostic procedures, CAEV-DNA or CAEV-RNA have been detected by PCR or RT-PCR base methods, these procedures required identification by agarose electrophoresis or sometimes by the capillary sequencing method [13-15]. Up until now, these molecular tools have required a long time and can be expensive; in addition, they do not allow viral quantization; to overcome these problems, we used a real time PCR method for the detection and quantization of CAEV strains in different goat clinical samples. This could be a useful anti-CAEV prophylactic strategy controller or

used in experimental infection studies for monitoring, avoiding possible cross reactions with other strongly related virus such as maedi visna virus (MVV). The purpose of this study was to develop a real-time PCR assay able to detect/quantify CAEV DNA or RNA in goat/sheep clinical samples.

MATERIALS AND METHODOLOGY

Animals and Clinical Samples

Our studies were performed in two distinct Italian native goat populations: (I) Sardinian, (II) Alpine, Central Italy.

A total of thirty-seven goat samples were processed:

Sardinian goat population, 16 subjects from 2 herds, ten of these were culled (Table 1).

- 16 DNA extracts from blood
- 10 DNA extracts from intra-articular fluid

Table 1. PCR Real Time Results Relative to Samples Recruited from Sardinian Goats (Istituto Zooprofilattico Sperimentale Della Sardegna)

N	Sample	Organ	Sintomatology	AGID	PCR Real Time
1	K88	BLOOD	PRESENT	POSITIVE	POSITIVE
1	K88	IAF	PRESENT	POSITIVE	NEGATIVE
2	K199	BLOOD	ABSENT	NEGATIVE	NEGATIVE
3	K163	BLOOD	ABSENT	POSITIVE	POSITIVE
4	K138	BLOOD	ABSENT	NEGATIVE	NEGATIVE
5	K134	BLOOD	PRESENT	POSITIVE	POSITIVE
5	K134	IAF	PRESENT	POSITIVE	NEGATIVE
6	K133	BLOOD	ABSENT	NEGATIVE	POSITIVE
7	K120	BLOOD	PRESENT	POSITIVE	POSITIVE
7	K120	IAF	PRESENT	POSITIVE	NEGATIVE
8	G06	BLOOD	ABSENT	POSITIVE	POSITIVE
8	G06	IAF	ABSENT	POSITIVE	NEGATIVE
9	G02	BLOOD	ABSENT	POSITIVE	POSITIVE
9	G02	IAF	ABSENT	POSITIVE	NEGATIVE
10	A29	BLOOD	ABSENT	POSITIVE	POSITIVE
10	A29	IAF	ABSENT	POSITIVE	NEGATIVE
11	A23	BLOOD	PRESENT	POSITIVE	POSITIVE
11	A23	IAF	PRESENT	POSITIVE	NEGATIVE
12	A02	BLOOD	ABSENT	POSITIVE	NEGATIVE
12	A02	IAF	ABSENT	POSITIVE	NEGATIVE
13	61	BLOOD	ABSENT	POSITIVE	POSITIVE
13	61	IAF	ABSENT	POSITIVE	NEGATIVE
14	51	BLOOD	ABSENT	NEGATIVE	NEGATIVE
15	286445	BLOOD	ABSENT	NEGATIVE	NEGATIVE
16	213835	BLOOD	PRESENT	POSITIVE	POSITIVE
16	213835	IAF	PRESENT	POSITIVE	NEGATIVE

JAF = intra-articular fluid. Positive samples showed a titre ranging from 10^2 to 10^3 *emv*-molecules/PCR. The detection limit was 10^2 *emv*-molecules/PCR.

- 10 RNA extracts from intra-articular fluid
- Clinical evaluation for CAEV symptoms was observed for these subjects [16].

Alpine goat population, 21 subjects from 1 herd

- 21 DNA extracts from blood
- 21 RNA extracts from serum

These herds occasionally showed serological CAEV positivity by the Agar Gel Immuno diffusion (AGID) technique [17], or by ELISA [18] see Tables 1 and 2.

