การสำรวจทางซีรั่มวิทยาและศึกษาผลกระทบของการติดเชื้อไวรัสข้ออักเสบและสมองอักเสบต่อสมรรถภาพการสืบพันธุ์ของแพะในภาคตะวันตกประเทศไทย

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A SEROLOGICAL SURVEY AND STUDY ON THE EFFECTS OF CAPRINE ARTHRITIS-ENCEPHALITIS VIRUS INFECTION ON REPRODUCTIVE PERFORMANCE OF GOATS IN THE WESTERN PART OF THAILAND

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A thesis submitted in partial fulfillment of the requirements

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Department of Obstetrics, Gynaecology and Reproduction

Faculty of Veterinary Science

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การศึกษาซีรัมวิทยาของการติดเชื้อไวรัสโรคข้ออักเสบและสมองอักเสบของแพะ (Caprine Arthritis Encephalitis Virus Infection, CAEV) จาก 3 จังหวัดในภาคตะวันตกของประเทศไทย ระหว่างเดือนพฤศจิกายน 2552 – มกราคม 2554 โดยการสุ่มคัดเลือกฟาร์ม จำนวน 74 ฟาร์ม เก็บตัวอย่างเลือดแพะจำนวน 1,129 ตัวอย่าง เพื่อวิเคราะห์หาภูมิคุ้มกันต่อเชื้อ CAEV โดยวิธี competitive enzyme-linked immunosorbent assay (cELISA) และใช้แบบสอบถามสำรวจเก็บข้อมูลจากเจ้าของฟาร์มเพื่อนำมาใช้ในการวิเคราะห์ทางสถิติโดยวิธีไคสแควร์ หาความสัมพันธ์ระหว่าง CAEV กับปัจจัยที่เกี่ยวข้อง และใช้การวิเคราะห์แบบถดถอยพหุลอจิสติกเพื่อหาปัจจัยเสี่ยงของฟาร์มต่อ CAEV พบว่าแพะจำนวน 67 ตัวจากฟาร์มแพะ 23 ฟาร์ม ให้ผลบวกต่อ CAEV คิดเป็นร้อยละ 31 และพบว่าในระดับฟาร์มปัจจัยเสี่ยงต่อ CAEV ของฟาร์ม ได้แก่ ชนิดของฝูง (p = 0.034; OR = 5.026; 95% CI = 1.130-22.360) ขนาดฝูง (p = 0.006; OR = 24.065; 95% CI = 2.466-234.788) และการติดต่อจากแพะจากฝูงอื่น (p = 0.008; OR = 8.526; 95% CI= 1.762 - 41.25) การเพิ่มแพะใหม่ในฝูง (p = 0.044, OR = 4.396; 95% CI = 1.044 – 18.51) ในระดับตัวแพะปัจจัยเสี่ยงต่อ CAEV ได้แก่ แพะอายุตั้งแต่ 3 ปีขึ้นไป (p = 0.001, OR = 4.288, 95% CI = 1.809 – 10.163) ขนาดฝูง (p < 0.001, OR = 17.971, 95% CI = 7.787 – 41.475) และการเพิ่มแพะใหม่ในฝูงเป็นปัจจัยเสี่ยงที่สัมพันธ์กับความชุกของโรคไวรัสข้ออักเสบและสมองอักเสบในแพะ ผลการศึกษาพบว่าปัจจัยเสี่ยงต่อ CAEV ของฟาร์มแพะในภาคตะวันตกเป็นสิ่งที่ควรต้องระมัดระวัง

การศึกษาด้านระบบสืบพันธุ์ของแพะในฟาร์มที่มีผลบวกต่อ CAEV แบ่งแพะในฟาร์มเป็น 2 กลุ่ม คือ กลุ่มที่มีผลบวก และ กลุ่มที่มีผลลบต่อ CAEV ระหว่างเดือนมกราคม 2553 ถึง เดือนกุมภาพันธ์ 2554 พบว่ามีลูกแพะแรกเกิดในกลุ่มที่มีผลลบ (1.63) มากกว่ากลุ่มที่มีผลบวก (1.50) อัตราการผสมติดครั้งแรกของกลุ่มที่มีผลบวกน้อยกว่าอย่างมีนัยสำคัญทางสถิติ (p < 0.05) ส่วนอัตราการผสมติดรวมของกลุ่มที่มีผลลบ (82.6%) มากกว่ากลุ่มที่มีผลบวก (50%) อัตราการผสมไม่ติดภายหลังการผสมเทียมติดต่อกัน 2 ครั้ง ของกลุ่มที่มีผลบวก (25%) สูงกว่ากลุ่มที่มีผลลบ (13%) ผลการศึกษาพบว่าระบบสืบพันธุ์ของแพะที่มีผลบวกต่อ CAEV มีผลกระทบต่อประสิทธิภาพของระบบสืบพันธุ์ แต่ผลกระทบจาก CAEV เป็นอย่างไรนั้นควรที่จะมีการศึกษาในเชิงลึกต่อไป

ภาควิชา ..สูติศาสตร์ เธนุเวชวิทยาและวิทยาการสืบพันธุ์.. ลายมือชื่อนิสิต……

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THANT NYI LIN: A SEROLOGICAL SURVEY AND STUDY ON THE EFFECTS OF CAPRINE ARTHRITIS-ENCEPHALITIS VIRUS INFECTION ON REPRODUCTIVE PERFORMANCE OF GOATS IN THE WESTERN PART OF THAILAND.
ADVISOR: PROFESSOR MONGKOL TECHAKUMPHU, D.V.M., Doctorat de 3e cycle,
CO-ADVISOR: ASSOCIATE PROFESSOR PRACHIN VIRAKUL, D.V.M., Ph.D., 77 pp.

During the period from November 2009 to January 2011, a cross-sectional serological survey was carried out in three western provinces of Thailand. A total of 1,129 serum samples from 74 randomly selected goat farms were collected and seroprevalence of antibodies to CAEV infection was determined using competitive enzyme-linked immunosorbent assay (cELISA) test. Semi-structural questionnaires were developed and presented to farm owners. Univariable analysis by Chi-square test was employed to find out the association between CAEV infection and each exposed factor. Multivariable logistic regression model was run to clarify the risk factors. A total of 67 goats (5.9%) were found seropositive with true prevalence of 5.5%. 23 farms out of 74 were found seropositve showing the prevalence of 31%. On herd prevalence, herd type (p= 0.034; OR=5.026; 95% CI=1.130-22.360), herd size (p=0.006; OR=24.065; 95% CI= 2.466- 234.788), contact with goats from other herds (p=0.008; OR=8.526; 95% CI= 1.762 - 41.25), and addition of new goats into herd (p=0.044, OR=4.396; 95% CI=1.044 – 18.51) were observed as risk factors to CAEV infection. On individual prevalence, age of 3 years and above (p=0.001, OR=4.288, 95% CI=1.809 – 10.163), herd size (p< 0.001, OR=17.971, 95% CI=7.787 – 41.475), and addition of new goats into herd were detected as risk factors. The results showed that CAEV infection prevailed among the goat herds in the western part of Thailand, with some risk factors to be aware of.

A comparative study between two groups of animal, a seropositive and a seronegative group, regarding their reproductive performance in response to CAEV infection was performed in a CAEV seropositive dairy goat farm during the period from January 2010 to February 2011. Average number of offspring born to each group was higher in seronegative group as compared to seropositive group (1.63 vs 1.50). A significantly low (p<0.05) first conception rate was observed in seropositive group, and total conception rate was higher in seronegative group than in seropositve group (82.6% vs 50.0%). Failure to conceive during two consecutive AI was more frequent in seropositive group than in seronegative group (25% vs 13%). These findings suggested some evidence of adverse effects of CAEV infection on reproductive performance of goats and further in-depth studies with greater sample size are deemed necessary to define the influencing effects of CAEV infection on reproduction in goats more precisely.

Department: Obstetrics, Gynaecology and Reproduction
Field of Study: Theriogenology
Academic Year: 2011

Student's Signature .....
Advisor's Signature.....
Co-advisor's Signature .....

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LIST OF ABBREVIATIONS

°C	Degree Celsius
AGID	Agarose Gel Immunodiffusion
AI	Artificial Insemination
AP	Apparent Prevalence
CAE	Caprine Arthritis-Encephalitis
CAEV	Caprine Arthritis-Encephalitis Virus
cELISA	Competitive Enzyme-Linked Immunosorbent Serologic Assay
CI	Confidence Interval
DLD	Department of Livestock Development, Thailand
DNA	Deoxyribonucleic acid
ELISA	Enzyme-Linked Immunosorbent Serologic Assay
et al.	et alia, and other
FAO	Food and Agriculture Organization
g	a unit force equal to force exerted by gravity
HIV	Human Immunodeficiency Virus
IP	Immunoprecipitation
LBVD	Livestock Breeding and Veterinary Department
min	min
ml	milliliter
MVV	Maedi-Visna Virus
OD	Optical Density
OR	Odds Ratio
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
Se	Sensitivity of the test
SE	Standard error
Sp	Specificity of the test

SPSS	Statistical Package for Social Sciences
SRLV	Small Ruminant Lentiviruses
TP	True Prevalence
UV	Ultraviolet
VLG	Viral Leukoencephalomyelitis of Goats

Chapter I
INTRODUCTION

Goat farming, with its virtue of turning low quality forage into highly nutritious milk and meat (Lombardi, 2005), has long been playing an important socio-economic role in many countries all over the world (Ruiz et al., 2009; Castle et al., 2010). According to FAO, total number of goats in the world, which was 590.1 million in 1990, had gradually increased by about 1% to 4% each year, and reached 861.9 million in 2008 (FAOSTAT, 2008), showing the worldwide significance of goat farming (Morand-Fehr and Lebbie, 2004). However, despite its socio-economic importance in many countries, goat farming, on the other hand, has not attained its optimum success partly due to many infectious diseases, including caprine arthritis-encephalitis (CAE) (Greenwood, 1995[a]; Lilenbaum et al., 2007; Leitner et al., 2010).

Caprine arthritis encephalitis, previously known as viral leukoencephalomyelitis of goats (VLG) (Kusza et al., 2004), is an important viral disease of domestic goats, which induces negative impacts on reproductive performance as well as on the production efficiency of goats (Rodriguez et al., 2005; Leitner et al., 2010). The adverse effects of CAE on the health of infected individuals (Al-Ani and Vestweber, 1984; Greenwood, 1995[a]) cause substantial economic losses to goat industry (Peterhans et al., 2004; Aslantas et al., 2005).

The disease was first reported by *Cork* and his associates in 1974 (Cork et al., 1974; Elfahal et al., 2010) as a nervous disease that caused leucoencephalomyelitis in young goats. Later on, it was found that arthritis could also result from the same disease causing leucoencephalomyelitis in goats, and therefore it was named Caprine Arthritis-Encephalitis (CAE) (Kusza et al., 2004). It is a slowly-progressive multi-systemic inflammatory disease, of chronic in nature, characterized by insidious onset, long incubation period, protracted clinical course and persistent infection with no apparent

recovery (Al-Ani and Vestweber, 1984). As the name implies, CAE is usually seen as leukoencephalomyelitis in kids, especially in those younger than 6 months, and polyarthritis in adult goats; however, other clinical manifestation such as indurative mastitis, interstitial pneumonia, chronic weight loss and debilitation are also common (Lamara et al., 2002; Rodriguez et al., 2005; Gregory et al., 2009).

CAE is also an economically significant disease (Aslantas et al., 2005; Le Jan et al., 2005). It gives rise to several problems on reproductive performance and productivity of affected herds (Reina et al., 2009). Economic losses due to CAE usually come from reproductive failure, poor production efficiency and premature culling of the goats (Peterhans et al., 2004; Aslantas et al., 2005).

Moreover, other inferior reproductive performances, such as decreased number of offspring at each generation, increased mortality rate before weaning, reduced conception rate in seropositive does, decreased birth weight and delayed weight gain in kids, as well as reduced lactation period, retarded growth rate after weaning, and poor survival of the kids are also found subsequent to CAE in affected herds (Greenwood, 1995[a]; Peterhans et al., 2004; Cruz et al., 2009; Reina et al., 2009).

In addition, the use of indirect indicator such as somatic cell counts can be impaired in CAE-affected herds due to increased cellularity caused by infection (Sanchez et al., 2001; Le Jan et al., 2005) and, furthermore, a long-term effects of increasing health problems within the herd will result in poor milk quality and lower milk yield (Le Jan et al., 2005; Gufler et al., 2007[a]; Bandeira et al., 2009; Leitner et al., 2010). There also will be an inferior genetic progress, followed by the premature culling of goats, causing a considerable economic loss (East et al., 1993; Travassos et al., 1999).

From zoonotic point of view, although there is no evidence that CAE can develop in human (MacDiarmid, 1983), it has been noticed that a strong cross reactivity exists

between the causative virus of CAE in goats and that of HIV in human (Tesoro-Cruz et al., 2003; Kusza et al., 2004), as they are of the same kind of virus with different serotypes coming from the same family (Bouzar et al., 2007; Denner, 2007), and therefore false positive reactions to HIV in some people have been postulated as a result of prior consumption on milk from the CAE-affected goats (Tesoro-Cruz et al., 2003), suggesting that drinking unpasteurized goat milk should be avoided in order to reduce zoonotic risk of CAE to human (Rowe et al., 1991).

Over the time, much attention has been paid to the control of CAE because of its massive consequences on livestock productivity and negative impacts on the economics of goat farming (Nord et al., 1998[a]; Peterhans et al., 2004). Several control and eradication programs against CAE (Nord et al., 1998[a;] Brinkhof et al., 2009; Reina et al., 2009) including the restriction of live animal trading, a principal risk factor for the spread of CAE (Blacklaws et al., 2004; Rodriguez et al., 2005; Elfahal et al., 2010), and regular check testing and purchasing of goats only from CAE negative herds have been implemented in many countries (MacDiarmid, 1983; Greenwood et al., 1995[b]; Cruz et al., 2009).

Basically, CAE is considered not only risky to health of animals, but it is also termed as animal welfare issue due to its significant impact on animal's well-being, caused by lifelong pain and disability, mainly due to bilateral swelling of carpal joints (deMaar et al., 1995; Peterhans et al., 2004; Leitner et al., 2010), and it is therefore alternatively called big knee disease (MacDiarmid, 1983).

Although it is worldwide in distribution (Aslantas et al., 2005; Leitner et al., 2010), prevalence is remarkably higher in those countries where dairy goat farming is highly industrialized (East et al., 1993; Lamara et al., 2002; Ali Al Ahmad et al., 2008[a]). In some countries, CAE has been found endemic to particular areas with high level of incidence (Klevjer-Anderson and Anderson, 1982; Al-Ani and Vestweber, 1984).

