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Seroprevalence of caprine arthritis and encephalitis virus (CAEV) infection in goats from three districts of Cagayan, Philippines using nested PCR

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Abstract

Caprine arthritis and encephalitis virus (CAEV) is poorly documented in the Philipines and is considered an emerging and re-emerging disease. With the increased and unmonitored importation of goats by the private sector, there is limited data on the prevalence of CAEV in the caprine populations in the Cagayan Valley Region. This study aimed to detect the presence of caprine arthritis and encephalitis virus (CAEV) from selected farms in the three districts of Cagayan Province, Philippines for baseline data. A nested polymerase chain reaction (PCR) was employed since this is highly specific for demonstrating infection positivity. A total of 285 serum samples were randomly taken from goats of varying ages, sexes, and breeds. Based on the results CAE virus infection is confirmed to be present in the area as substantiated by 5.26 % overall prevalence among the samples surveyed. However, the overall chi-square test signified that there is no statistically significant difference in infection rates across the three districts of Cagayan Province (p-value= 0.132).

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Introduction

Caprine Arthritis Encephalitis Virus (CAEV) is a lentivirus characterized by inflammation of the bronchus in small ruminants encompassing sheep and goats irrespective of breed and age.

Caprine Arthritis Encephalitis Virus (CAEV) and Visna/maedi virus (VISNA) are genetically distinct but antigenically correlated pathogens of goats and sheep (Feitosa, 2010).

Lateral transmission or via the colostrum from adults to their offspring is one of the primary routes. Other means of transfer have been reported like transmission via aerosol and animal-to-animal contact. According to Carrozza *et al.* (2023), Small Ruminant Lentivirus (SRLV) may exist in the copulatory organs and semen of infected animals in three forms: incorporated in the cells (proviral DNA), as virions and as a free virus released by cell lysis. Once the virus occupies the system, the animal remains a carrier for life.

CAEV is endemic worldwide and is a chief hindrance to milk production among goat dairy herds. Contaminated colostrum and milk ingestion are considered to be the topmost source of infection for kids in which the virus is disseminated down the generations (Ravazollo *et al.*, 2006). It is thought that mastitis may diminish milk production by 10%. These losses tortuously disturb progeny weight gain because of the reduction in milk. Other economic consequences of CAE include interference with the well-being and quality of life of the affected animal (Paula, 2009). The adult form of the syndrome is manifested by chronic proliferative synovitis and periarthritis, whereas in neonates, it is indicated by acute afebrile leucoencephalomielitis. The disease induces an asymptomatic contagion in most goats. Signs of disease comprise progressive inflammation in one or more organ or tissue systems including the joints, bursae, brain, spinal cord, lungs, and udder (Nord and Adnoy, 1997).

According to the report of Turchetti *et al.* (2013), CAEV can cause four basic forms of the disease: nervous, characterized by leukoencephalomyelitis, arthritic, respiratory, characterized by interstitial pneumonia, and mammary. The disease results in decreased production and economic losses in goat herds.

PCR is specific for CAEV proviral DNA detection in infected animals because the crossed reaction has not been identified with other ruminant retroviruses such as Maedi Visna Virus (MVV), bovine immunodeficiency virus, or bovine leukemia virus (Paula, 2009).

It is indispensable to comprehend all conceivable ways of transmission of the disease since there is no effective vaccine or treatment for CAEV, thus control is based on preventing infection through culling and sacrificing the animal.

The geographical distribution and prevalence of CAE infection in the Cagayan Valley Region remain insufficiently documented. In 1999, reports indicated that two stock farms were infected with the said illness, exhibiting both subclinical and clinical symptoms, which led to mass culling of their populations at the same time.

Thus, this study aimed to report on the presence of CAEV and the possibility of re-emergence in the province of Cagayan, Philippines. The findings generated can be used as baseline information for concerned agencies for possible policy formulation that can be integrated with the program of the Department of Agriculture, Region 02 to prevent the possible spread that is detrimental to the income of farmers.