In addition, 15 other sheep, serologically negative for CAEV and recruited from a herd from central Italy, were used for CAEV/MMV experimental infections (see later).

Viral Strains

Two different viral clinical isolates (CAEV and MMV) were used for sheep experimental infection and as positive controls, in order to check the reliability of the real time PCR procedure described in this paper.

1. The first strain, originally isolated by explantation of the synovial membrane from an arthritic goat, (It-Pi1) was identified as a CAEV- like genotype B by traditional PCR procedures [19, 20].

2. The second Visna maedi- like strain (It-651), obtained by sheep tissue explantation, was identified as genotype A [20, 21].

Serological Analysis

Serological analysis was performed by the Agar Gel Immuno diffusion (AGID) technique and by Elisa procedures described by other authors in previous publications [17, 20]. In particular, two commercially available ELISAs were used. One whole virus ELISA (hereafter named WVcomELISA) was from Bommeli Diagnostics (Checkit MVV/CAEV) and a recombinant based ELISA (named RcomELISA) was made by the Institut Pourquier, employing the recombinant capsid antigen and trans membrane protein. Both tests were carried out following the manufacturer's instructions.

Experimental Infection

Experimental infection was carried out during a previous study at Perugia's *Istituto Zooprofilattico Sperimentale* (IZSP) and a set of immunological results with some samples has already been published, Lacerenza *et al.*, 2006 [20].

Briefly, 15 sheep from the Appennine population were selected from a long term negative flock and housed in

Table 2. PCR Real Time Results Relative to Samples Recruited from Alpine Goats (Istituto Zooprofilattico sperimentale delle Regioni Lazio e Toscana)

N°	Sample	Elisa	Real Time PCR Blood	RT-Real Time PCR Serum
1	433	Negative	Positive	Negative
2	364	Negative	Negative	Negative
3	429	Negative	Negative	Negative
4	439	Negative	Negative	Negative
5	415	Negative	Negative	Negative
6	435	Negative	Negative	Negative
7	334	Negative	Negative	Negative
8	347	Negative	Negative	Negative
9	CL	Positive	Positive	Negative
10	437	Positive	Positive	Negative
11	379	Positive	Positive	Negative
12	420	Positive	Positive	Negative
13	373	Positive	Positive	Negative
14	413	Positive	Positive	Positive
15	414	Positive	Positive	Positive
16	402	Positive	Positive	Positive
17	401	Positive	Positive	Positive
18	351	Positive	Positive	Positive
19	353	Positive	Negative	Positive
20	388	Positive	Negative	Positive
21	396	≤10 ³	Negative	Negative

Positive samples showed a titre ranging from 10² to 10³ *env*-molecules/PCR. The detection limit was 10² *env*-molecules/PCR.

Oligonucleotide Primers Design

The PCR target region was identified by multiple sequence alignment programs (ClustalW, and Geneious Biomatters Ltd.) by means of the complete CAEV and visna Maedi proviral genomes, available in the DNA data Bank (GenBank). The *env* gene sequences, codifying for CAEV viral envelope polypeptide, showed a good conserved region inside the CAEV group, even though a mean difference was observed with the Visna Maedi virus (Fig. 1). The PCR primers, used to perform real time PCR, were designed from a 114 bp fragment, corresponding to nucleotide positions from 5960 to 6073 in the published CAEV genome sequence identified with GenBank accession n. NC_001463. The theoretical melting temperatures of the primers (T_m), the formation of oligonucleotide dimers and self-complementarity were evaluated using Oligo Program version 4 (MedProbe, Oslo, Norway) and by module 1 of the DNA Hybridisation prediction algorithm program, HYTHER <http://ozone.chem.wayne.edu/> [22, 23]. Parameters set as hybridisation conditions were a monovalent cation concentration of 0.05 mol/L, Mg^{2+} at 0.004 mol/L, a concentration of probe and target of 10^{-7} mol/L and a hybridisation temperature of 50°C. This fragment was chosen for its high similarity in all CAEV genotypes. The positions of the PCR primers and the comparative matches with the visna maedi (MVV) *env* region are shown in Fig. (1).