Outbreaks of CAE reported from many countries have covered numerous parts of the world, showing a wide range of variation over the prevalence rates. It ranged from a very high percentage, as high as 82% in Australia, 73% in USA and 56% in Wales, through some moderate levels of around 42% in Norway, 23% in Jordan and 14.1% in Brazil, to a very low percentage of as low as 4.0% in Italy, 3.6% in Mexico and 1.9% in Turkey (Nord et al., 1998[b]; Al-Qudah et al., 2006; Gufler and Baumgartner, 2007[b]).

In Thailand, along with the increasing number of goats under the development of small ruminant farming (Rukkwamsuk et al., 2008), presence of CAE has been reported on occasions (Tantaswasdi et al., 1985), and prevalence from a fresh finding was found to be standing at 12.4% (Ratanapob et al., 2009).

1.1 Research questions

- What is the seroprevalence of CAEV infection among the goat population in the Western part of Thailand?
- What are the risk factors associated with the prevalence of CAEV infection in the study area?
- What are the effects of CAEV infection on reproductive performance of goats?

1.2 Research objectives

- To determine the seroprevalance of CAEV infection among the goat herds from three Western provinces (Ratchaburi, Petchaburi and Kanchanaburi provinces) of Thailand.
- To detect the potential risk factors associated with the prevalence of CAEV infection in the study area.
- To study the effects of CAEV infection on the reproductive performance of goats.

1.3 Research hypothesis

- Caprine arthritis encephalitis virus may have been spread over among the population of goats in three Western provinces (Ratchaburi, Phetchaburi and Kanchanaburi provinces) of Thailand, and there must be some potential risk factors related to that occurrence of infection.
- The presence of CAEV infection in herds will produce a negative impact on the reproductive performance of the herds due to the adverse effects of infection, and there must be some differences between seropositive and seronegative does on their reproductive performances against CAEV infection.

Chapter II
LITERATURE REVIEW

2.1 Etiologic agent

CAE is caused by a virus called Caprine Arthritis-Encephalitis virus (CAEV) (Klevjer-Anderson and Anderson, 1982; Al-Ani and Vestweber, 1984), which is closely related to the maedi-visna virus (MVV) of sheep (Kwang et al., 1995; Plaza et al., 2009). It is one of the only two lentiviruses currently known to be associated with infections in sheep and goats (Denner, 2007; Ghanem et al., 2009). CAEV can be defined as a part of small ruminant lentivirus (SRLV), which comprises a blend of maedi-visna virus (MVV) in sheep and caprine arthritis-encephalitis virus (CAEV) in goats (de Andrés et al., 2005; Gufler et al., 2007[a]; Brinkhof et al., 2009), both of which are considered genetically similar (Kwang et al., 1995; Chebloune et al., 1996) having 60-70% nucleotide sequence homology (Eltahir et al., 2006) and capable of producing evolving complex syndrome (Narayan et al., 1980; Archambault et al., 1988) causing multisystemic inflammatory diseases in sheep and goats (Blacklaws et al., 2004; Logan et al., 2004; Reina et al., 2009).

2.2 Virus morphology

CAEV is the single-stranded RNA Lentivirus belonging to the subfamily Lentivirinae of the family Retroviridae (Klevjer-Anderson and Anderson, 1982; Archambault et al., 1988; Tesoro-Cruz et al., 2003). It is magnesium-dependent and has a RNA-dependent DNA polymerase (Al-Ani and Vestweber, 1984). Cross-reactivity between CAEV and other lentiviruses, including HIV in human, have been reported (Tesoro-Cruz et al., 2003). Similar to the other lentiviruses, CAEV is relatively resistant to UV light and irradiation (Nord et al., 1998[a]), but not to drying, heating and sunshine (Narayan et al., 1982). Just like other lentiviruses, CAEV cannot survive long, and does

not stay infectious, outside the host (Nord et al., 1998[a]). The virus has a particular affection on leucocytes, especially with monocytes and tissue macrophages (Narayan et al., 1983; Bouzar et al., 2007).

2.3. Species affected

Goats of any age, sex and breed, including crossbreeds, are susceptible to CAEV and can be readily infected (Al-Ani and Vestweber, 1984; Cortez-Moreira et al., 2005), despite the fact there have been a few reports on the description of natural resistance of some indigenous breeds to CAEV infection (Torres-Acosta et al., 2003; Cruz et al., 2009). Another statement is that the CAEV infection is more prevalent with imported goats (Peterhans et al., 2004; Elfahal et al., 2010), and also that it is more common in dairy goats than in meat goats (MacDiarmid, 1983; deMaar et al., 1995; Gufler et al., 2007[a]; Ali Al Ahmad et al., 2008[b]). In terms of breeds, Saanen is the most susceptible breed known to be infected by CAEV infection (Rodriguez et al., 2005).

Although CAEV infection is primarily confined to goats (Nord et al., 1998[a]), cumulative findings have suggested that trans-species transmission between sheep and goats (Torres-Acosta et al., 2003; Gufler et al., 2007[a]; Ghanem et al., 2009), which may be bidirectional, either from goat to sheep or from sheep to goat (MacDiarmid, 1983; Reina et al., 2009), may exist. Some reports say that Maedi-visna virus (MVV) of sheep can transmit to goats, and, vice versa, CAEV of goats can transmit to sheep (Denner, 2007).

2.4 Transmission

Transmission of CAEV infection most commonly occurs via the ingestion of virus-containing colostrums or milk (East et al., 1993; Peterhans et al., 2004), and therefore transmission of infection from dam to kid through the colostrums is accepted as the principal mode of transmission (Blacklaws et al., 2004; Le Jan et al., 2005). However, since the CAEV has a particular tropism for monocytes, macrophages, fibroblasts and endothelial cells (Le Jan et al., 2005; Bouzar et al., 2007), all bodily secretion and excretion containing white blood cells can be considered as possible sources of infection (Narayan et al., 1983; Kusza et al., 2004; Ali Al Ahmad et al., 2008[b]) and the virus can therefore be transmitted through any other possible routes, other than ingestion of virus-infected milk or colostrums, such as direct contact with infected animals, in contact with urogenital secretion or saliva, and through the blood and contaminated utensils such as milking machine and tattooing equipments (Al-Ani and Vestweber, 1984; East et al., 1993; Travassos et al., 1999; Logan et al., 2004).

Although transmission primarily takes place by the ingestion of colostrums or milk between dam and kid, vertical transmission through intrauterine infection or transplacental transmission remains unclear (MacDiarmid, 1983; Peterhans et al., 2004; Gufler et al., 2007[a]), nevertheless some suggest there is a possibility of vertical transmission from dam to offspring (Nord et al., 1998[a]; Blacklaws et al., 2004). Transmission through embryo transfer is considered unlikely to happen (Lamara et al., 2002; Ali Al Ahmad et al., 2008[a]). Aerosol transmission can also exist between the animals kept close to each other (Rowe et al., 1991; Blacklaws et al., 2004), and this route of transmission may be significant over a distance of several meters within a herd, particularly under intensive management systems (Peterhans et al., 2004).

Unlike other retroviruses, CAEV is not well defined for sexual transmission (MacDiarmid, 1983; Travassos et al., 1999; Blacklaws et al., 2004). But then again, the

presence of CAEV proviral DNA in the semen of naturally infected bucks (Bandeira et al., 2009) and the evidence of a positive correlation between the positivity of CAEV in blood and its presence in the semen of infected bucks suggest that transmission through semen is not unlikely (Rodriguez et al., 2005; Ali Al Ahmad et al., 2008[b]; Cruz et al., 2009). It may depend on the presence of infected cells in the genital tracts of bucks and release of infected cells through contaminated semen (Nord et al., 1998[a]; Rodriguez et al., 2005; Ali Al Ahmad et al., 2008[b]). However, to date, very little information has been available for such route of transmission (Rodriguez et al., 2005; Bandeira et al., 2009). In general, males are considered as main reservoirs of CAEV infection and females, in turn, are considered to be principal distributors of disease in a herd (Rodriguez et al., 2005; Bandeira et al., 2009).

2.5 Pathogenesis

Once it invades the body, CAEV is absorbed by the gastrointestinal tract, from where it continues the process of invasion, and finally reaches into the peripheral blood mononuclear cells (Al-Ani and Vestweber, 1984; Logan et al., 2004). Like other lentiviruses, CAE virus has a great affection to monocytes and macrophages (Chebloune et al., 1996; Bouzar et al., 2007) and usually stays latent in them (Narayan et al., 1983; Ali Al Ahmad et al., 2008[b]). It infects the targeted cells, attacking on the monocyte-macrophage lineage (Chebloune et al., 1996; Logan et al., 2004), and a viral infection is subsequently distributed throughout the body along with the dissemination of infected macrophages via blood stream (Le Jan et al., 2005).

Following the dissemination CAEV-infected leucocytes, after the virus has reached to other tissues, a variety of chronic inflammatory lesions are observed in the brain, spinal cord, lungs (Storset et al., 1997), mammary glands and joints of the infected animals (Al-Ani and Vestweber, 1984), resulting from the hyperplasia of lymphoid follicles and progressive infiltration of mononuclear inflammatory cells into the

parenchyma of targeted tissues in the affected organs (Karanikolaou et al., 2005; Gregory et al., 2009). This is particularly happened to the tissue macrophages of central nervous system, lungs, mammary glands and synovium because of the predilection of virus to attack the mononuclear cells (Chebloune et al., 1996; Bouzar et al., 2007).

CAEV can replicate in the epithelial cells of mammary glands, genital tissues and other several tissues, and also it can infect several endothelial cells (Le Jan et al., 2005; Rodriguez et al., 2005; Ali Al Ahmad et al., 2008[b]). Both humoral and cell-mediated immune response induced by animals after onset of the infection (Archambault et al., 1988; de Andrés et al., 2005) are strong, but, not fully protective against the CAEV infection and therefore, unable to stop the infection (Karanikolaou et al., 2005). Maternal antibody in colostrums, as well, is not strong enough to protect the offspring from infection (Logan et al., 2004).

2.6 Clinical signs

In general, goats usually get infected with CAEV early in their life (Karanikolaou et al., 2005) through the ingestion of infected cells-containing colostrums or milk (Lamara et al., 2002; Le Jan et al., 2005). However, due to the delayed seroconversion rate (Eltahir et al., 2006; Ali Al Ahmad et al., 2008[a]), they become clinically evident only after a long period of time (Archambault et al., 1988), which may be somewhere between a few to several months after infection (de Andrés et al., 2005) or only after two or three years of infection (MacDiarmid, 1983; Karanikolaou et al., 2005).

Major clinical signs colligated with CAEV infection are as polyarthritis, chronic synovitis, chronic intestinal pneumonia, acute or chronic indurative mastitis, progressive weight loss in adult goats, and leukoencephalitis in younger goats, especially in kids between 2 to 6 months of age (Al-Ani and Vestweber, 1984; Lamara et al., 2002; Logan et al., 2004; Gregory et al., 2009). Among these, arthritis form is the most common

manifestation of disease and it is more frequently observed in adults older than 6 months of age, whereas encephalitis is more abundantly seen in younger animals, while chronic progressive weight loss can co-exist with any other forms of the diseases at any age interval (Cortez-Moreira et al., 2005; Bandeira et al., 2009).

In addition to these signs, other clinical features such as decreased birth weights, delayed weight gain, and increased mortality before weaning are often seen in offspring (Peterhans et al., 2004; Reina et al., 2009). However, severity of disease or clinical signs may vary between individuals and some infected animals may not even show any obvious signs of the disease (Archambault et al., 1988; Kusza et al., 2004).

Being asymptomatic, only 25 to 30% of infected total develop clinical illness, mainly due to chronic infection of joints, and become incapacitated (Lamara et al., 2002; Karanikolaou et al., 2005; Eltahir et al., 2006). With long incubation of virus and delay seroconversion rate, clinical signs are usually subtle and it may take years to develop in some (Gufler et al., 2007[a]; Leitner et al., 2010). However, despite the fact that only a few of total infected animals develop clinical illness (Gufler et al., 2007[a]; Plaza et al., 2009), all of them, once infected, become subclinical carriers, even if asymptomatic (Nord et al., 1998[a]; Al-Qudah et al., 2006), and continue shedding the virus throughout their lifetime, transmitting the infection to the others (East et al., 1993; Peterhans et al., 2004).

2.7 Effect of CAEV on reproductive performance and productivity

CAEV produces several effects on reproductive performance and productivity of infected animals, thereby affecting the production efficiency of an entire herd (Greenwood, 1995[a]; Cruz et al., 2009). One of the most obvious effects seen is decreased milk yield in affected goats with increased somatic cell counts and recurrent udder infection (Sanchez et al., 2001; Gregory et al., 2009; Leitner et al., 2010). Indurative mastitis due to CAEV has been reported and subclinical intramammary bacterial infection with reduced lactation length and lower milk fat content has also been recorded in CAE affected herds (Al-Ani and Vestweber, 1984; Sanchez et al., 2001; Le Jan et al., 2005; Ali Al Ahmad et al., 2008[a]).

Moreover, decreased birth weight, delayed weight gain and increased mortality rate before weaning are more frequently observed in CAE affected goat herds, where overall conception rate is relatively low in infected dams (Aslantas et al., 2005; Reina et al., 2009). In addition, average number of offspring born in each gestation is lower in CAE affected herds, with which the average lifespan of infected individuals become shorter due to premature culling that affects the productivity of the herd (Peterhans et al., 2004).

2.8 Diagnosis

Diagnosis can be achieved through a combination of history, clinical signs, histopathological lesions and serology test, for example: examination of the mononuclear cell counts in synovial fluid and measuring of the metacarpal diameter or joint enlargement in the animals with arthritis form of disease (Al-Ani and Vestweber, 1984; Lilenbaum et al., 2007), immunohistochemistry of tissue sections from the synovial membrane and connective tissue surrounding the joints (Storset et al., 1997; Bouzar et al., 2007), radiography of the lungs in animals with respiratory form (Al-Ani and

Vestweber, 1984; Logan et al., 2004), and mammary gland evaluation in the animals with indurative mastitis (Sanchez et al., 2001; Gregory et al., 2009).

However, since most of the infected goats remain asymptomatic and antibodies titer indicating the evidence of the virus are higher in blood (East et al., 1993; Plaza et al., 2009), diagnosis of CAEV is preferably based on serology (Lilenbaum et al., 2007) and usually performed by such serological tests as agarose gel immunodiffusion (AGID), immunoprecipitation test (IP), and enzyme-linked immunosorbent assay (ELISA) (Cortez-Moreira et al., 2005; Ghanem et al., 2009).

From several experiments, it was observed that the sensitivity value of ELISA to CAEV is higher than that of AGID to CAEV, suggesting that (ELISA) is more sensitive and can give greater accuracy than (AGID) (Cortez-Moreira et al., 2005). Therefore ELISA is favourably used for the detection of antibodies against CAEV in serum and milk (Plaza et al., 2009). Nowadays, competitive ELISA (cELISA) that provide higher sensitivity and can detect lower titers of CAEV antibodies more precisely than indirect ELISA, thereby rendering more accurate diagnosis, has been widely used for detection of antibodies to CAEV in serum (Aslantas et al., 2005; Ghanem et al., 2009). However, there are times when antibodies may fail to develop in some infected animals due to delayed seroconversion, and in kids younger than 6 months of age (Lamara et al., 2002; Rodriguez et al., 2005; Elfahal et al., 2010).

