



NOTE

Virology



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Received: 25 July 2016 Accepted: 7 November 2016 Published online in J-STAGE: 4 December 2016 **ABSTRACT.** In this study, a large-scale serological survey of caprine arthritis encephalitis virus (CAEV) infection was conducted between March 2011 and October 2012. 3,437 goat blood or milk samples were collected from 65 goat farms throughout Taiwan. A commercial ELISA kit was used to detect antibodies against CAEV. The overall seropositive rate was 61.7% (2,120/3,437) in goats and in 98.5% (64/65) of goat farms. These results provide the first large-scale serological evidence for the presence of CAEV infection, indicating that the disease is widespread in Taiwan.

KEY WORDS: caprine encephalitis virus, goat, serological survey, Taiwan

Caprine arthritis encephalitis virus (CAEV) belongs to the genus *Lentivirus* of the family *Retroviridae* and causes a persistent and progressive inflammatory disease in goats [6]. CAEV infections deteriorate the goat productivity, causing great impacts of economic and animal welfare in goat industries [10, 15]. According to the latest 2015 survey, the goat population in Taiwan is 156,045 and consists of an estimated two- thirds for meat goats and one-third for dairy goats (Statistics numbers of goats were provided by Council of Agriculture, Executive Yuan, Republic of China (R.O.C.); http://agrstat.coa.gov.tw/sdweb/public/ book/Book.aspx). Although the first CAEV case was identified by transmission electron microscopy in 1993 [12], the exact CAEV prevalence rate in Taiwan is still largely unknown [23]. Therefore, the aim of the study is to conduct a large-scale CAEV serological survey in Taiwan goat farms using commercial CAEV enzyme-linked immunosorbent assay (ELISA) kit.

Blood samples from the goat's jugular vein were collected into a clot activator syringe. Blood samples were transported to the laboratory, and serum was isolated and stored at -20°C until examined. Milk and serum samples provided by the Goat Farmer Association of R.O.C., as well as local Animal Disease Control Centers in Taiwan, were also included in this study. A commercial ELISA kit (CHEKIT CAEV/MVV Antibody ELISA Test Kit, IDEXX Laboratories, Bern, Switzerland) was used to detect antibodies against CAEV in the samples. The wells of microtiter plates were precoated with the inactive viral antigen, capsid protein P28, which can be recognized by the primary antibodies present in the serum or milk. Horseradish peroxidase (HRP)-labeled anti-ruminant IgG antibodies were added to detect the primary goat antibodies, followed by adding tetramethylbenzidine (TMB)-containing substrate to form a soluble blue reaction product. The titer of anti-CAEV antibodies was measured at a wavelength of 450 nm. The OD value was averaged and analyzed with the formula:

Value (%)=[(OD_{sample}-OD_{negative})/(OD_{positive}-O_{negative})] × 100%

A value greater than or equal to 40% was considered a positive result, and a value less than 30% was considered a negative result. A value between 30 and 40% was considered a suspect result. All statistical analyses were processed using commercial statistical software (SPSS, version 22). The Pearson's chi-square test was used for comparing the serological CAEV infection status between different types of goat farms (dairy, meat and breeding goats). Differences among the three types of goat farms were identified by the Scheffe test. A *P*-value less than 0.05 was considered statistically significant.

Between March 2011 and October 2012, 3,437 samples, including serum and milk, were collected from 65 goat farms that were located in the northern, central, southern and eastern regions of Taiwan. The goat farms were further divided into three categories, dairy goat farm (n=49), meat goat farm (n=14) and breeding goat farm (n=2). Our survey indicates that there was a high CAEV

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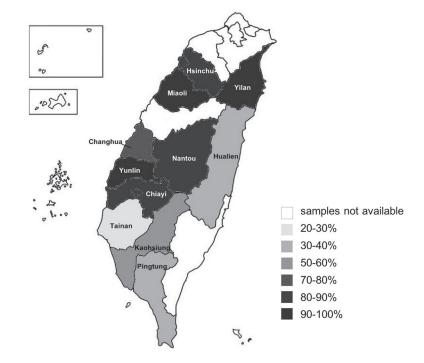