Positive Quantitative Standard

A synthetic 114 bp DNA fragment (MM4) was used as a positive control. This fragment, corresponding to the PCR amplicon was synthesized by eurofins MWG/operon (www.eurofinsdna.com). Serial 10 fold dilutions of this target, ranging from 10^6 molecules/2µl, served as a standard quantification curve.

Optimal Amounts of Template DNA for PCR Reaction

Proviral CAEV DNA is normally represented as the smallest percentage in the sample compared to goat eukaryotic DNA. This aspect could compromise PCR reaction as a false negative result [24]. The clinical sample analyzed in this study showed a total DNA concentration ranging from 5 to 43 µg/ml. For this reason, we performed a real time PCR reaction containing a serial dilution of mixed goat DNA, from 50 to 0.78 µg/ml, with a synthetic CAEV target MM4 *env* molecules/PCR at a concentration of 1×10^5 (Table 1). The DNA amount was calculated by a Qubit® 2.0 Fluorometer (Invitrogen) following the manufacturer's instructions.

Real Time RT-PCR

In this work we used a version 1.2 LightCycler Instrument, (Roche Diagnostics Mannheim, Germany).

Real Time RT-PCR for CAEV RNA

PCR was performed with a LightCycler-RNA Amplification kit SYBR Green I (Roche Diagnostics Mannheim Germany) according to the manufacturer's instructions. The 20 µl final volume contained: 2.5 mM $MgCl_2$, 0,5 µM of each primer and 1 U of heat-labile Uracyl-DNA-Glycosylase (UDG, Roche Molecular Biochemicals), 2µl of RNA extract. The PCR program was as follows: (i) an initial reverse

transcription at 55°C for 10 min. (ii) denaturation at 95°C for 30 sec. and (iii) 40 cycles of 0 sec at 95°C, 10 sec at 50 °C, 24 sec at 72°C and 3 sec at 79 °C (vi) Melting curve: performed for 0 seconds at 95°C, 45°C, 95°C. Transition rates were: 5 °C/s in 72°C segment, 0.1 °C/s in 45° C segment and 20°C/s for another step. Fluorescence was detected at the end of the 79°C segment in the PCR step (single mode) and at the 45°C segment in the melting step (continuous mode) in the F1 channel.

Real Time PCR for CAEV Proviral DNA

PCR was performed with a LightCycler-DNA Amplification kit SYBR Green I (Roche Diagnostics Mannheim Germany) according to the manufacturer's instructions. The 20 µl final volume contained: 2.5 mM $MgCl_2$, 0,5 µM of each primer, 2µl of DNA extract. The PCR program was the same as described above without the reverse transcription step. The quantitative real time PCR or real time RT-PCR, performed with the LightCycler (Roche Diagnostics Mannheim, Germany), required 30 minutes.

RESULTS

Magnesium Optimization

Magnesium concentration is a critical factor affecting the performance of Taq DNA polymerase. PCR reaction components can affect the amount of free magnesium. In the absence of adequate magnesium, Taq DNA polymerase is ineffective. On the contrary, excess free magnesium reduces enzyme fidelity and may increase the level of nonspecific amplification [24]. For this reason, we prepared a reaction series containing 3-5.5 mM, Mg^{2+} in 0.4 mM increments by adding 0-0.4-0.8-1,2-1.6-2 µl of a 25 mM $MgCl_2$ stock to 20 µl reactions as described in the "LightCycler® DNA/RNA SYBR Green I" Kit instruction manuals. The most sensitive combination was observed with $MgCl_2$ 3.5 mM, with a shift in the PCR threshold cycle (CT) from 32 to 12.