Materials and methods

Study area

This study was conducted in the three districts of Cagayan encompassing the selected municipalities for each district: District I (Alcala, Baggao, Buguey, Gattaran, and Lallo); District II (Piat, Rizal and Sto. Niño); and District III (Enrile, Solana, Peñablanca, Tuao, and Tuguegarao City). Testing of the blood samples was performed at the Regional Animal Disease Diagnostic Laboratory (RADDL), Department of Agriculture, Region 2, located at Carig Sur,

Tuguegarao City, Philippines. A total of 285 blood samples were randomly collected from sheep and goats on various farms irrespective of sex, age, and breed. Three (3) milliliters of whole blood were drawn via venipuncture from the disinfected jugular vein. Withdrawn samples were placed on a vacutainer.

DNA extraction procedure

In a 1.5 ml microtube, 500 *ul* whole blood/buffy coat was dispensed, and 1000 *ul* cell lysis solution was added. Vortex thoroughly and centrifuged at 14,000 rpm for 2 minutes and then discarding the supernatant. The procedure is repeated twice. Three hundred (300) *ul* of nuclei lysis solution were added and vortex thoroughly. One hundred (100) *ul* of protein precipitation were added and vortex thoroughly for 1 minute. This was subjected to centrifugation at 14,000 rpm for 10 minutes. Supernatants were transferred in a new 1.5 ml microtube containing 500 *ul* of isopropanol. After which, it is mixed gently by inversion and centrifuged at 14,000 rpm for 2 minutes. The supernatant was discarded again and then 500 *ul* of 70% ethanol was added. This is mixed gently by inversion and centrifuged at 14,000 rpm for 2 minutes. The supernatant was discarded and the tubes containing the DNA were used in a Biosafety Cabinet for 30 minutes to an hour. The DNA is rehydrated and 30 *ul* of DNA rehydration solution was added, stored DNA at -20°C or 4°C.

Amplification by PCR

Two sets of CAEV primers were used in the study. Nested PCR was performed using extracted genomic DNA of samples. The first PCR components contain 10 uM each of primer (1st Forward and Reverse Primer), 0.5 mM of dNTPs, 1X Go Taq™ Green Buffer, 2.5mM $MgCl_2$, 0.025U Go Taq™ Flexi Polymerase, and 3ul of genomic DNA. The second PCR, contains PCR components of 10 uM each of primer (2nd Forward and Reverse Primer), 0.5mM of dNTPs, 1X Go Taq™ Green Buffer, 2.5mM $MgCl_2$, 0.025U Go Taq™ Flexi Polymerase, and 3ul of 1st nested PCR product. First and second nested PCR assays were performed on Simpliamp™ thermal cycler, with an initial denaturation of 95°C, 5 minutes; followed by 40 cycles of denaturation at 95°C, 30 seconds, annealing at 60°C, 30 seconds; Elongation at 72°C, 45 seconds; and a final elongation at 72°C, 10 minutes.

Table 1. Primer Sequences for diagnosis of caprine arthritis encephalitis (CAE) virus infection

Primer name		Primer sequence (5'- 3')	Product size(bp)
CAE P1-F1	F1	CAA GCA GCA GGA GGG AGA AGC	185 bp
CAE P2-R1	R1	TCC TAC CCC CAT AAT TTG ATC CAC	
CAE P3-F2	F2	GTT CCA GCA ACT GCA AAC AGT AGC AAT G	
CAE P3-R2	R2	ACC TTT CTG CTT CTT CAT TTA ATT TCC C	

The primers (Table 1) were designed based on the CAEV sequence detected in the Philippines using nested PCR (Padiernos *et al.,* 2014*)*.

Analysis

Amplified products were separated by agarose gel electrophoresis (2% agarose in 1x TAE) at 100V for 30 minutes and stained with GelRed™. DNA fragments were viewed by UV Gel Documentation system™ and photographed. Expected PCR product size is ~185 bp.

Statistical analysis

The association between the prevalence of CAE virus infection and the risk factors was analyzed by univariate analysis utilizing a chi-square test. The results were expressed as *p*-values at a 95% confidence interval (CI 95%).

Results and discussion

The study determined the occurrence of Caprine Arthritis and Encephalitis Virus (CAEV) in selected small ruminant farms in Cagayan with a 17.28 % positivity rate demonstrated through nested PCR. During the blood sampling, animals had not observed any manifestations of the illness which implies that asymptomatic carriers were tested. This corroborates the findings reported by Peterhans *et al.* (2004), who noted that not all infected animals show clinical

symptoms and that asymptomatic carriers can transmit disease within a herd. Additionally, Shillcock *et al.* (2023) reported that CAEV can infect both through natural cross-species transmission, with both horizontal and vertical transmission occurring within herds having no signs.