Fig. 1. Geographic distribution of seropositive goats for anti-CAEV antibodies in Taiwan, 2011–2012 (n=3,437). Almost all the goats from each county in Taiwan we tested were seropositive for anti-CAEV antibodies. The seropositive rates ranged from 49.7 to 90.7% among four regions of Taiwan.

| Region of Taiwan | County | Total tested samples | Seropositive samples | CAEV positive rate (%) |
|------------------|-----------|----------------------|----------------------|------------------------|
| Northern | Hsinchu | 30 | 26 | 86.7 |
| | Miaoli | 120 | 110 | 91.7 |
| Central | Changhua | 507 | 392 | 77.3 |
| | Nantou | 536 | 456 | 85.1 |
| | Yunlin | 49 | 44 | 89.8 |
| Southern | Chiayi | 787 | 666 | 84.6 |
| | Tainan | 870 | 205 | 23.6 |
| | Kaohsiung | 84 | 50 | 59.5 |
| | Pingtung | 327 | 107 | 32.7 |
| Eastern | Yilan | 30 | 27 | 90.0 |
| | Hualien | 97 | 37 | 38.1 |

Table 1. Distribution of CAEV antibody positive goats in the regions of Taiwan

seroprevalence among the goat farms in Taiwan (98.5%, 64/65). Among the 65 farms that were chosen nationwide in our study, only one goat farm in Tainan tested negative for CAEV. All other farms tested positive for CAEV, with the northern, central, southern and eastern regions in Taiwan, being 90.7% (136/150), 81.7% (892/1,092), 49.7% (1,028/2,068) and 50.4% (64/127), respectively. The highest prevalence rate was found in Miaoli (91.7%, 110/120 goats) (Table 1) (Fig.1). We then analyzed the CAEV prevalence rates among the different types of goat farms, and we found that the goats from dairy farms showed a higher CAEV positive rate (82.0%, 1,669/2,035) than the goats from meat farms (35.1%, 399/1,138) or breeding farms (19.7%, 52/264) (Table 2), although the statistical analysis does not reveal a significant difference (Table 3).

We have noticed that the CAEV positive rate of dairy goat farms is higher than meat goat farms or breeding goat farms. The cause of this problem stems from issues in the nursing management. Nearly all the commercial dairy goat farms in Taiwan use incompletely pasteurized bulk milk to feed their lambs, whereas for meat goats, whose lambs are fed right from their mother. The former feeding strategy used in dairy goat farm may accelerate the spreading of diseases [13, 14]. Previous studies have suggested that the longer lifespan of dairy goats combined with their close proximity to each other may cause this high CAEV prevalence rate in dairy goat farms [1, 15]. Meat goats in Taiwan are often sold and slaughtered from twelve to fifteen months of age, so CAEV would be effectively eliminated from the farm due to total turnover of new meat goats. The lower infection rates in breeding goat farms are likely due to strict security measures and compliance towards CAEV monitoring and control programs, since these two

| Types of farms | n ^{a)} | Prevalence of CAEV ^{b)} | | | Chi-square | P value |
|------------------|-----------------|----------------------------------|------------|----------|---------------|----------|
| Types of failins | 11.77 | + | _ | +/- | · CIII-square | 1 value |
| Dairy goat | 2,035 | 1,669 (82.0) | 339 (16.7) | 27 (1.3) | 932.98 | < 0.0001 |
| Meat goat | 1,138 | 399 (35.1) | 731 (64.2) | 8 (0.7) | | |
| Breeding goat | 264 | 52 (19.7) | 212 (80.3) | 0 (0.0) | | |

| Table 2. | Prevalence of CAEV | infection status | by the types | s of goat farms |
|----------|--------------------|------------------|--------------|-----------------|
|----------|--------------------|------------------|--------------|-----------------|

a) n: sample size, the percentage in parenthesis. b) CAEV ELISA results: +: positive, -: negative, +/-: suspected positive.