Melting Curve and Procedure Specificity

The melting temperature of a DNA duplex, with SYBR green dye, gives information about DNA fragment length [25], which shows dissociation-characteristics of double-stranded DNA during heating and, normally we can observe two different melting peaks after real time PCR reaction: the first low temperature melting peak represents a possible primer-dimer PCR product; the second (at a higher temperature), represents the signal corresponding to PCR amplicon dissociation. Thus a negative result is represented with the first peak only. RT-PCR, generation of melting curves and fluorescence detection were all performed using: (i) viral strains, (ii) positive clinical strains for MMV and CAEV, (iii) CAEV/MMV negative goat samples. As described in Fig. (2), a 82°C melting Peak is shown in CAEV samples, even though a T_m peak shift (82-76°C) was detected in the case of MMV samples or in the negative control. These results confirm the prediction obtained by an *in silico* study described in Fig. (1).

Sensitivity

Real-time PCR, as evaluated here, has been shown to have a detection limit of 10^2 synthetic MM4 fragment/PCR. The wide linear range (10^7 - 10^3 MM4/PCR) as illustrated in

Fig. (3), Standard curves showed a good correlation regression coefficient R2 of 0.98 [26].

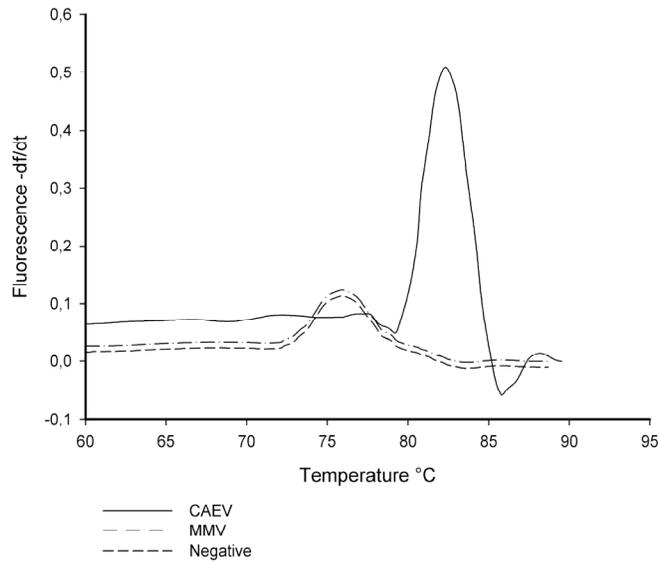


Fig. (2). Real time PCR melting curve in CAEV and MMV samples. Only the positive CAEV samples showed a Melting curve peak profile of 83°C.

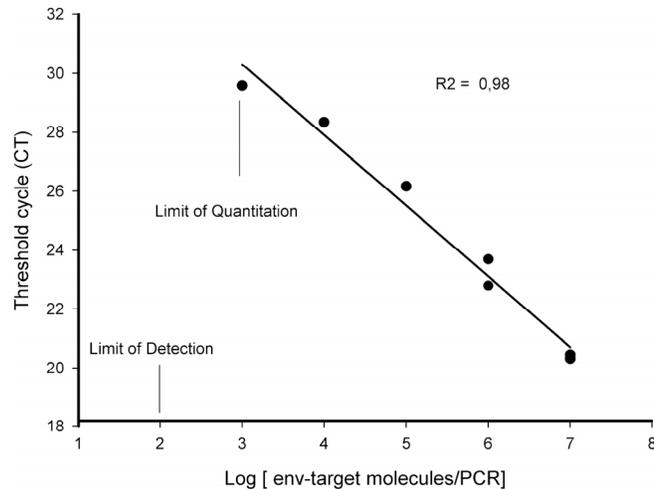


Fig. (3). Real time PCR standard curve obtained with serial dilution of synthetic MM4 CAEV target. The linear range of quantitation was 10^3 - 10^7 molecules/PCR. All CAEV positive samples showed a concentration ranging under quantification limit $10^2 \leq 10^3$ env-molecules/PCR, the detection limit was 10^2 env- molecules/PCR.