Virus isolation from synovial membrane, brain and genital tract tissues is also an alternative to diagnosis, but with little chance of success during infection (Lamara et al., 2002; Le Jan et al., 2005; Rodriguez et al., 2005; Ali Al Ahmad et al., 2008[b]). PCR (Polymerase chain reaction) detection of proviral DNA in blood mononuclear cells and targeted tissues is also possible, but sensitivity may be slighter lower than that of ELISA (Karanikolaou et al., 2005; Eltahir et al., 2006).

2.9 Treatment

To date, there has been no successful treatment or vaccination available for CAEV infection (Al-Ani and Vestweber, 1984; Cruz et al., 2009). Only symptomatic and supportive therapies, such as foot trimming, good pasture management, administration of anti-inflammatory drugs and antibiotic therapy for secondary bacterial infection, have been applied to CAEV infected animals (Logan et al., 2004). However, most of the infected animals are eventually culled since the infection is unstoppable and clinical signs usually become more exaggerated over time (Nord et al., 1998[a]).

2.10 Control and prevention

Without any treatment of choice, control and prevention with early diagnosis of disease become essential to eradication of CAE (Cruz et al., 2009). Control measure of CAE is mainly based on reduction of possible sources of infection (Rodriguez et al., 2005), such as restriction of live animal trading and introduction of new animals into herds, early diagnosis of disease by performing serological tests at regular period of time (Nord et al., 1998[a]; Aslantas et al., 2005), segregation of seropositive animals from seronegative individuals, quarantine and regular culling of infected animals (Al-Ani and Vestweber, 1984), isolation of newborn kids from seropositive dams before suckling and feeding them on heat-treated colostrums or pasteurized milk or milk replacer until weaning. (Rowe et al., 1991; Aslantas et al., 2005; Leitner et al., 2010), and reducing stocking density in the herds (Rowe et al., 1991; Blacklaws et al., 2004).

But, delayed seroconversion is sometimes a problem to control of CAE since the control program is usually based on early diagnosis of disease using serological tests. Segregation and culling of seropositive animals are essential to eradication of CAE from a seropositive herd (Al-Ani and Vestweber, 1984; East et al., 1993; Aslantas et al., 2005; Ghanem et al., 2009).

2.11 CAE in Thailand

Thailand has a large population of goats, around 390,000 heads and 36,000 herds, as of year 2010, stated by DLD (Department of Livestock Development, Thailand). Among these, more than 80% of the total population of goat is raised in western and southern parts of Thailand, mainly raised by small-holder farmers. This population comprises of several breeds, a variety of Thai native breeds and cross-breeds, raised either for meat or milk (DLD, unpublished data). In regard to outbreaks of CAE, two articles have been published to date, from which the prevalence of CAEV infection was reported to be around 12.0% (Tantaswasdi et al., 1985; Ratanapob et al., 2009). However, there was no study focusing in-depth on the potential risk factors for the prevalence of CAE in Thailand and also on the effect of CAEV infection on the reproductive performance of an affected herd.

In this study, it was tried to elucidate the potential risk factors associated with the seroprevalence of CAEV infection in goat herds in the western part of Thailand, as well as the effects of CAEV infection on the reproductive performance of goats in an affected herd.

Chapter III
MATERIALS AND METHODS

This study consisted of two different parts, the first part investigating over the seroprevalence of, and risk factors associated with, CAEV infection among the goat herds from three Western provinces of Thailand, namely Ratchaburi, Petchaburi, and Kanchanaburi, and the second part studying the effects of CAEV infection on reproductive performance of goats by comparing the differences between seropositive and seronegative does within a CAEV seropositive goat herd.

3.1 Study design

This study was based on two study designs. The first one was a cross-sectional study investigating the seroprevalence of CAEV infection and risk factors associated with the infection at a specific point of time. The second one was a comparative study between a seropositive and seronegative group of animals in a CAEV seropositive goat herd, in which reproductive performance of does from each group were compared.

3.2 Study area

Goat farms from Ratchaburi, Petchaburi and Kanchanaburi provinces (Figure 1), were included in this study. These three provinces were particularly chosen for the surveillance because there is a big population of goats raised in this area, and also that these provinces are situated on the outermost part of the country, closely adjacent to the borderline, and, passing through which a great flow of goat transport from the North to the Southern parts of country has been taking place for years. Therefore, thinking in terms of possibility, goat population in this area, three provinces as a whole part, is more

likely to be risk-affected, compared to other provinces which are far more inland.

Figure 1: The study area (Coloured), a combination of Ratchaburi (Red), Petchaburi (Blue) and Kanchanaburi (Black) provinces of Thailand

3.3 Goat population in study area

Goat population in these three provinces, as of 2009, was around 42,000 heads, which comprised of approximately 850 farms (DLD, unpublished data).

Table 1: Total number of goats in Ratchaburi, Petchaburi and Kanchanaburi provinces

Province	Head	Herd
Ratchaburi	7652	253
Petchaburi	9441	280
Kanchanaburi	24529	314
Total	**41622**	**847**

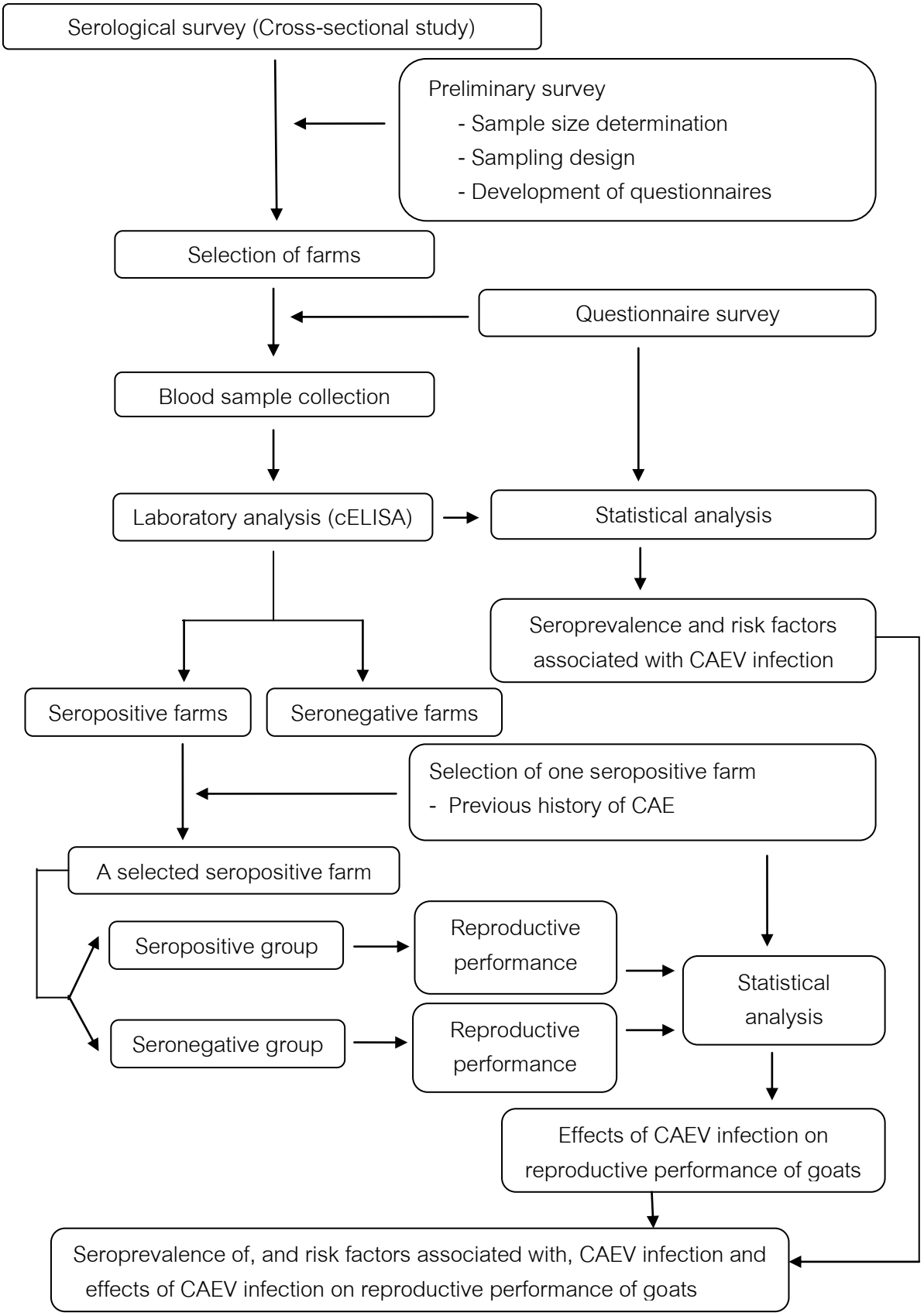

Figure 2: Conceptual framework of the study

3.4 Serological survey

3.4.1 Sample size calculation

Determination of sample size was performed in two stage sampling designs. First, number of animals to be sampled from the targeted population was calculated using the following formula:

$$n = \frac{Z^2 pq}{d^2} \quad \text{(Thrusfield, 2005)}$$

where,

n = number of animals to be sampled

Z = value for selected alpha level of 0.025 in each tail= 1.96

p = estimate of prevalence

d = margin of error

Therefore, by assuming the seroprevalence of CAEV infection in goats as 12.40% (Ratanapob et al., 2009), with an error of margin and confidence level respectively set at 0.02 and 95%, the required sample size of 1044, better adjusted as 1,100, was calculated.

Following the estimation of sample size, number of animals to be sampled from each herd to detect at least one positive animal was calculated using the formula for detecting the presence of disease (Al-Majali, 2005).

$$n = [1- (1- p_1)^{1/d}] [N – d/2] + 1 \quad \text{(Thrusfield, 2005)}$$

where,

n= number of animals to be sampled

N = herd size

d = within herd prevalence of infected animals

p_1 = probability of finding an infected animal in the herd

Therefore, as the herd size in this study area ranged from 5 to 200, by fixing the herd size to be 200 at its maximum, with the estimated within herd prevalence set as 15% and the probability of 95% confidence level, the minimum number of animals to be sampled from each herd was obtained; it was 18.

3.4.2 Classification of herd size

Because the herd size in the study area ranges from 5 to 200 animals, all farms were categorized into three groups; small (less than 50 animals), medium (51-100 animals) and large (above 100 animals).

3.4.3 Selection of farms and animals

All farms were chosen in random, and from each selected farm, 18 animals were sampled for blood. However, in those farms whose herd sizes were equal to or less than 18, all animals were sampled. Regardless of age, sex and breed, goats in every selected farm were chosen at random for blood sampling.

3.4.4 Blood (serum) sample collection

A total of 1,129 blood samples, randomly taken from 74 randomly selected herds, were collected during the period from November 2009 to January 2011. From each sampled animal, 5 ml of blood (serum sample) was collected from jugular vein using vacutainer tubes. Afterwards, all samples were centrifuged at 1000 g for 10 min and stored at -20°C until analysis.

3.4.5 Questionnaire survey

Semi-structural questionnaires (Appendix 1 and 2) were developed and asked to farm owners, shortly before or after collection of blood, to obtain necessary information relating to the background history and management practice of the farms, such as previous outbreaks of infectious diseases in the herd, breeding management and import of animals, control measure against infectious diseases and so on.

3.4.6 Laboratory analysis of collected serum samples

All collected sera were run for analysis to detect the presence of CAEV antibodies using a commercially available competitive enzyme-linked immunosorbent assay (c-ELISA) test kits (Caprine Arthritis Encephalitis Virus Anti-body Test Kit, cELISA, VMRD, Inc., Pullman, WA, USA). Specificity and sensitivity of VMRD cELISA kit are 100% and 99.6% respectively. It contains 96-well plates coated with CAEV antigen. The test kit also provides positive and negative control of the goat sera that come with the set.

3.4.6.1 Test preparation and procedures

Test preparation and procedures were carried out according to manufacturer's instruction (Appendix C).

3.4.6.2 Interpreting the results

Based on the optical density (O.D.) value of the samples obtained from microplate reader, the percent inhibitions of antibodies to the antigens were calculated as follow.

% inhibition = (100 – [Sample O.D. x100) ÷ (mean negative control O.D.)]

Samples producing the values $\geq$ 35% inhibition are defined positive while those producing $\leq$ 35% are defined negative.

3.4.7 Adjustment for true seroprevalence

Based on the results obtained from serological analysis, true individual seroprevalence was calculated from overall apparent prevalence using the following formula:

$$TP = \frac{Ap+Sp-1}{Se+Sp-1}$$ (Thrusfield, 2005)

where,

TP = true prevalence

AP = apparent prevalence

Se = sensitivity of the test

Sp = specificity of the test

3.4.8 Risk factors analysis

Using the serological results obtained from laboratory analysis of serum samples and information received from questionnaire survey, risk factors analysis was carried out in two-fold process. First, presence of association between serological status of animals and each hypothesized risk factor, on both herd and individual levels, was examined in case-control design, where seropositive and seronegative groups were compared in terms of exposure to hypothesized risk factors (Abo-Shehada and Abu-Halaweh, 2010) by using Chi-square test.

All hypothesized risk factors that showed significant association with the seropositivity of CAEV infection on univariate analysis, at two-tailed level ($p<0.05$), were then advanced to multivariable logistic regression model for further analysis of risk factors. Hosmer and Lemeshow's goodness of fit test was applied, and backward-stepwise elimination process was performed to filter the variables. Variables significant

($p<0.05$) in final logistic regression model were defined as risk factors associated with CAEV infection in the study population.

3.5 Comparative study on the effects of CAEV infection on reproductive performance of goats between seropositve and seronegative groups in a CAEV infected herd

3.5.1 Selection of one seropositve farm

One CAEV seropositive dairy goat farm in Suan Phueng district, Ratchburi province was selected for this part of study, out of the several seropositive farms detected seropositive to CAEV infection during the year 2009. This farm was selected because it was a dairy farm, where the prevalence was usually higher in dairy goat than in meat goat (deMaar et al., 1995), and all breeds in this farm were Saneen crossbreed, the most susceptible breed to CAEV infection among various goat breeds (Rodriguez et al., 2005). In addition, it was a close type farm, into which no new goats had been added for years. Again, this farm has been established for almost ten years, and, previous cases of CAEV seropositivity have been reported in the farm from time to time. Furthermore, this farm practiced artificial insemination and therefore previous records of the reproductive performance for recent years were most available compared to other farms that performed natural mating. The study on the reproductive performances of goats between two groups in the herd was carried out from January 2010 to February 2011.

3.5.2 Separation of animals into two groups

Blood samples were collected from all adult females, older than one year of age, and, run for laboratory analysis using the same test kit (VMRD cELISA test kit) and laboratory procedure as it was performed in seroprevalence study. Eight adult female

goats were found seropositive to CAEV infection, while seronegative group contained twenty three adult females.