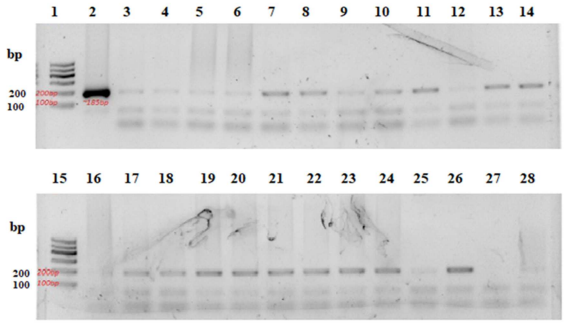

Fig. 1. Nested PCR amplification of the gag-genes of CAE

Fig. 1 illustrates CAEV Nested Polymerase Chain Reaction (PCR) products. Samples were electrophorised at 100volts, in a 2% agarose gel, 1X TrisAcetate EDTA buffer, stained with Gel Red, visualized by UV-illumination and photographed. Lane 1 is the molecular marker at 185bp while lane 2 for the positive control. On the other hand, lane 3 up to lane 26 is the sample that resulted out to be positive for CAEV and lane 27 for the negative control (double distilled water). A total of 15 blood samples resulted out positive which is denoted as a bright band on the gel at the level of 185 to 200bp.

Table 2. Overall summary seroprevalence of CAE virus infection in the 1st district of Cagayan province

District 1	Number of samples	CAEV positive	% of CAEV infection
Alcala	20	2	10%
Baggao	13	0	0
Buguey	10	0	0
Gattaran	6	1	16.66%
Lallo	12	1	8.33%
Total	61	4	7%

The overall seroprevalence of CAEV infection in District 1 (Table 2) was 7%, with 4 out of 61 tested samples being positive for CAEV antibodies. The contagion rates varied suggestively across various municipalities. The generated results show a marked disparity in CAEV infection rates across the sampled farms. The municipalities of Alcala and Gattaran reported the highest infection rates, at 10% and 16.66%, respectively. Municipalities of Baggao and Buguey revealed no cases of CAEV infection, suggesting that certain areas may have more active control procedures or environmental aspects that limit viral spread. Lallo municipality indicated a moderate infection rate of 8.33%. This variability in infection rates may be influenced by several factors, including differences in herd management, biosecurity practices, or herd density as supported by Jesse *et al.* (2018) and Han *et al.* (2019).

Table 3. Overall summary seroprevalence of CAE virus infection in the 2nd district of Cagayan province

District 2	Number of samples	CAEV positive	% of CAEV infection
Piat	21	4	19.04%
Rizal	14	0	0%
Sto. Niño	4	1	25%
Total	39	5	12.5%

The overall seroprevalence of CAEV infection in District 2 (Table 3) was at 12.5%, with 5 out of 39 tested samples being positive for CAEV antibodies. However, when disaggregated by site, the results divulge unpredictability in infection rates. The distribution of CAEV infection across District 2 specifies probably limited risk aspects or managing practices influencing transmission. The 19.04% infection rate is in Piat and 25% in Sto. Niño is suggestively higher than in Rizal, where no infection was detected. This suggests that environmental, husbandry, or biosecurity differences may be contributing to these disparities. Denser goat populations, as possibly seen in Piat, may surge the probability of viral transmission through close interaction (Peng *et al.*, 2024).

The findings underline the need for targeted intervention strategies in regions with higher seroprevalence, such as Piat and Sto. Niño. It is also critical to conduct further inquiries to comprehend the absence of contagion in Rizal, which could offer an understanding of effective disease management practices that could be employed in another place.