Optimal Amount of Total DNA Concentration

The PCR reaction was performable at all serial dilutions of goat DNA tested. Early CT values were around 18 and they were observed under 12.5 $\mu\text{g/ml}$ of total DNA, corresponding to the samples diluted $\frac{1}{4}$ fold, (data not shown).

Infected Sheep

As described in the Materials and Methods section: a cohort of three different groups of sheep were used, with 5 subjects each of which were :

- (i) infected with a CAEV strain (It-Pi1),
- (ii) infected with a Visna maedi strain (It-651),

(iii) non- infected.

For serological analysis and nucleic acid extraction, we took serum and blood specimens for each sheep at 4, 8, 15, 23, 28, 42, 58, 71 days after infection. Real time PCR and RT-real time PCR showed that:

MMV infected and non infected sheep resulted PCR negative (data not shown).

Viral RNA in all infected sheep was undetectable by RT real time PCR (data not shown).

As shown in Fig. (4a), proviral DNA was detectable in blood, 15 days after infection for all CAEV infected sheep. The titres (the mean for 5 sheep, calculated as a MM4 CAEV fragment/PCR) ranged from 9×10^2 (15th day PI) to 1×10^4 (71th day PI). 1×10^3 fragment/PCR, corresponding to about 5×10^4 virus particles/ml blood.

Fig. (4b) represents the percentage of positivity of CAEV antigens obtained with two different Elisa manufacturer's kits (Bommelli and Pourquier). Significant positivity (> 50%) was detected after 60 days PI. The Bommelli kit resulted positive around 20 days PI but it showed a lower positivity percentage (< 20%).

Clinical Samples

A total of thirty seven goat samples were processed. All positive specimens analyzed showed a very low viral titre (≤ 103 C env-DNA copies/ml).

Sardinian Goats

We analyzed a total of 16 subjects from 2 herds. All symptomatic goats (5/16 subjects) and 6 asymptomatic subjects resulted Blood DNA CAEV positive (Table 1), no intrarticular fluid (IAF) resulted positive for CAEV DNA or RNA.

Central Italy Goats (Alpine).

Of the 21 goats analyzed, Table 2, only two samples showed a discrepancy between the serological (Pourquier) and molecular analysis. Sample n. 1 resulted CAEV DNA positive but serologically negative, while sample n. 21 was Elisa positive with CAEV DNA/RNA negative. Considering only the Elisa positive specimens, 77% were Blood CAEV DNA positive and 53% were serum RNA positive. Real time PCR (DNA+RNA) revealed 92% serological positive samples (12/13) CAEV positive and 25% Elisa negative samples.

DISCUSSION AND CONCLUSION

Caprine arthritis-encephalitis (CAE) is a disease in goats and sheep caused by a retrovirus (CAEV). This is a persistent lentivirus infection and is often grouped with the Maedi visna (MVV) virus which is a small ruminant lentivirus, SRLV [27]. It is an emerging problem throughout Europe, particularly in the Mediterranean regions. The disease tends to spread widely if left uncontrolled and most affected animals are culled because these animals are sources of infection and their clinical signs worsen over time, causing high economic loss. At the present time, there are no biological products such as vaccines for CAEV prophylaxis; in fact, the only choice is infection prevention by early