3.5.3 Collection of secondary data

General information of the farm, such as farm management practice, reproductive problems and previous cases of CAEV infection within the herd, were collected.

3.5.4 Observation over reproductive performance of animals

Date of each Artificial insemination was documented with each animal from both groups, and, following that, every animal in each group were watched over for any differences in reproductive performance. Herd prolificacy rate, first service conception rate, total conception rate, gestation period, and failure to conceive during two consecutive AI, frequency of abortion, neonatal mortality and other reproductive problems with each group were accordingly recorded.

3.5.4.1 The prolificacy of each goat herd was determined by the total number of kids born over the total number of does kidded during the period of study multiplied by 100.

$$\text{Herd prolificacy rate} = \frac{\text{Number of kids born}}{\text{Number of does kidded}} \times 100$$

3.5.4.2 The first service conception rate was determined by the number of does becoming pregnant from the first AI over the total number of does inseminated during the period of study multiplied by 100.

$$\text{First service conception rate} = \frac{\text{Number of does pregnant from first AI}}{\text{Number of does inseminated for first time AI}} \times 100$$

3.5.4.3 The total conception rate was determined by the number of does becoming pregnant over the total number of does inseminated during the period of study multiplied by 100.

$$\text{Total conception rate} = \frac{\text{Number of does becoming pregnant}}{\text{Numbers of does inseminated}} \times 100$$

3.5.4.4 Gestation period of each doe was determined by the period starting from the date of AI to the date of delivery.

3.5.5 Comparison of the results obtained from two groups

Recorded data on reproductive performance obtained from two different groups were then analyzed. The numbers of kids born per doe per parturition and gestation length from each group were compared using two-tailed Student's t-test. First service conception rate, total conception rate, failure to conceive during two consecutive AI, frequency of abortion and neonatal mortality rate from each group were analyzed using Chi-square test.

3.6 Statistical analysis and softwares used in this study

Microsoft office excel 2007 and statistic program for social science (SPSS for windows) version 16.0 (SPSS Inc., Chicago, USA) were used for data analysis in this study.

In seroprevalence study, univariate analysis using Chi-square test was applied to find out the association between the CAEV seropositivity and each hypothesized risk factors. Fisher's exact test was employed where the number of observation in one cell was less than 5. Confidence level was set at 95%. With variables showing *p*value less than 0.05 ($P<0.05$, two-tailed) on univariate analysis, multivariable logistic regression model was used for determination of significant risk factors associated with CAEV infection. Hosmer and Lemeshow's goodness of fit test, with confidence level of 95%, was applied, and least significant variables were filtered out by backward-stepwise elimination process.

In comparative study, Chi-square test was used for comparison of categorical variables, and two tailed student's t-test for continuous variables. Presence of any significant difference between two groups of animal regarding their reproductive performance was determined by *p*value ($p<0.05$) with confidence level set at 95%.

Chapter IV
RESULTS

4.1 Serological survey

4.1.1 Individual and herd level seroprevalence of CAEV infection

From serological analysis, 67 out of the total of 1,129 goats were found seropositive to CAEV infection, showing the apparent seroprevalence of 5.9% and true individual seroprevalence of 5.52%. On herd level analysis, 23 farms out of 74 in total were found seropositive (Table 2).

Table 2: Individual and herd seroprevalence of CAEV infection in goats

Prevalence level	Positive	Negative	Total
Individual	67 (5.9%)	1,062 (94.1%)	1,129 (100%)
Herd	23 (31%)	51 (69%)	74 (100%)

4.2 Univariate analysis

4.2.1 Seroprevalence of CAEV infection with sex and age

It was observed that seroprevalence was higher in male, with 16 positive cases (9.4%) from 171 total, compared to female showing 51 positive cases (5.3%) from 958 total samples tested.

Regarding age, seroprevalence was found highest (10.1%) in the oldest group, 3 years and above, with which 34 seropositives were detected from 336 total samples

tested, and, becoming decreased with age, it was found lowest (3.2%) in the youngest group, less than 1 year of age, in which 7 out of 218 goats were found seropositive to CAE virus infection. With different levels of age, seroprevalence ranged from 3.2% to 10.1%.

Table 3: Seroprevalence of CAEV infection with sex

Sex	Positive	Negative	Total
Male	16 (9.4%)	155 (90.6%)	171 (100%)
Female	51 (5.3%)	907 (94.7%)	958 (100%)

Table 4: Seroprevalence of CAEV infection with age

Age	Positive	Negative	Total
Less than 1 year	7 (3.2%)	211 (96.8%)	218 (100%)
1 to 2 years	11 (3.4%)	308 (96.6%)	319 (100%)
2 to 3 years	15 (5.9%)	241 (94.1%)	256 (100%)
3 years and above	34 (10.1%)	302 (89.9%)	336 (100%)

Both sex and various levels of age showed significant association with the seroprevalence of CAEV infection on univariate analysis by Chi-square test, with (p=0.04) and (p=0.001) respectively.

4.2.2 Seroprevalence of CAEV infection with herd type

In regards to herd type, higher seroprevalence was detected in dairy goats, on both herd and individual levels, where 27 (9.3%) out of 291 goats as individual and 10 (55.6%) out of 18 as herd level prevalence, were found seropositive to CAEV infection. In meat type goats, seroprevalence was 4.8%, 40 out of 838, on individual level and 23.2%, 13 out of 56, on herd level prevalence.

Table 5: Seroprevalence of CAEV infection with herd type

Herd type	Individual prevalence			Herd prevalence		
	Positive	Negative	Total	Positive	Negative	Total
Meat	40 (4.8%)	798 (95.2%)	838 (100%)	13 (23.2%)	43 (76.8%)	56 (100%)
Dairy	27 (9.3%)	264 (90.7%)	291 (100%)	10 (55.6%)	8 (44.4%)	18 (100%)

Herd type showed a significant association with the seroprevalence of CAEV infection, at both herd (p=0.01) and individual (p=0.005) levels of prevalence.

4.2.3 Presence of other animals in the farm

Sheep, cattle and dogs were observed in some farms. Among them presence of sheep and cattle were documented.

4.2.3.1 Presence of sheep in the farm

Of 74 goat farms surveyed, 3 farms had sheep raised together with goats in the herd. But, none of them were found seropositive to CAEV infection. And no significant association was observed between the presence of sheep and seroprevalence of CAEV infection.

4.2.3.2 Presence of cattle in the farm

Cattle were present in 26 farms and, from them, 8 farms were found seropositive to CAEV infection, while with the rest 48 farms, which did not have cattle in them, 15 farms were found seropositive. There was no significant association observed between the presence of cattle and seroprevalence of CAEV infection.

4.2.4 Seroprevalence of CAEV infection with different breeds of goat

On herd level prevalence, 1 farm out of 8 (12.5%) in native breed, 11 out of 49 (22.4%) in crossbreed and 11 out of 17 (64.7%) in Saanen crossbreed were seropositive to CAEV infection. On individual level, seroprevalence was observed as 1.4% (1 out of 69) in native breed, 4.9% (38 out of 777) in crossbreed and 9.9% (28 out of 283) in Saanen crossbreed. With different breeds of goats, seroprevalence ranged from 12.5% to 64.9% on herd level and from 1.4% to 9.9% on individual level.

Table 6: Seroprevalence of CAEV infection with different breeds of goat

Breeds	Individual prevalence			Herd prevalence		
	Positive	Negative	Total	Positive	Negative	Total
Native breed	1 (1.4%)	68 (98.6%)	69 (100%)	1 (12.5%)	7 (87.5%)	8 (100%)
Crossbreed	38 (4.9%)	739 (95.1%)	777 (100%)	11 (22.4%)	38 (77.6%)	49 (100%)
Saanen crossbreed	28 (9.9%)	255 (90.1%)	283 (100%)	11 (64.7%)	6 (35.3%)	17 (100%)

It was observed that difference in breed of goats was significantly associated with the seroprevalence of CAEV infection, on both herd (p=0.003) and individual (p=0.003) levels.

4.2.5 Seroprevalence of CAEV infection with difference in herd size

Of 74 farms, 55 were farms with less than or equal to 50 goats in them, 13 were those having between 50 to 100 goats, and the other 6 containing more than 100. Serological findings showed that 13 farms out of 55 in small herd size group, 6 farms from the total of 13 in medium herd size group and 4 farms out of 6 in large herd size group were seropositive to CAEV infection on herd level prevalence.

On individual level, 22 goats were found seropositive from the total number of 787 goats included in small herd size group, while 28 out of 234 goats in medium herd size group and 17 out of 108 goats in large herd size group were seropositive to CAEV infection. With differences in herd size, herd level seroprevalence ranged from 23.6% to 66.7%, while individual seroprevalence lied within the range of 2.8% to 15.7%.

Table 7: Seroprevalence of CAEV infection with difference in herd size

Herd size	Individual prevalence			Herd prevalence		
	Positive	Negative	Total	Positive	Negative	Total
Small (1 - 50)	22 (2.8%)	765 (97.2%)	787 (100%)	13 (23.6%)	42 (76.4%)	55 (100%)
Medium (51 - 100)	28 (12.0%)	206 (88.0%)	234 (100%)	6 (46.2%)	7 (53.8%)	13 (100%)
Large (> 100)	17 (15.7%)	91 (84.3%)	108 (100%)	4 (66.7%)	2 (33.3%)	6 (100%)

A significant association between the seroprevalence of CAEV infection and variation in herd size was discovered, on both levels of prevalence, with (p=0.042) and (p=0.001) respectively.

4.2.6 Association between farm management system and seroprevalence of CAEV infection

Relationship between seroprevalence of CAEV infection and farm management system, which included rearing system, use of pasture, contact with goats from other herds, separation of male and female, addition of new goats into herd, farm replacement policy, use of disinfectants, use of vaccines, presence of veterinary services and breeding methods, as well as relationship between seroprevalence of CAEV infection and other hypothesized risk factors, such as previous cases of CAE, knowledge of farm owner towards CAE and presence of other goat farms within 1km distance, were evaluated, both on herd and individual levels. These variables were compared as binary data, having only two categories, with “Yes” or “No” outcomes.

4.2.6.1 Rearing system (intensive vs. semi-intensive)

Higher seroprevalence was detected in goats raised on intensive rearing system on both herd (38.5% vs. 29.5%) and individual (9.0% vs. 5.1%) prevalence levels, but a significant association between rearing system and seropositivity of CAEV infection was observed only on individual prevalence level (p=0.027).

4.2.6.2 Use of pasture (used vs. not used)

On herd level, seroprevalence was higher in those farms that practiced use of pasture or grazing outside, but not significant (37.0% vs. 27.7%). However, in contrast, on individual level, higher seroprevalence, significantly associated with the seropositivity of CAEV infection, was seen in those farms that do not use pasture (4.1% vs. 7.3%, p=0.026).

4.2.6.3 Contact with goats from other herds (in contact vs. not in contact)

On individual level, goats that do not have contact with goats from other herds showed higher seroprevalence, but no significant association was observed (6.1 % vs. 5.5%). However, on herd level, seroprevalence was almost double in those farms that were in contact with goats from other herds, with a significant association to seropositivity of CAEV infection (53.3% vs. 25.4%, p=0.037).

4.2.6.4 Presence of other goat herds within 1km distance (present vs. absent)

On herd level, higher seroprevalence was observed with those farms which have other goat herds within 1 km distance from them (34.5% vs. 28.9%). But, on individual level, it was reversely seen, as higher seroprevalence was detected with farms that do not have any other goat herds within 1 km distance (4.5% vs. 6.9%). On either level of prevalence, a significant association to CAEV infection was not present.

4.2.6.5 Male-female separation (separated vs. not separated)

With those farms that separate male and female apart, lower seroprevalence was observed on both herd (28.6% vs. 32.1%) and individual (4.2% vs. 6.7%) levels, but no significant association to CAEV infection was produced on either level.

4.2.6.6 Addition of new goats into herd (added vs. not added)

Higher seroprevalence was observed in those farms that practiced addition of new goats into herd on both herd (47.8% vs.23.5%, p=0.037)) and individual (8.3% vs. 5.0%, p= 0.036)) levels, where adding new goats into herd was found to be significantly associated with the seropositivity of CAEV infection on both levels of prevalence.

4.2.6.7 Replacement policy (all-in-all-out vs. not all-in-all-out)

Out of 74 farms included in this study, there were only four farms that practiced all-in-all-out replacement policy, with which lower seroprevalence was detected on both herd (25.0% vs. 31.4%) and individual (1.7% vs. 6.2%) levels, but not significantly associated with CAEV infection.

4.2.6.8 Use of disinfectants (used vs. not used)

With those farms that regularly used disinfectants, seroprevalence was found lower on herd level (30.4% vs. 33.3%). However, on individual level, it was contrarily lower in those farms that do not regularly use disinfectants (5.3% vs. 6.1%). On either level of prevalence, no significant association existed regarding the use of disinfectants and seropositivity of CAEV infection.

4.2.6.9 Practice of FMD vaccination (vaccinated vs. not vaccinated)

On herd level, seroprevalence was lower with those farms that practice vaccination against FMD (30.2% vs. 32.3%). However, on the other hand, seroprevalence was found higher in vaccinated farms on individual level (6.3% vs. 5.2%). But, neither of the differences on both levels was significantly associated with CAEV infection.

4.2.6.10 Presence of veterinary service (presence vs. absence)

On herd level, seroprevalence was lower in those farms with which veterinary service was present (22.2% vs. 33.9%). But, on individual level, seroprevalence was found lower with those where veterinary service was not provided (6.5% vs. 5.7%). No significant association between practice of veterinary service and seropositivity of CAEV infection was observed on either level of prevalence.

4.2.6.11 Method of breeding (AI vs. Natural mating)

On both levels, seroprevalence was found lower in those farms that practiced AI, where it was detected as (28.6% vs. 31.3%) on herd level prevalence and (2.5% vs. 6.3%) on individual level prevalence. But, no significant association was observed between the method of breeding and seropositivity of CAEV infection on both levels of prevalence.

4.2.6.12 Previous case of CAE (previous outbreak vs. no previous outbreak)

On both levels, higher seroprevalence, seen as 8.0% vs. 5.0% on individual level and 47.8% vs. 23.5% on herd level, was observed with those farms in which CAEV infection had taken place in the past. It was also noticed that previous occurrence of CAEV infection was significantly associated with the seropositivity of CAEV infection on both herd (p=0.037) and individual (p=0.042) levels of prevalence.

4.2.6.13 Knowledge of farm owner towards CAE (without knowledge vs. with knowledge)

Knowledge of farm owner towards CAE did not show any significant association with the seropositivity of CAEV infection in goats. However, with those farms whose owners have no knowledge towards CAE, seroprevalence was found higher, where it was detected as 6.3% vs. 5.0% on individual level and 32.1% vs. 27.8% on herd level.