Table 4. Overall summary seroprevalence of CAE virus infection in the 3rd district of Cagayan province

District 3	Number of samples	CAEV positive	% of CAEV infection
Enrile	15	0	0%
Peñablanca	10	0	0%
Solana	92	0	0%
Tuao	46	6	13.04%
Tuguegarao city	22	0	0%
Total	185	6	3.24

CAEV infection in District 3 (Table 4) was 3.24%, with 6 out of 185 samples testing positive for CAEV antibodies. Remarkably, only one farm site, Tuao, displayed contagion, while all other municipalities reported zero incidences. The data demonstrates a contrast between Tuao, where 13.04% of the samples were positive for CAEV, and the other four municipalities (Enrile, Peñablanca, Solana, and Tuguegarao City), all of which showed 0% contamination rates. This denoted that the contagion may be localized to specific areas within District 3, with Tuao being the only place exhibiting active CAEV transmission. The presence of CAEV in Tuao may indicate that the place has surroundings that favor the survival or spread of the virus. Such inference is supported by the attribution of McGuire *et al.* (1990).

Table 5. Comparison of the seroprevalence of caprine arthritis encephalitis virus (CAEV) infection between three districts of Cagayan province

Comparison	Chi-square statistic	p-value	Interpretation
District 1 vs 2	0.50	0.478	No significant difference
District 1 vs 3	3.73	0.054	Marginally significant difference
District 2 vs 3	0.27	0.600	No significant difference

Comparing District 1 vs District 2 ($\chi^2 = 0.50$, $p = 0.478$), the statistic of 0.50 and p-value of 0.478 indicate no significant difference in CAEV seroprevalence (Table 5). The *p*-value is well above the standard significance threshold of 0.05, signifying that the contagion rates in these districts are statistically analogous. This result shows that similar risk factors, management practices, or environmental conditions may influence CAEV transmission in Districts 1 and 2. The comparison between District 1 and District 3 ($\chi^2 = 3.73$, $p = 0.054$) yields are marginally significant. Although the *p*-value is slightly above the conventional 0.05 threshold, it suggests a potential difference in CAEV seroprevalence between these districts. This marginal result might warrant further investigation, as District 3 had notably lower infection rates overall compared to District 1. The difference in seroprevalence might be due to varying levels of biosecurity measures, herd management practices, or other localized factors that impact CAEV transmission. The chi-square statistic of 0.27 and a *p*-value of 0.600 show no significant difference between District 2 and District 3. With a *p*-value far exceeding the 0.05 threshold, this result implies that CAEV infection rates in these districts are statistically similar, further reinforcing the notion that factors influencing disease spread in District 2 and District 3 may be alike.

The lack of significant differences between Districts 1 and 2 suggests that CAEV prevalence is relatively uniform across these regions. This may point to similar risk profiles, such as herd management practices, goat population density, or exposure to infected animals (Tabet *et al.*, 2015). Disease control measures could be applied similarly in these regions, focusing on general prevention strategies, such as improved biosecurity protocols and regular testing.

The marginally significant difference between Districts 1 and 3 could highlight important epidemiological distinctions between these areas. District 1, with its higher prevalence, may benefit from intensified interventions to control CAEV spread, including more frequent testing, isolation of seropositive animals, and educating farmers on improved management practices. District 3, on the other hand, may reflect regions with lower infection rates, where existing control measures could be more effective or less animal movement occurs.

The statistical similarity between District 2 and District 3 may reflect shared management practices or geographic characteristics that minimize CAEV transmission. These findings could inform targeted surveillance and control programs, ensuring resources are directed toward areas with elevated risk, like District 1 (Peterson *et al.*, 2022).

Conclusion

The results of this study emphasize the critical need for continuous surveillance, targeted interventions, and further inquiry into the epidemiological drivers of CAEV (Caprine Arthritis Encephalitis Virus) transmission within the goat populations of Cagayan, Philippines. The substantial health inferences for goat farmers dictate the employment of inclusive control approaches. Key measures such as regular serological testing, selective culling of infected animals, and rigorous biosecurity procedures are crucial in reducing viral transmission rates and mitigating economic losses. Moreover, the necessity for localized research is evident, as it is crucial for understanding the full scope of CAEV prevalence and risk factors in these districts. Tailored intervention programs, based on specific epidemiological data, are essential for enhancing the efficacy of prevention strategies. The insights generated from this study offer critical data that will aid the Department of Agriculture in formulating a scientifically grounded, evidence-based animal health program for small ruminants. This program will specifically target the prevention and control of CAEV, contributing to the sustainable management of the disease and the long-term viability of goat farming in the Cagayan Valley region.

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