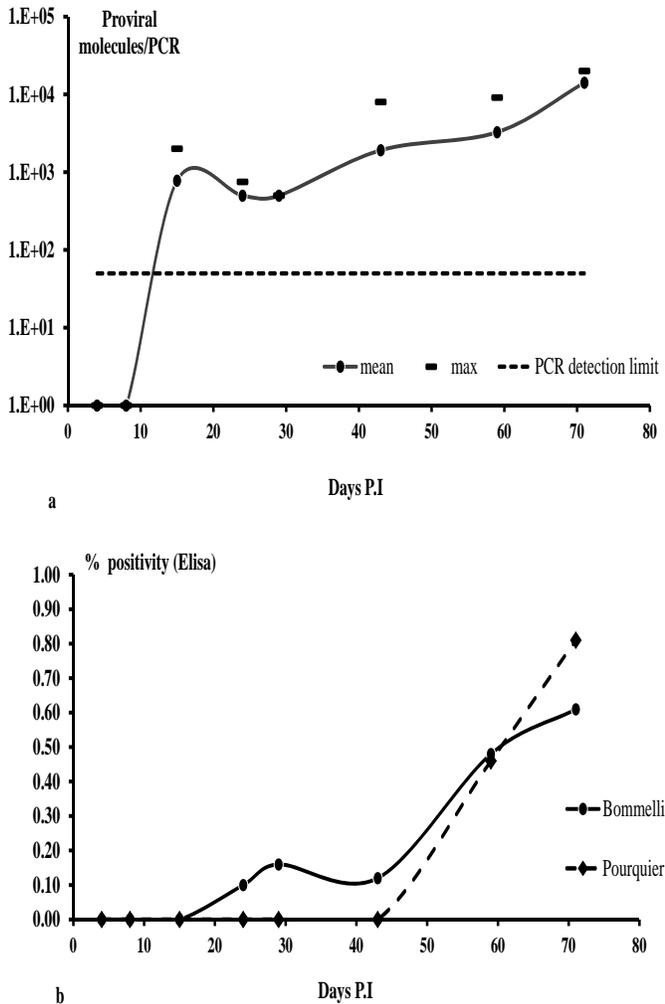


Fig. (4). (a) Titre of proviral CAEV DNA measured in infected sheep at 71 days post infection. The represented titre was the geometrical mean in five subjects. (b) Serological Elisa results for the same specimens are described in figure.

identification and isolation of CAEV-infected subjects. Most infected subjects possess detectable specific antibodies and a number of serological test are available for CAEV infection diagnosis. The two most commonly used are the agar gel immuno diffusion test (AGID) and the enzyme-linked immunosorbent assay, ELISA [11, 28]. In a previous work, Adams *et al.* [29] demonstrated that Virus-inoculated goats produced a CAEV-specific antibody that reached a maximum level between 49 and 77 days post inoculation. In this work, we also observed that a significant positivity (>50%) could be detected after around 60 days PI in infected sheep. Following other literature data, the time required for seroconversion following infection can be relatively prolonged and unpredictable, measured in months rather than in weeks [17, 30-32]; this “window period” could cause prevention strategies to fail with a subsequent spread of infection. Direct virus isolation by co-cultivating peripheral blood or milk leukocytes with appropriate caprine cell cultures, such as synovial membrane cell isolation and subsequent confirmation by immunolabelling and electron microscopy is very specific but showed variable sensitivities

[33]; in addition, this method requires specialised laboratories.

Direct laboratory diagnosis by CAEV nucleic acid recognition for detecting CAEV virus or provirus has already been described [6, 13-15, 34-40]. These procedures represent *in situ* hybridization methods or PCR (polymerase chain reaction) methods. PCR base methods are now routinely being used in different laboratories, and their specificity is confirmed by amplicons or cloning products.

Traditional PCR is inexpensive, easy to use but current molecular methods do not allow simultaneous CAEV detection/quantization. We have described a fast molecular procedure (around 1.5-2 hours) with good sensitivity and specificity, able to detect and quantify CAEV in the viral or proviral form.