Table 8: Association between seroprevalence of CAEV infection and different exposed factors on individual level prevalence univariate analysis (n=1,129)

Category	Total	Positive (%)	Negative (%)	*p*value
Rearing system				
Intensive	234	21 (9)	213 (91)	0.027*
Semi-intensive	895	46 (5.1)	849 (94.9)	
Use of pasture				
Yes	467	19 (4.1)	448 (95.9)	0.026*
No	662	48 (7.3)	614 (92.7)	
Contact with goats from other herds				
Yes	254	14 (5.5)	240 (94.5)	0.746
No	875	53 (6.1)	822 (93.9)	
Presence of other goat herds within 1 km distance				
Yes	443	20 (4.5)	423 (95.5)	0.105
No	686	47 (6.9)	639 (93.1)	
Male-female separation				
Yes	356	15 (4.2)	341 (95.8)	0.097
No	773	52 (6.7)	721 (93.3)	
Addition of new goats into herd				
Yes	324	27 (8.3)	297 (91.7)	0.03*
No	805	40 (5.0)	765 (95.0)	
Replacement policy				
All-in-all-out	60	1 (1.7)	59 (98.3)	0.254
Not all-in-all-out	1069	66 (6.2)	1003 (93.8)	

Table 8 (continued): Association between seroprevalence of CAEV infection and different exposed factors on individual level prevalence univariate analysis (n=1,129)

Use of disinfectants				
Yes	923	56 (6.1)	867 (93.9)	0.686
No	206	11 (5.3)	195 (94.7)	
Practice of FMD vaccination				
Yes	726	46 (6.3)	680 (93.7)	0.443
No	403	21 (5.2)	382 (94.8)	
Veterinary service				
Yes	306	20 (6.5)	286 (93.5)	0.602
No	823	47 (5.7)	776 (94.3)	
Breeding methods				
AI	121	3 (2.5)	118 (97.5)	0.089
Natural mating	1008	64 (6.3)	944 (93.7)	
Previous case of CAE				
Yes	362	29 (8.0)	333 (92.0)	0.042
No	767	38.0 (5.0)	729 (95.0)	
Knowledge of owner towards CAE				
With knowledge	319	16 (5.0)	303 (95.0)	0.412
Without knowledge	810	51 (6.3)	759 (93.7)	

*****pvalue significant

Table 9: Association between seroprevalence of CAEV infection and different exposed factors on herd level prevalence univariate analysis (n=74)

Category	Number	Positive	Negative	*p*value
Rearing system				
Intensive	13	5 (38.5)	8 (61.5)	0.527
Semi-intensive	61	18 (29.5)	43 (70.5)	
Use of pasture				
Yes	27	10 (37.0)	17 (63.0)	0.401
No	47	13 (27.7)	34 (72.3)	
Contact with goats from other herds				
Yes	15	8 (53.3)	7 (46.7)	0.037*
No	59	15 (25.4)	44 (74.6)	
Presence of other goat herds within 1 km distance				
Yes	29	10 (34.5)	19 (65.5)	0.612
No	45	13 (28.9)	32 (71.1)	
Male-female separation				
Yes	21	6 (28.6)	15 (71.4)	0.769
No	53	17 (32.1)	36 (67.9)	
Addition of new goats into herd				
Yes	23	11 (47.8)	12 (52.2)	0.037*
No	51	12 (23.5)	39 (76.5)	
Replacement policy				
All-in-all-out	4	1 (25.0)	3 (75.0)	0.787
Not all-in-all-out	70	22 (31.4)	48 (68.6)	

Table 9 (continued): Association between seroprevalence of CAEV infection and different exposed factors on herd level prevalence univariate analysis (n=74)

Use of disinfectants				
Yes	56	17 (30.4)	39 (69.6)	0.812
No	18	6 (33.3)	12 (66.7)	
Practice of FMD vaccination				
Yes	43	13 (30.2)	30 (69.8)	0.853
No	31	10 (32.3)	21 (67.7)	
Veterinary service				
Yes	18	4 (22.2)	14 (77.8)	0.351
No	56	19 (33.9)	37 (66.1)	
Breeding methods				
AI	7	2 (28.6)	5 (71.4)	0.879
Natural mating	67	21 (31.3)	46 (68.7)	
Previous case of CAE				
Yes	23	11 (47.8)	12 (52.2)	0.037*
No	51	12 (23.5)	39 (76.5)	
Knowledge of owner towards CAE				
With knowledge	18	5 (27.8)	13 (72.2)	0.728
Without knowledge	56	18 (32.1)	38 (67.9)	

**p*value significant

4.3 Multivariable analysis

From univariate analysis, nine variables (age, sex, herd type, herd size, breed, rearing system, use of pasture, addition of new animals into herd, and previous outbreak of CAE) showed significant association ($p<0.05$) with seropositivity of CAEV infection on individual level, whereas on herd level analysis, six variables (herd type, herd size, breed, contact with goats from other herds, addition of new animals into herd, and previous outbreak of CAE) were found to be significantly associated ($p<0.05$)with seropositivity of CAEV infection. All significant variables from univariate analysis were therefore transferred to multivariable logistic regression model.

From multivariable analysis, final regression model revealed three variables (age of three years and above, herd size, and addition of new animals into herd) as significant risk factors ($p<0.05$) associated with the seropositivity of CAEV infection on individual prevalence level (Table 9). It was seen that keeping animals older than three years of age in herd would increase the chances for occurrence of CAEV infection to 4.2 times that of others, younger than three years of age, would do. With herd size of the farm, which was another risk factor, it was observed that an increase in herd size would increase the likelihood of CAEV infection to take place, where, as shown in the table 9, the possibility of CAEV infection to occur was 8.6 times higher in medium herd size farms and 17.9 times higher in large herd size farms, compared to those of small herd size containing less than 50 animals. Another finding was that the addition of new animals into herd, revealed as a risk factor, would intensify the possibility of occurrence of CAEV infection 5.5 times higher than that of a close herd, into which no new animals were added.

Table 10: Risk factors associated with seroprevalence of CAEV infection on individual level prevalence multivariable analysis

Risk factor	β	SE	Wald	95% CI	Odds	*p*value
Age (3 years and above)	1.456	0.440	10.935	1.809-10.163	4.288	0.001
Herd size						
Small (1-50)					1	
Medium (51-100)	2.153	0.337	40.814	4.448-16.671	8.612	< 0.001
Large (> 100)	2.889	0.427	45.832	7.787-41.475	17.971	< 0.001
Addition of new goats	1.715	0.337	25.935	2.873-10.758	5.559	< 0.001

β = regression coefficient

SE= standard error

Wald= Wald's statistical value

On herd level analysis, multivariable final model showed that four variables (herd type, herd size, addition of new animals into herd, and contact with goats from other herds) were significant risk factors ($p<0.05$) associated with the seropositivity of CAEV infection (Table 10). From the following table, it was seen that CAEV infection was 5.026 times more likely to take place in dairy goat herds than in meat goat herds. Concerning herd size, the chance of infection to occur was 5.186 times higher in medium herd size farms and 24.065 times higher in large herd size farms, compared to small herd size farms. With those farms that were in contact with goats from other herds, occurrence of CAEV infection would increase 8.526 times as much as that of isolated farms. With those farms that added new goats into herd, the chance of getting infected by CAEV infection was 4.396 times higher compared with that of a closed herd.

Table 11: Risk factors associated with seroprevalence of CAEV infection on herd level prevalence multivariable analysis

Risk factor	β	SE	Wald	95% CI	Odds	*p*value
Herd type	1.615	0.762	4.496	1.130-22.360	5.026	0.034
Herd size						
Small (1-50)					1	
Medium (51-100)	1.646	0.823	4.001	1.034-26.021	5.186	0.045
Large (> 100)	3.181	1.162	7.490	2.466-234.788	24.065	0.006
Contact with goats from other goat herds	2.143	0.804	7.100	1.762-41.250	8.526	0.008
Addition of new goats	1.481	0.734	4.074	1.044-18.510	4.396	0.044

β = regression coefficient

SE= standard error

Wald= Wald's statistical value

4.4 Comparative study on the effects of CAEV infection on reproductive performance of goats between seropositve and seronegative groups in a CAEV infected herd

4.4.1 Herd prolificacy rate

Of the whole herd, the number of kids born per doe kidded was 1.63 (163%), 39 offspring from 24 dams. The number of kids born per doe kidded did not significantly differ between two groups. It was 1.65 in seronegative group and 1.50 in seropositive group, showing the respective herd prolificacy rates of 163% and 150% (Table 11).

Table 12: Comparison of herd prolificacy rate between seropositive and seronegative groups

Groups	Number of kids born			T test		
	Single	Twin	Triplet	*mean*	*t*value	*p*value
Seronegative does	9	9	2	1.65	0.416	0.583
Seropositive does	2	2	0	1.50		
Overall (entire herd)	10	12	2	1.65		

4.4.2 First conception rate

First conception rate of the herd was 58.1%, with 18 does conceived from first AI from the total of 31 does serviced. Between two groups, first conception rate was higher in seronegative group, in which 16 does from the total of 23 became conceived from their first time AI, whereas in seropositive group, only two does from the total of eight became conceived from first time AI (69.6% vs 25%). A significant association was observed between the seropositivity of CAEV infection and first conception rate in does ($p<0.05$) (Table 12).

Table 13: Comparison of first conception rate between seropositive and seronegative groups

Groups	Conception at first AI			Fisher's exact test
	Conceived	Failed	Total	*p*value
Seronegative does	16 (69.6%)	7 (30.4%)	23 (100%)	0.043
Seropositive does	2 (25%)	6 (75%)	8 (100%)	
Overall (entire herd)	18 (58.1%)	13 (41.9%)	31 (100%)	

4.4.3 Total conception rate

Total conception rate of the herd was 77.4%, with 24 does becoming pregnant from the total of 31 does AI-serviced over 14 months period. Between two groups, total conception rate was higher in seronegative group, which was 87.0%, with 20 does becoming pregnant from the total of 23 does AI-serviced, whereas in seropositive group, it was 50%, with four does becoming pregnant from the total of eight does AI-serviced. However, two groups did not significantly differ from each other on total conception rate, and no significant association was observed between the seropositivity of CAEV infection and total conception rate in does (Table 13).

Table 14: Comparison of total conception rate between seropositive and seronegative groups

Groups	Total conception rate			Fisher's exact test
	Conceived	Failed	Total	*p*value
Seronegative does	20 (87.0%)	3 (13.0%)	23 (100%)	0.053
Seropositive does	4 (50.0%)	4 (50%)	8 (100%)	
Overall (entire herd)	24 (77.4%)	7 (25.8%)	31 (100%)	

4.4.4 Failure to conceive during two consecutive AI

Of 31 does serviced over 14 months period, 13 (41.9%) failed to conceive at their first service with AI. Ten of them were serviced again and six of them became pregnant on second AI while the other four still failed to conceive. Failure to conceive through two consecutive AI was therefore 40% in the herd. Between two groups, the percentage of failure to conceive through two consecutive AI was lower in seronegative group (33.3%), with only two does from the total of six failed to conceive on the second time AI. In seropositive group, it was as high as 50%, in which two does from the total of four that had been serviced for two consecutive AI failed to get conception. No significant association was observed between the seropositivity of CAEV infection and failure to get conception during two consecutive AI.

4.4.5 Gestation length

Average gestation length of the herd was 149.67 days, ranging from 147 to 152 days. It was 149.7 days in seronegative group and 149.5 days in seropositive group, where the range lied between 147 to 152 days in both groups. There was no significant association between the seropositivity of the does and length of gestation (Table 14).

Table15: Comparison of gestation length between seropositive and seronegative groups

Groups	Gestation length in days			T test		
	< 150	150	< 150	*mean*	*t*value	*p*value
Seronegative does	8	6	6	149.70	0.244	0.433
Seropositive does	2	0	2	149.50		
Overall (entire herd)	10	6	8	149.67		

Chapter V
DISCUSSION

5.1. Serological survey

Overall seroprevalence of CAEV infection in the western part of Thailand from this study, 5.9%, was relatively low, compared to the previous finding by Ratanapob et al (2009), in which seroprevalence rose up to 12.4%. This can be possibly due to the fact that the number of infected animals within the herds has been annually reduced by eradication programs carried out by DLD (Department of Livestock Development, Thailand) and achievement of success with the control measure against animal smuggling and live animal trading in this area.

However, similar to that previous study, seroprevalence in this study was significantly higher in dairy goats than in meat goats (9.3% vs. 4.8%). Although it has been generally accepted that infection rate is much higher in dairy goats (deMaar et al., 1995), findings from this study suggested that farm management practice and replacement policy could also be a reason to the higher seroprevalence rate in dairy goats. It was observed that a vast population of meat goats is seasonally sold out while dairy goats are usually kept for longer-term purposes, increasing the chance of transmission among the goats within the herds. Moreover, the fact that the majority of dairy farms included in this study practiced intensive rearing system and seroprevalence rate was found higher with those farms that practiced intensive rearing system is also a reason to be considered. Therefore, rearing system could be a confounding factor to the higher prevalence of CAEV infection in goats.

Compared to some others reports of CAE from other countries around the world, individual true prevalence in this study, 5.52%, was lower than those reported in Somalia (6.0%) (Ghanem et al., 2009), Jordan (8.9%) (Al-Qudah et al., 2006), Brazil (14.1% and

8.2% respectively) (Lilenbaum et al., 2007; Bandeira et al., 2009), America (31%) (Cutlip et al., 1992) and Norway (42%) (Nord et al., 1998[b]). However, on the other hand, it was higher than those reported in Mexico (0.4%) (Tesoro-Cruz et al., 2003), Saudi Arabia (0.08%) (Alluwaimi et al., 1990), Turkey (1.03% and 1.9% respectively) (Burgu et al., 1994; Aslantas et al., 2005) and Italy (4.0%) (Gufler and Baumgartner, 2007[b]).

Across the world, on herd level, seroprevalence from this study, 31%, was relatively high, compared to that of 3.6% in Mexico (Torres-Acosta et al., 2003), 10.3% in Great Britain (Dawson and Wilesmith, 1985), and 23.3% in Jordan (Al-Qudah et al., 2006). However, in contrast, it was much lower than those reported in Somalia (71%) (Ghanem et al., 2009), USA (73%) (Cutlip et al., 1992), and Norway (86%) (Nord et al., 1998[b]). Therefore, seroprevalence seems to vary depending on the geographical distribution of different countries and different regions of the world.

In this study, seroprevalence of CAEV infection tended to increase with the age, where it gradually increased in goats from less than one year (3.2%) to 3 years of age (5.9%). However, in goats with the age of 3 years onwards, seroprevalence rose up to almost double (10.1%), and it was significantly higher ($p= 0.001$; OR= 4.288; CI 95%= 1.809-10.163) than that of others less than 3 years of age. This finding was similar to the previous report from Somalia (Ghanem et al., 2009) which described that goats of 3 years and above were more likely to be seropositive. Other studies also indicated that seroprevalence of CAEV infection increased with the age (Cutlip et al., 1992), and it was significantly higher in goats older than 3 years of age (Al-Qudah et al., 2006). This can be explained by the fact that CAEV infection is prone to infect any age of goats (Al-Ani and Vestweber, 1984) and older animals, with higher possibility of exposure to risk factors, are therefore more likely to be at risk, get infected and remain infected for life since CAEV is persistent and can produce lifelong infection in host (Knight and Jokinen, 1982). However, this finding was contradictory to another study (Dawson and Wilesmith, 1985) that said the prevalence was highest in yearlings. As in Thailand, a previous study

by Ratanapob *et al* (2009) stated that the seroprevalence was significantly higher in adult goats.