 Table 3. Comparison of the CAEV-positive rate in the different types of goat farms in Taiwan

| Farms | Meat goat | Breeding goat |
|------------|-------------------------------|-----------------|
| Diary goat | 0.12034 (0.281) ^{a)} | 0.57520 (0.008) |
| Meat goat | | 0.45486 (0.058) |
| | a : | |

a) the *P* value in parenthesis.

farms are government owned and operated.

Our study is the first large-scale serological survey of CAEV in Taiwan. Based on our findings, CAEV is widespread in Taiwan, as 61.7% (2,120/3,437) of goats from 98.5% (64/65) of tested farms in Taiwan tested positive for CAEV. CAEV is widespread around the world. Serological surveys have shown that the prevalence rates of total population were 31%, 42%, 8.2% and 5.1% in U.S.A. [4], Switzerland [9], Brazil [2] and Sultanate of Oman [21], respectively. Besides, very high herd seropositivity for CAEV has been reported in the U.S.A. (73%) [4], Australia (82%) [5] and Brazil (35%) [2]. Among the neighboring countries of Taiwan, the seroprevalence of CAEV was 10% in a total of 857 goat serum samples in Japan [7], compared to only 2.73% of goats in South Korea [14]. In China, the CAEV seropositive rates varied from 0.2 to 30% [16]. The seroprevalence of CAEV in Taiwan is much higher than the neighboring areas, such as Japan, China and Korea. Worth noting is that most of the frozen semen imported into Taiwan is from France and that most goats are imported from New Zealand. Both France and New Zealand are not CAEV-free. Although the seroprevalence of the disease in New Zealand is low [17, 18, 20], France is higher than 65% [11]. The quarantine requirements in Taiwan, however, do not require a CAEV-free certification when importing goats and goat products. From the results we observed, there are some areas for improvement in our quarantine policy for CAEV disease management.

Neither effective treatment nor vaccine against CAEV has been available until now, and preventing infection is the best strategy to halt the spread of this virus [17, 22]. To minimize the risk of CAEV infection in the future, the owner of the sole CAEV negative farm that we identified in Tainan has thoroughly followed the infection control protocol provided by the Council of Agriculture, Executive Yuan, R.O.C. Our disease control and eradication program is composed of three procedures. The first procedure is neonate isolation; the newborn kids will be removed from their dams right after birth, to minimize the vertical transmission. Furthermore, neonates will be artificially fed with heat-treated colostrum (56°C, an hour) to avoid lactogenic transmission [8, 13, 19]. The second procedure is segregation of infected animals; goats over three months old will be periodically tested for CAEV every three to six months. Upon diagnosis, all CAEV seropositive goats will be segregated from the CAEV negative goats, and culling is suggested if the infected goats show signs of CAEV [3, 8, 17, 19]. As for biosecurity measures, feeding and farm cleaning should begin from the stables housing CAEV negative goats, followed by CAEV positive stables. The last procedure is selection of imported animal; goats and semen samples must be obtained from certified CAEV-free goat farms. Several studies have identified that both live animal trading and artificial insemination expedite the spread of CAEV [15, 17, 22]. World Organization for Animal Health (OIE) has provided the following recommendations for importing goat from other countries: (1) the goats should show no clinical signs of CAEV on the day of shipment; (2) goats over a year old should be tested negative for CAEV, 30 days prior to shipment, or (3) CAEV must be neither clinically nor serologically diagnosed at the origin of the goat flocks during the past three years, and importing goats from a flock of CAEV-infected or of unknown health status are prohibited during this three year period (OIE, http://www.oie.int).

In conclusion, the high occurrence of CAEV seropositivity in Taiwan indicates an urgent need to implement a CAEV control and eradication program in goat farms nationwide. In addition, further studies are needed to determine the economic impact on the goat industries. Moreover, to ascertain the epidemic status of CAEV in Taiwan, routine monitoring should be carried out for CAEV prevention and diagnosis.

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