We designed a real time PCR protocol by using a primer couple flanking the *env* gene region representative for all CAEV sequences deposited in the GeneBank but containing many mismatch regions, in comparison with the Visna Maedi (MVV) isolate. With MVV *env* sequences, simulation analysis *in silico* by Hyther and mfold servers showed an increase in Gibbs energy (delta G), with a subsequent *Tm* decrement positioned from 7 to 50 °C for upper and lower primers respectively (Fig. 1). This observation was confirmed by a reconstruction experiment using a Visna maedi strain (It-651) as a DNA/RNA direct template for PCR real time or in experimentally infected sheep; these results suggest an absence of false CAEV positive results with samples containing MVV, demonstrating the optimal specificity of this molecular approach.

In this work we used an intercalating dye as a fluorescent probe (Sybr Green I). This procedure required a melting curve at the end of the amplification cycle to determine if the product is pure (amplicon only) or if there are non specific products like primer-dimers [41]. In our experiment, shown in Fig. (2), melting curves are localized on the graph in two distinct areas: (i) a 78°C peak region due to primer dimer products in negative samples (such as MVV positive samples), (ii) a 83° C peak region in positive CAEV samples; for this reason we positioned the fluorescence detection point at 80°C when the aspecific product signal is the lowest (for PCR and RT PCR), thereby avoiding specificity errors due to inappropriate fluorescence signals (Fig. 2).

By using a synthetic *env* gene fragment reproducing the PCR amplicon, this real time PCR showed a 5 log linearity range, from 10^2 to 10^7 *env* fragments/PCR with a regression factor (R^2) of 0.98. Viral enumeration using the present procedure in clinical or laboratory samples (such as virus or provirus) could be useful for *viraemia* or proviral evaluation during anti-CAEV prophylactic measures or in laboratory experiments (such as cell culture infection). A comparative evaluation between serological and molecular procedures is shown by experimental infections and by biological samples recruited in subjects coming from infected areas.

First of all, experimental infections suggested a complete absence of fluorescence signals in samples recruited in sheep infected with MVV, despite the fact that a mean of 10^3 proviral molecules PCR is detectable in CAEV infected sheep at 15 day PI, a correlative serological positive signal

is detected around 40-60 day PI, this *datum* suggests that with this real time PCR we could “gain a time” of around 25-45 days in diagnostic procedures, which (as discussed above) could be useful in an early prophylactic anti CAEV procedure. CAEV RNA was not detectable in these sheep until 71 days PI; this result could be due to low *viraemia* during this period. In clinical samples, only 7 Alpine goats were CAEV RNA positive, and their simultaneous positivity for CAEV DNA could indicate a very old infection (Table 2). All symptomatic goats (5 subjects) resulted proviral real time PCR positive (Table 1). The prognostic effect of this molecular approach on diagnosis for CAE is represented by Sardinian goats monitored serologically by the AGID procedure and the Alpine type monitored by Elisa. Two serologically negative samples resulted PCR positive (Tables 1 and 2), these subjects could be in a “window period”. However some discrepancies were revealed in these specimens, i.e. sample 396 (Table 2) and A02 and K133 where serological positivity did not correspond to the molecular signal. This could be due to different causes: (I) specificity mistakes with serological methods, (ii) PCR sensitivity mistakes (target < 10² env/PCR or PCR inhibitors, in this case an internal amplification control (i.e. on 18S rRNA gene) could be useful. The blood for proviral detection represented the best procedure in laboratory diagnosis for CAEV infection in goats/sheep.

In conclusion, we tested and validated the feasibility of a real time PCR such as PCR or RT PCR as a method for the detection and simultaneous quantisation of Caprine Arthritis-Encephalitis Virus in naturally-infected goats or in experimentally infected sheep. This method showed a good sensitivity (10² env fragment/PCR) and a good specificity (100%) when this protocol was applied in experimentally infected subjects or goats with a clinical diagnosis of Caprine Arthritis-Encephalitis (CAE).

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CONFLICT OF INTEREST

The authors declares that they have no financial/commercial conflicts of interest.

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