With reference to sex, seroprevalence was remarkably higher in male. Higher prevalence in male has also been reported in some other studies as well (Aslantas et al., 2005; Bandeira et al., 2009). However, the difference was sometimes not significant (Gufler et al., 2007[a]). In the previous study in Thailand by Ratanapob *et al* (2009), higher prevalence was observed in female. In this study, higher seroprevalence in male might have been reflected by the male-female ratio in the herds, from which comparatively few numbers of male were available to be included in this study. A larger number of male animals, if included, might have changed the seroprevalence ratio.

Regarding breeds, similar to previous findings (Rodriguez et al., 2005; Gufler et al., 2007[a]), higher seroprevalence was observed in crossbreed goats, especially in Saanen crossbreed. In this study, most of the dairy goat farms were raised on Saanen crossbreed and, vice versa, all native breeds in this study were kept for meat purposes. Therefore it can be said that higher prevalence in dairy farms may be partly due to the breeds they raised on.

Herd size was found to be a risk factor to seroprevalence of CAEV infection. From this study, it was observed that an increase in herd size was directly proportional to an increase in odds ratio. A similar finding was reported in previous studies (Ghanem et al., 2009). This can be mainly due to the stocking density of the herd, which could increase the likelihood of transmission within herd (Greenwood et al., 1995[b]; Aslantas et al., 2005). But, this finding was not in agreement with a previous study which reported that herd size could have no effect on serological status of the herd (Al-Qudah et al., 2006). Most of the farmers in this study were small-holders and therefore majority of the farms were of small size containing less than 50 animals in them. However, relatively low

seroprevalence was observed with them and this was in contrast to a previous study that said seroprevalence was higher in small-sized farms (Cutlip et al., 1992).

Not similar to previous reports that described intensive rearing as a risk factor for CAEV infection (Aslantas et al., 2005; Gufler et al., 2007[a]), rearing system did not show any significant association with seropositivity of CAEV infection on risk factor analysis. However, though not significant in multivariable analysis, higher seroprevalence was detected in goats raised on intensive management on both herd and individual levels, and a significant association on univariate analysis was also observed between the rearing system and seropositivity of goats on individual level analysis. Therefore, intensive rearing should be taken into account in the consideration of farm management practice against CAEV infection.

Addition of new goats into herds, with significant association in multivariable analysis, was found to be a risk factor to CAEV infection. Similar findings had been published in others studies as well (Al-Qudah et al., 2006; Bandeira et al., 2009). However, it may not be appropriate to say addition of new goats into herd will always be a potential risk factor to CAEV infection unless the farm management practice is being thought about. In some commercial farms, where serological diagnosis is regularly performed and intake of new animals into herd is based on prior investigation and purchasing only from CAE-negative herds, addition of new goat may not involve this much in the prevalence of CAE. But, in this study, since the majority of farms belonged to small holders, who paid little attention towards regular testing of CAE, with low tendency to check the serological status of animal before taking it into herd, addition of new goats was promptly seen as a potential risk factor to CAEV infection.

Use of pasture was not a risky issue to CAEV infection. This can be because most of the farms that used pasture in this study were of meat type with low seroprevalence rate, where dairy goats were usually kept intensive. Another reason was

that not all farms that used pasture had their goats in contact with goats from other herds because many of the farms were not close to each other.

However, contact with goats from other herds, including the use of common bucks, which was previously described as a risk factor in some studies (Torres-Acosta et al., 2003; Al-Qudah et al., 2006), was found significant in this study, too. But, the presence of two or more farms within one kilometer area has no effect on seroprevalence results. This affirmed the statement that CAEV infection through aerosol transmission could take place only over a close distance within herd (Rowe et al., 1991; Blacklaws et al., 2004) .

Presence of sheep in the farms, which had been described as a risk factor in a previous study (Ghanem et al., 2009), did not have any significant association on risk factor analysis. However, the number of farms that raised sheep and goats together included in this study was only three and also that sheep in this study were not checked for serological status. Therefore, another in-depth study focusing on such relationship between sheep and goats towards CAEV infection should be conducted.

Regarding preventive measures, use of disinfectants, practice of FMD vaccination and presence of veterinary service showed no significant relationship with seroprevalence of CAEV infection. Majority of the farms used disinfectants, but infection rate was not much different from those that did not use disinfectants, nevertheless prevalence rate was slightly lower in disinfected farms. This is because route of transmission for CAEV infection is primarily from dams to kids and other routes of transmission such as through the utensils and contaminated feed trough are less efficient. Therefore, sanitation may not involve much in transmission of CAEV infection.

With vaccination, since no vaccine against CAEV has been available yet (Al-Ani and Vestweber, 1984; Logan et al., 2004), use of vaccination against FMD was studied

instead. With FMD vaccination, farms with or without the practice of vaccination were not much different from each other in regards to the prevalence of CAEV infection. However, on individual prevalence, FMD vaccinated animals were found more common to CAEV infection. This suggested that transmission of disease within herd is possible through the use of contaminated syringe and needle.

Although not significant, a relatively low rate of infection was observed with those farms that had veterinary service with them. However, in proportion, only a few farms had veterinary service with them. This may be mainly because the majority of farmers in this area are small holders, not willingly to spend extra money for health care issue, and in many farms, it was observed that farmers did every medication process, for example vaccination against FMD and deworming, by themselves.

With breeding methods, a slight reduction in seroprevalence was observed in the farms that used AI. However, a firm conclusion cannot be drawn since the difference was not significant and, in addition, not many of the bucks from seropositive farms using natural mating were seropositive to CAEV infection. In some farms, there were no bucks for breeding purposes. They just shared a common buck or hire one when needed. Therefore, more detailed focus on male involvement would be necessary for further evaluation of breeding methods in regards to CAEV infection. However, according to this study, though not significant, with reduced seroprevalence of infection observed and its exclusion of the male involvement, AI seemed to be more appropriate for minimizing CAEV infection in farms.

It was interesting that seroprevalence was significantly higher on univariate analysis with those farms in which CAEV infection had taken place in the past. This suggested that infection could be recurrent or persistent in the herds unless a proper eradication program is introduced. But, on the other hand, this association was no longer significant in multivariable analysis and so it could not be defined as a risk factor.

However, a significant difference in seroprevalence rate reminded that a particular attention should be paid with the history of CAEV infection in the herds.

Knowledge of farm owner towards CAE, supposedly helpful for better health status of the herds, did not relate much with the seroprevalence of CAEV, where infection rate were more or less the same in two different groups of owner. Having knowledge provides better condition was observed in this study, with lower seroprevalence in those farms whose owners have knowledge of CAE. But knowledge alone could not solve the problem was also seen.

To sum up with risk analysis, age of three years and above, large herd size, addition of new animals into herd, contact with other goats from other herds and herd type were found as significant risk factors to CAEV infection from this study.

However, in this study, relationship between seropositivity of CAEV infection and some hypothesized risk factors were unable to be judged. For example, feeding system, drinking system, deworming practice of the farms were not analyzed since all farms practiced shared feeding, shared drinking and regular deworming scheme. Furthermore, feeding the kids on pasteurized milk or milk replacer was also not analyzed since rearing on pasteurized milk was not practiced in studied farms. This is because most of the farms herein studied are small farms with low economic status. A detailed study with these criteria in some commercial farms should be further conducted.

5.2. Comparative study on the effects of CAEV infection on reproductive performance of goats between seropositve and seronegative groups in a CAEV infected herd

Regarding the comparative study between two groups of animals in a selected seropositive farm, not much significant difference was observed between seropositive and seronegative does during the study period of 14 months. It may be partly because limited numbers of animals, particularly adult female goats, were included in this study since it was undertaken in a semi-commercial farm having a moderate herd size, from which relatively small sample size was available, compared to other studies carried out in big commercial farms (Greenwood, 1995[a]; Leitner et al., 2010)

From this study it was seen that the first conception rate was significantly lower in seropositive group than in seronegative group. A similar finding was reported in previous studies saying that reduced conception rate was observed in seropositive does (Peterhans et al., 2004; Reina et al., 2009). It could be explained by the fact that CAEV infection is immunosuppressive to infected goats. With a great affection to leucocytes, CAEV infects monocytes and tissue macrophage and, as a consequence of which, metabolic processes may become impaired as the time goes on. With altered macrophage function and acquired immunodeficiency (Klevjer-Anderson and Anderson, 1982; Narayan et al., 1983), nutrient intake by goats will not become fully useable for reproductive performance, being partitioned by body mechanism for the compensatory function processed for other impaired systems, and it could result in poor reproductive performance with a decrease in conception rate. The viral infection activates a massive immune response, as a result of which arrays of inflammatory mediators are generated and that leads to viral arthropathy. This inflammatory condition activates self-reactive lymphocytes where autoimmune response is released and immune system starts to attack itself by mistake (Suhrbier and Mahalingam, 2009). A similar suggestion was presented in a previous study describing that longer-term consequence of CAEV

infection may partition the nutrients, taking them away from normal metabolic usage (Greenwood, 1995[a]).

In this study, seropositive does seemed less efficient in converting nutrient intake into energy and looked more susceptible to any extrinsic factor as their body condition scores on average were relatively lower compared to those of seropositive individuals. It was more clearly seen when any change in ration was made. A similar assumption had been described in a study which reported that the impact of reduction in supplementary feed was found larger in seropositive does than in seronegative does (Greenwood, 1995[a]).

However, with total conception rate, no significant difference was observed between two groups. Some does, which failed to conceive at the first AI, became pregnant on the following AI. Therefore total conception rate between each group was not significantly different, but it was still noticeably lower in seropositive group. This may be due to the fact that seropositive does, being suffered from immunodeficiency, could be more easily affected by any change in ration or climate as it was found that conception rate elevated more perceptibly in seropositive does in winter time, and, vice versa, more drastically in summer.

There were also few cases in which does failed to become pregnant over two consecutive AI in both groups. The rate was higher in seropositive group (50% vs. 33.3%), but not significant. A similar finding was reported in a previous study which said there was no significant difference between seropositive and seronegative does in regards to reproductive failure rate, but it was found higher in seropositive does (Greenwood, 1995a). Again, it added that reproductive failure was more common in multiparous does. However, in this study, the differences between multiparous and primiparous does were unable to analyze since all animals studied were multiparae.

Those goats that failed to conceive during two consecutive AI should not be assumed infertile as the study period was confined to 14 months. Perhaps, they may become pregnant if time and frequency extended, for example, to the third time AI. It was observed that even with the same animals of the same herd, pregnancy rate widely varied from time to time according to previous records of the farm. It was sometimes merely 40% or lesser, and sometimes almost up to 90%, being different at times. Many other factors such as age of animal, nutrition provided, change in climate and skill of the practitioner should be taken into account. Therefore, a more detailed study should be conducted to determine the relationship between infertility and CAEV infection.

Average number of kids born to each doe was higher in seronegative group, but very slightly (1.65 vs.1.50). This was similar to previous findings which stated that there was no significant difference between seropositive and seronegative does in terms of the number of kids born (Greenwood, 1995[a]; Leitner et al., 2010). Though not significant, this finding also agreed with a previous statement that said the average number of offspring born to each gestation was lower in seropositive does (Peterhans et al., 2004). But, in this study, since the difference was very slight, it could be deduced that CAEV infection has no interference with the number of offspring born.

Average gestation lengths from two groups were not significantly different. It was 149.7 days in seronegative group and 149.5 in seropositive group. *Greenwood* (1995[a]) also found that gestation length was not significantly different between seropositive and seronegative groups. From this study, a conclusion can be drawn that CAEV infection has no influencing effect over the length of gestation. This finding, however, was contradictory to a previous statement which said that CAEV infection could have influenced length of gestation (Zink et al., 1987). However, there was one thing to be considered that gestation length could vary depending on the sex and number of offspring.

In this study, no abortion cases were observed since all of the goats that became pregnant were able to carry their pregnancy to full term. But then, tracing back to the history of the farm, it was realized that abortion cases were also rare in the past. This could be because good management was practiced and pregnant does were well looked after by farm owners in their antenatal period.

One more interesting thing from this study was that kids born from seropositive dams were found seronegative to CAEV infection on subsequent serological tests. Because, in this farm, seropositive does were kept alive for their reproductive performance and milk production, while newborn kids were separated from their dams shortly after delivery and raised on other seronegative goats' milk. This finding suggested that a proper management could be a way to eradication of CAEV infection in seropositive herds. However, a similar strategy had been previously reported that total segregation of seronegative and seropositive individuals was of vital importance in the control of CAEV infection (Nord et al., 1998[a]).

5.3 Conclusion and suggestion

This study was conducted to elucidate risk factors for the seroprevalence of CAEV infection in goat herds in the Western parts of Thailand and to study the effects of CAEV infection on reproductive performances of goats in a CAE affected herd. Following the study designs, a cross-sectional serological survey with questionnaire interview and a case-control study over 14 month's period were performed.

Results showed that seroprevalence was higher in the case of herd prevalence than that of individual. Age of three years and above, herd size of greater than 50 animals, addition of new goats into herds, and contact with goats from other herds were found as significant risk factors for the prevalence of CAEV infection in studied goat population.

With significant reduction in first conception, lower total conception rate and fewer numbers of kids born to each doe in seropositive does, the comparative study suggested that CAEV infection may have influenced the reproductive performance of infected goats in some way.

Findings from this study provided additional information concerning the prevalence of CAEV infection and risk factors associated with it, as well as the effects of CAEV infection on reproductive performance and health of dairy goats. However, due to some constraints and unfavourable situations, some farms in the study area had not been explored into detail and some parts of the comparative study were unable to be analyzed more precisely. Further epidemiological studies with more in-depth investigation of CAEV infection and its effects on reproductive performance of goats are therefore deemed necessary for a better understanding of the nature of CAEV infection, as well as its involvement in reproductive performance and physiology of goats, and risk factors associated with the prevalence of CAEV infection in goats in Thailand.

REFERENCES

Abo-Shehada, M.N. and Abu-Halaweh, M.M. 2010. Flock-level seroprevalence of, and risk factors for, Neospora caninum among sheep and goats in northern Jordan. Preventive. Vet. Med. 93: 25-32.

Al-Ani, F.K. and Vestweber, J.G.E. 1984. Caprine arthritis-encephalitis syndrome (CAE): a review. Vet. Res. Comm. 8: 243-253.

Al-Majali, A.M. 2005. Seroepidemiology of caprine brucellosis in Jordan. Small. Rumin. Res. 58: 13-18.

Al-Qudah, K., Al-Majali, A.M. and Ismail, Z.B. 2006. Epidemiological studies on caprine arthritis-encephalitis virus infection in Jordan. Small. Rumin. Res. 66: 181-186.

Ali Al Ahmad, M.Z., Cheboune, Y., Bouzar, B.A., Baril, G., Bouvier, F., Chatagnon, G., Leboeuf, B., Pepin, M., Guibert, J.M., Russo, P., Manfredi, E., Martin, J. and Fieni, F. 2008[a]. Lack of risk of transmission of caprine arthritis-encephalitis virus (CAEV) after an appropriate embryo transfer procedure. Theriogenology. 69: 408-415.

Ali Al Ahmad, M.Z., Fieni, F., Pellerin, J.L., Guiguen, F., Cherel, Y., Chatagnon, G., Bouzar, A.B. and Cheboune, Y. 2008[b]. Detection of viral genomes of caprine arthritis-encephalitis virus (CAEV) in semen and in genital tract tissues of male goat. Theriogenology. 69: 473-480.

Alluwaimi, A.M., Abu Elzein, E.M. and Hassanein, M.M. 1990. Caprine arthritis-encephalitis antibodies in indigenous sheep in Saudi Arabia. Rev. Elev. Med. Vet. Pays. Trop. 43: 444-445.

Archambault, D., East, N., Perk, K. and Dahlberg, J.E. 1988. Development of an enzyme-linked immunosorbent assay for caprine arthritis-encephalitis virus. J. Clin. Microbiol. 26: 971-975.

Aslantas, O., Ozyoruk, F., Pinar, D. and Gungor, B. 2005. Serological survey for caprine arthritis-encephalitis virus in Damascus and Kilis goats in Hatay, Turkey. Revue. Méd. Vét. 156: 402-404.

Bandeira, D.A., de Castro, R.S., Azevedo, E.O., de Souza Seixas Melo, L. and de Melo, C.B. 2009. Seroprevalence of caprine arthritis-encephalitis virus in goats in the Cariri region, Paraiba state, Brazil. Vet. J. 180: 399-401.

Blacklaws, B.A., Berriatua, E., Torsteinsdottir, S., Watt, N.J., de Andres, D., Klein, D. and Harkiss, G.D. 2004. Transmission of small ruminant lentiviruses. Vet. Microbiol. 101: 199-208.

Bouzar, B.A., Rea, A., Hoc-Villet, S., Garnier, C., Guiguen, F., Jin, Y., Narayan, O. and Cheblounе, Y. 2007. Activation/proliferation and apoptosis of bystander goat lymphocytes induced by a macrophage-tropic chimeric caprine arthritis encephalitis virus expressing SIV Nef. Virology. 364: 269-280.

Brinkhof, J.M.A., Moll, L., van Maanen, C. and Houwers, D.J. 2009. Use of serology and polymerase chain reaction for the rapid eradication of small ruminant lentivirus infections from a sheep flock: A case report. Res. Vet. Sci. 88: 41-43.

Burgu, I., Akca, Y., Alkan, F., Ozkul, A., Karaoglu, T. and Cabalar, M. 1994. Antibody prevalence of caprine arthritis encephalitis virus (CAEV) in goats in Turkey. Dtsch. Tierarztl. Wochenschr. 101: 390-391.

Castle, J.M., Ruiz, F.A., Mena, Y. and Sanchez-Rodriguez, M. 2010. Present situation and future perspectives for goat production systems in spain. Small. Rumin. Res. 89: 207-210.

Cheblounе, Y., Sheffer, D., Karr, B.M., Stephens, E. and Narayan, O. 1996. Restrictive type of replication of ovine/caprine lentiviruseses in ovine fibroblast cell cultures. Virology. 222: 21-30.

Cork, L.C., Hadlow, W.J. and Crawford, T.B. 1974. Infectious leukoencephalomyelitis of young goats. J. Infect. Dis. 129: 134-141.

Cortez-Moreira, M., Oelemann, W.M.R. and Lilenbaum, W. 2005. Comparison of serological methods for the diagnostic of caprine arthritis-encephalitis (CAE) in Rio de Janeiro, Brazil. Braz. J. Microbiol. 36: 48-50.

Cruz, J.C.M., Gouveia, A.M.G., Souza, K.C., Braz, G.F., Teixeira, B.M., Heinemann, M.B., Leite, R.C., Reis, J.K.P., Pinheiro, R.R. and Andrioli, A. 2009. Caprine arthritis-encephalitis virus (CAEV) detection in semen of endangered goat breeds by nested polymerase chain reaction. Small. Rumin. Res. 85: 149-152.

Cutlip, R.C., Lehmkuhl, H.D., Sacks, J.M. and Weaver, A.L. 1992. Prevalence of antibody to caprine arthritis-encephalitis virus in goats in the United States. J. Am. Vet. Med. Assoc. 200: 802-805.

Dawson, M. and Wilesmith, J.W. 1985. Serological survey of lentivirus (maedi-visna/caprine arthritis-encephalitis) infection in British goat herds. Vet. Rec. 117: 86-89.

de Andrés, D., Klein, D., Watt, N.J., Berriatua, E., Torsteinsdottir, S., Blacklaws, B.A. and Harkiss, G.D. 2005. Diagnostic tests for small ruminant lentiviruses. Vet. Microbiol. 107: 49-62.

deMaar, T.W., Blumer, E.S. and Sherman, D.M. 1995. Failure of horizontal transmission of caprine arthritis encephalitis virus to non-dairy breeds of goats. Small. Rumin. Res. 17: 197-198.

Denner, J. 2007. Tansspecies transmissions of retroviruses: new cases. Virology. 369: 229-233.

East, N.E., Rowe, J.D., Dahlberg, J.E., Theilen, G.H. and Pederson, N.C. 1993. Modes of transmission of caprine arthritis-encephalitis virus infection. Small. Rumin. Res. 10: 251-262.

Elfahal, A.M., Zakia, A.M. and El-Hussien, A.M. 2010. First report of caprine arthritis-encephalitis virus infection in Sudan. J. Anim. Vet. Advances. 9: 736-740.

Eltahir, Y.M., Dovas, C.I., Papanastassopoulou, M., Koumbati, M., Giadinis, N., Verghese-Nikolakaki, S. and Koptopoulos, G. 2006. Development of a semi-nested PCR using degenerate primers for the generic detection of small ruminant lentivirus proviral DNA. J. Virol. Methods. 135: 240-246.

FAOSTAT. 2008. [Online]. Available: http://faostat.fao.org/default.aspx.

Ghanem, Y.M., El-Khodery, S.A., Saad, A.A., Elragaby, S.A., Abdelkader, A.H. and Heybe, A. 2009. Prevalence and risk factors of caprine arthritis encephalitis virus infection (CAEV) in Northern Somalia. Small. Rumin. Res. 85: 142-148.

Greenwood, P.L. 1995[a]. Effects of caprine arthritis-encephalitis virus on productivity and health of dairy goats in New Suth Wales, Australia. Preventive. Vet. Med. 22: 71-87.

Greenwood, P.L., North, R.N. and Kirkland, P.D. 1995[b]. Prevalence, spread and control of caprine arthritis-encephalitis virus in dairy goat herds in New South Wales. Aust. Vet. J. 72: 341-345.

Gregory, L., Junior, E.H.B., Lara, M.C.C.S.H., Angelini, M., Araujo, W.P., Rizzo, H., Maiorka, P.C., Castro, R.S., Kiraly, A.C.M., Benesi, F.J. and Birgel, E.H. 2009. Clinical features of indurative mastitis caused by caprine arthritis encephalitis virus. Braz. J. Vet. Pathol. 2: 64-68.

Gufler, H. and Baumgartner, W. 2007[b]. Overview of herd and CAEV status in dwarf goats in South Tyrol, Italy. Vet. Q. 29: 68-70.

Gufler, H., Gasteiner, J., Lombardo, D., Stifter, E., Krassnig, R. and Baumgartner. 2007[a]. Serological study of small ruminant lentivirus in goats in Italy. Small. Rumin. Res. 73: 169-173.

Karanikolaou, K., Angelopoulou, K., Papanastasopoulou, M., Koumpati-Artopiou, M., Papadopoulos, O. and Koptopoulos, G. 2005. Detection of small ruminant lentiviruses by PCR and serology tests in field samples of animals from Greece. Small. Rumin. Res. 58: 181-187.

Klevjer-Anderson, P. and Anderson, L.W. 1982. Caprine arthritis-encephalitis virus infection of caprine monocytes. J. Gen. Virol. 58: 195-198.

Knight, A.P. and Jokinen, M.P. 1982. Caprine arthritis-encephalitis virus Compend. Cont. Educ. Pract. Vet. . 4: 263-269.

Kusza, S., Bosze, Z., Kukovics, S. and Javor, A. 2004. Genetic assay of caprine arthritis-encephalitis in the Hungarian goat herd. South. African. J. Anim. Sci. 34: 13-16.

Kwang, J., Keen, J., Cutlip, R.C., Kim, H.S. and de la Concha-Bermejillo, A. 1995. Serological diagnosis of caprine lentivirus infection by recombinant immunoassays. Small. Rumin. Res. 16: 171-177.

Lamara, A., Fieni, F., Mselli-Lakhal, L., Chatagnon, G., Bruyas, J.F., Tainturier, D., Battut, I., Fornazero, C. and Chebloune, Y. 2002. Early embryonic cells from in vivo-produced goat embryos transmit the caprine arthritis-encephalitis virus (CAEV). Theriogenology. 58: 1153-1163.

Le Jan, C., Bellaton, C., Greenland, T. and Mornex, J.F. 2005. Mammary transmission of caprine arthritis encephalitis virus: a 3D model for in vitro study. Reprod. Nutr. Dev. 45: 513-523.

Leitner, G., Krifucks, O., Weisblit, L., Lavi, Y., Bernstein, S. and Merin, U. 2010. The effect of caprine arthritis encephalitis virus infection on production in goats. Vet. J. 183: 328-331.

Lilenbaum, W., de Souza, G.N., Ristow, P., Cortez-Moreira, M., Fráguas, S., da Silva Cardoso, V. and Oelemann, W.M.R. 2007. A serological study on brucella abortus, caprine arthritis-encephalitis virus and leptospira in dairy goats in Rio de Janeiro, Brazil. Vet. J. 173: 408-412.

Logan, S., Tarpley, H.L. and Latimer, K.S. 2004. "Caprine arthritis encephalitis virus" [Online]. Available: http:// www.vet.uga.edu.

Lombardi, G. 2005. Optimum management and quality pastures for sheep and goat in mountain areas. options mediterr. 67: 19-29.

MacDiarmid, S.C. 1983. Survey suggests low prevalence of caprine arthritis encephalitis. Surveillance. 10: 4-8.

Morand-Fehr, P. and Lebbie, S.H.B. 2004. Proposal for improving the research efficiency in goats. Small. Rumin. Res. 51: 145-153.

Narayan, O., Clements, J.E., Strandberg, J.D., Cork, L.C. and Griffin, D.E. 1980. Biological characterization of the virus causing leukoencephalitis and arthritis in goats. J. Gen. Virol. 50: 69-79.

Narayan, O., Kennedy-Stoskopf, S., Sheffer, D., Griffin, D.E. and Clements, J.E. 1983. Activation of caprine arthritis-encephalitis virus expression during maturation of monocytes to macrophages. Infect. Immun. 41: 67-73.

Narayan, O., Wolinsky, J.S., Clements, J.E., Strandberg, J.D., Griffin, D.E. and Cork, L.C. 1982. Slow virus replication: the role of macrophages in the persistence and expression of visna viruses of sheep and goats. J. Gen. Virol. 59: 345-356.

Nord, K., Løken, T. and Orten, Å. 1998[a]. Control of caprine arthritis-encephalitis virus infection in three Norwegian goat herds. Small. Rumin. Res. 28: 109-114.

Nord, K., Rimstad, E., Storset, A.K. and Loken, T. 1998[b]. Prevalence of antibodies against caprine arthritis-encephalitis virus in goat herds in Norway. Small. Rumin. Res. 28: 115-121.

Peterhans, E., Greenland, T., Badiola, J., Harkiss, G., Bertoni, G., Amorena, B., Eliaszewicz, M., Juste, R.A., Krassnig, R., Lafont, J.P., Lenihan, P., Petursson, G., Pritchard, G., Thorley, J., Vitu, C., Mornex, J.F. and Pepin, M. 2004. Routes of transmission and consequences of small ruminant lentiviruses (SRLVs) infection and eradication schemes. Vet. Res. 35: 257-274.

Plaza, M., Sánchez, A., Corrales, J.C., De la Fe, C. and Contreras, A. 2009. Caprine arthritis encephalitis virus diagnosed by ELISA in lactating goats using milk samples. Small. Rumin. Res. 81: 189-192.

Ratanapob, N., Rukkwamsuk, T. and Jala, S. 2009. Seroprevalence of caprine arthritis encephalitis virus infection in goats raised in the central part and western part of Thailand. The 47th Kasetsart University Ann. Con., Bangkok, Thailand.

Reina, R., Berriatua, E., Luján, L., Juste, R., Sánchez, A., de Andrés, D. and Amorena, B. 2009. Prevention strategies against small ruminant lentiviruses: An update. Vet. J. 182: 31-37.

Rodriguez, H.A.M., Alvarez, H.R., Perez, J.T., Setien, A.A., Farina, G.I.G. and Crespo, J.A.M. 2005. Effect of the caprine arthritis encephalitis virus in the reproductive system of male goats. Vet. Mex. 36: 159-176.

Rowe, J.D., East, N.E., C.Thurmond, M. and Franti, C.E. 1991. Risk factors associated with caprine arthritis-encephalitis virus infection in goats on California dairies. Am. J. Vet. Res. 52: 510-514.

Ruiz, F.A., Mena, Y., Castle, J.M., Guinamard, C., Bossis, N., Caramelle-Holtz, E., Contu, M., Sitzia, M. and Fois, N. 2009. Dairy goat grazing systems in Mediterranean regions: A comparative analysis in Spain, France and Italy. Small. Rumin. Res. 85: 42-49.

Rukkwamsuk, T., Karnjanamala, W., Nuamjit, M., Supa, P., Phokrasung, P. and Chakmongkhol, S. 2008. A study on antibody against brucella melitensis infection in meat goats. The 15th congress of FAVA, Bangkok, Thailand.

Sanchez, A., Contreras, A., Corrales, J.C. and Marco, J.C. 2001. Relationships between infection with caprine arthritis encephalitis virus, intramammary bacterial infection and somatic cell counts in dairy goats. Vet. Rec. 148: 711-714.

Storset, A.K., Evensen, O. and Rimstad, E. 1997. Immunohistochemical identification of caprine arthritis-encephalitis virus in paraffin-embedded specimens from naturally infected goats. Vet. Pathol. 34: 180-188.

Suhrbier, A. and Mahalingam, S. 2009. The immunobiology of viral arthritides. Pharmacol. Therapeut. 124: 301-308.

Tantaswasdi, U., Wattanavijarn, W. and Pinyochon, W. 1985. Caprine arthritis-encephalitis like virus infection in saanen goats. The 12th ann. vet. con., Bangkok, Thailand.

Tesoro-Cruz, E., Hernandez-Gonzalez, R., Kretschmer-Schmid, R. and Aguilar-Setien, A. 2003. Cross-reactivity between caprine arthritis-encephalitis virus and type 1 human immunodeficeincy virus. Archives. Med. Res. 34: 362-366.

Thrusfield, M. 2005. Veterinary Epidemiology. Great Britain: Blackwell Science Ltd. 584 pp pp.

Torres-Acosta, J.F.J., Gutierrez-Ruiz, E.J., Butler, V., Schmidt, A., Evans, J., Babington, J., Bearman, K., Fordham, T., Brownlie, T., Schroer, S., Cámara-G, E. and Lightsey, J. 2003. Serological survey of caprine arthritis-encephalitis virus in 83 goat herds of Yucatan, Mexico. Small. Rumin. Res. 49: 207-211.

Travassos, C.E., Benoit, C., Valas, S., da Silva, A.G. and Perrin, G. 1999. Caprine arthritis-encephalitis virus in semen of naturally infected bucks. Small. Rumin. Res. 32: 101-106.

Zink, M.C., Narayan, O., Kennedy, P.G.E. and Clements, J.E. 1987. Pathogenesis of visna-maedi and caprine arthritis-encephalitis: new leads on the mechanism of restricted virus replication and persistent inflammation. Vet. Immunol. Immunopathol. 15: 167-180.

APPENDICES

APPENDIX A

Questionnaire

การสำรวจปัจจัยเสี่ยงที่มีความเกี่ยวข้องต่อการติดเชื้อ CAEV **และผลกระทบของการติดเชื้อ** CAEV **ต่อประสิทธิภาพการสืบพันธุ์ และสภาวะด้านสุขภาพของฟาร์มแพะ**

1. ชื่อ-นามสกุล เจ้าของฟาร์ม .....
2. ระดับการศึกษา ประถมศึกษา ☐ มัธยมศึกษา ☐
   อนุปริญญา ☐ ปริญญาตรี ☐ อื่นๆ ☐
3. หมายเลขทะเบียนฟาร์ม .....
4. ที่ตั้งของฟาร์ม .....
   .....
5. จำนวนแรงงานในฟาร์ม .....
6. รูปแบบของฟาร์ม: ☐ แพะนม ☐ แพะเนื้อ ☐ แบบผสม
   ถ้าเป็นแพะนม กรุณาระบุปริมาณผลผลิตน้ำนมรายวันของฟาร์ม .....
7. จำนวนแพะภายในฟาร์ม .....
   แพะเพศผู้ .....
   แพะพ่อพันธุ์ .....
   แพะเพศเมีย .....
   แม่แพะให้นม .....
   ลูกแพะ .....
8. พันธุ์แพะที่เลี้ยงในฟาร์ม _____
9. ระบบการเลี้ยง: ☐ เลี้ยงแบบขังคอก ☐ เลี้ยงแบบกึ่งปล่อย ☐ เลี้ยงแบบปล่อยแปลง
10. มีการแยกแพะเพศผู้และเพศเมียหรือไม่: ☐ มี ☐ ไม่มี
11. มีฟาร์มเลี้ยงสัตว์ข้างเคียงในระยะ 1 กิโลเมตรหรือไม่: ☐ มี ☐ ไม่มี
    ถ้ามี ให้ระบุระยะห่างจากฟาร์มดังกล่าวถึงฟาร์มของท่าน _____
12. มีการเลี้ยงแกะร่วมด้วยหรือไม่: ☐ มี ☐ ไม่มี
13. มีการนำแพะจากฟาร์มอื่นเข้ามาในฟาร์มหรือไม่: ☐ มี ☐ ไม่มี
    ถ้ามี ให้ระบุที่มา และพันธุ์ของแพะที่นำเข้ามา _____

14. ระบบการทดแทนแพะเป็นแบบ All in all out หรือไม่ ☐ ช่ ☐ ไม่ใช่

15. ระบบการให้น้ำดื่มสำหรับแพะ: ☐ ให้ดื่มร่วมกัน

☐ ให้ดื่มแยกเป็นรายตัว

16. การให้สารเสริมในน้ำดื่ม: ให้น้ำดื่มเพียงอย่างเดียว ☐ ใช่ ☐ ไม่ใช่

ถ้าไม่ใช่ ให้ระบุว่าชนิดของสารเสริม: _____

17. การเสริมอาหารข้น: _____

18. ระบบการให้อาหาร: ☐ ให้กินร่วมกัน

☐ ให้กินแยกเป็นรายตัว

19. การให้นมน้ำเหลือง (ภายใน 24 ชม. หลังคลอด) ☐ ใช่ ☐ ไม่ใช่

ถ้ามีการให้นมน้ำเหลือง: ☐ นมน้ำเหลืองธรรมชาติ ☐ นมน้ำเหลืองสังเคราะห์

20. การน้ำนมแก่ลูกแพะ : (A) ☐ ดื่มจากนมแม่ (B) ☐ รายตัว

☐ ดื่มจากขวดนม ☐ ให้ดื่มร่วมกัน

☐ ทั้งสองแบบ ☐ ทั้งสองแบบ

21. ได้มีการใช้ประโยชน์ของพืชอาหารสัตว์หรือไม่: ☐ มี ☐ ม่มี

ถ้ามี มีการพบกับแพะจากฟาร์มอื่นหรือไม่ (แปลงหญ้าสาธารณะ): ☐ มี ☐ ไม่มี

22. วิธีการผสมพันธุ์: ☐ การผสมเทียม

ถ้าใช้วิธีการผสมเทียม ให้ระบุแหล่งที่มาของน้ำเชื้อ _____

☐ การผสมแบบธรรมชาติ

23. ระบบการรีดนม: ☐ รีดด้วยมือ

☐ รีดด้วยเครื่องรีดนม

24. มีการใช้ยาฆ่าเชื้อหรือไม่: ☐ มี ☐ ไม่มี

25. ได้ใช้บริการของสัตวแพทย์หรือไม่: ☐ ใช้ ☐ ม่ใช้

มีการใช้วัคซีนป้องกันโรค FMD หรือไม่ ☐ มี ☐ ไม่มี

ถ้ามีการใช้วัคซีน - เริ่มใช้เมื่อไหร่ _____

- ความถี่ของการใช้วัคซีน _____

- การใช้วัคซีนครั้งสุดท้าย _____

การใช้วัคซีนอื่นๆ _____

ผู้ฉีดวัคซีน ☐ เกษตรกร ☐ สัตวแพทย์ ☐ อื่นๆ

26. มีการถ่ายพยาธิหรือไม่: ☐ มี ☐ ไม่มี

ถ้ามี ให้ระบุ - ชนิดของยาถ่ายพยาธิ _____

- ระยะห่างของการถ่ายพยาธิ _____

27. มีโปรแกรมการให้ยาปฏิชีวนะ หรือเสริมวิตามินเป็นประจำหรือไม่: ☐ มี ☐ ไม่มี

ถ้ามี ให้ระบุว่าเสริมอะไรบ้าง _____

28. เคยได้รับการตรวจโรค CAE หรือไม่: ☐ เคย ☐ ไม่เคย

ถ้าเคย ให้ระบุช่วงเวลาที่ตรวจและผลของการตรวจ _____

29. พบแพะมีอาการขาเจ็บหรือไม่: ☐ มี ☐ ไม่มี

ถ้ามี ให้ระบุช่วงเวลาที่พบอาการดังกล่าว _____

30. พบแพะมีอาการเต้านมอักเสบหรือไม่: ☐ มี ☐ ไม่มี

ถ้ามี ให้ระบุช่วงเวลาที่พบอาการดังกล่าว _____

31. พบแพะมีอาการป่วยด้วยโรคอื่นๆ หรือไม่: ☐ มี ☐ ไม่มี

ถ้ามี ให้ระบุช่วงเวลาที่พบ และป่วยด้วยโรคอะไร _____

32. พบแพะที่เป็นโรค Brucellosis หรือไม่: ☐ มี ☐ ไม่มี

ถ้ามี ให้ระบุช่วงเวลาที่พบ _____

33. มีสัตว์ชนิดอื่นๆ ภายในฟาร์มหรือไม่: ☐ มี ☐ ไม่มี

ถ้ามี ให้ระบุว่ามีสัตว์ชนิดใดบ้าง _____

34. มีโคภายในฟาร์มหรือไม่: ☐ มี ☐ ไม่มี

35. เคยได้รับการตรวจโรค Brucellosis หรือไม่ - ในโค: ☐ คย ☐ ไม่เคย

- ในแพะ: ☐ เคย ☐ ไม่เคย

ถ้าเคย ให้ระบุช่วงเวลาที่ตรวจและผลของการตรวจ _____

36. มีการใช้วัคซีนป้องกันโรค Brucellosis หรือไม่ - ในโค: ☐ ใช้ ☐ ไม่ใช้

- ในแพะ: ☐ ใช้ ☐ ไม่ใช้

ถ้ามีการใช้วัคซีน - เริ่มใช้เมื่อไหร่ _____

- ความถี่ของการใช้วัคซีน _____

- การใช้วัคซีนครั้งสุดท้าย _____

37. พบปัญหาผสมไม่ติดหรือไม่: ☐ มี ☐ ไม่มี

ถ้าพบ ให้ระบุช่วงเวลาและพบบ่อยครั้งแค่ไหน _____

38. พบปัญหาการแท้งลูกภายในฟาร์มหรือไม่: ☐ มี ☐ ไม่มี

ถ้าพบ ให้ระบุช่วงเวลาและพบบ่อยครั้งแค่ไหน _____

39. พบปัญหาการตายแรกคลอดหรือไม่: ☐ มี ☐ ไม่มี

ถ้าพบ ให้ระบุช่วงเวลาและพบบ่อยครั้งแค่ไหน _____

40. พบปัญหาด้านระบบสืบพันธุ์อื่นๆ หรือไม่: ☐ มี ☐ ไม่มี

ถ้าพบ ให้ระบุปัญหาดังกล่าว _____

41. พบปัญหาสุขภาพอื่นๆ หรือไม่: ☐ มี ☐ ไม่มี

ถ้าพบ ให้ระบุปัญหาดังกล่าว _____

42. ข้อคิดเห็น และคำแนะนำ: _____

APPENDIX B

Questionnaire

Investigation of risk factors associated with the prevalence of CAEV infection and the effects of CAEV infection on reproductive performance and health status of goat herds

1. Herd Owner _____
2. Education of Herd Owner Primary school ☐ High school ☐ Diploma ☐
   Graduate ☐ None of them ☐
3. Herd Code _____
4. Address _____
   _____
5. Number of Workers _____
6. Herd Type: Dairy ☐ Meat ☐ Mixed ☐
   If dairy, please specify daily milk yield of the farm _____
7. Number of Goats _____
   Male _____
   Breeder male _____
   Female _____
   Lactating does _____
   Neonatal kids _____
8. Breed or Breeds _____
9. Rearing system: Intensive ☐ Semi-intensive ☐ Extensive ☐
10. Separation of male and female YES ☐ NO ☐
11. Presence of neighborhood farms within 1 km: YES ☐ NO ☐
    If YES, please specify the estimated distance _____
12. Presence of sheep: YES ☐ NO ☐
13. Addition of new goats: YES ☐ NO ☐

If yes, please specify the source and breed of new goats, _____

14. Replacement policy: All in all out system: YES ☐ NO ☐

15. Drinking system: Shared drinking ☐

Separated drinking ☐

16. Supply for Drinking: Water only YES ☐ NO ☐

If NO, please describe what the additives are: _____

_____

17. Supply for feeding: _____

18. Feeding system: Shared feeding ☐

Separated feeding ☐

19. Colostrum feeding (within 24 hrs after birth) YES ☐ NO ☐

If YES, Natural colostrums ☐ Artificial colostrums ☐

20. Milk Feeding to kids: (A) By Mother ☐ (B) Individual ☐

By Bottle Shared ☐ ☐

Both Both ☐ ☐

21. Utilization of pasture: YES ☐ NO ☐

If YES, is there contact with goats from other farms (Common pasture):

YES ☐ NO ☐

22. Method of breeding: Artificial Insemination ☐

If AI, please specify the source of frozen semen, _____

Natural Mating ☐

23. Methods of milking: Hand Milking ☐

Machine Milking ☐

24. Utilization of disinfectants: YES ☐ NO ☐

25. Presence of veterinary services: YES ☐ NO ☐

Vaccination against FMD YES ☐ NO ☐

If YES - When it was started _____

- How often and _____

- When it was last performed _____

Other Vaccinations _____

Vaccinator Farmer ☐ Veterinarian ☐ Others ☐

26. Deworming: YES ☐ NO ☐

If YES, please specify - the list of deworming drugs _____

- the interval of deworming _____

27. Are there other routine supportive treatment (Antibiotics, Vitamins etc.)

YES ☐ No ☐

If YES, please describe what they are. _____

28. Have your herd ever been tested on CAE: YES ☐ NO ☐

If YES, when and what was the result? _____

29. Previous cases of lameness: YES ☐ NO ☐

If YES, please specify when. _____

30. Previous cases of mastitis: YES ☐ NO ☐

If YES, please specify when. _____

31. Previous cases of other diseases: YES ☐ NO ☐

If YES, please specify when and what. _____

32. Previous cases of Brucellosis: YES ☐ NO ☐

If YES, please specify when. _____

33. Presence of other animals in the farm: YES ☐ NO ☐

If YES, please specify what they are. _____

34. Presence of cattle in the farm: YES ☐ NO ☐

35. Have you ever tested Brucellosis - in cattle: YES ☐ NO ☐

- in goats: YES ☐ NO ☐

If YES, when and what was the result? _____

36. Vaccination against Brucellosis - in cattle: YES ☐ NO ☐

- in goats: YES ☐ NO ☐

If YES - when it was started _____

- how often and _____

- when it was last performed: _____

37. Failure to conceive: YES ☐ NO ☐

If YES, when and how often. _____

38. Any cases of abortion in the herd: YES ☐ NO ☐

If YES, when and how often. _____

39. Any cases of neonatal mortality: YES ☐ NO ☐

If YES, when and how often. _____

40. Any other reproductive problems: YES ☐ NO ☐

If YES, please enumerate. _____

41. Any other health problems: YES ☐ NO ☐

If YES, please enumerate. _____

42. Comments and suggestions: _____

APPENDIX C

Test preparation and procedure

1. Test preparation

- First, serum samples, reagents and 96 well CAEV antigen-coated plates were all brought to room temperature before analysis.
- After that, both the positive and negative controls were run in duplicate on every plate.
- And then, the CAEV antigen-coated plate was removed from the foil pouch.
- Following that, IX antibody-peroxidase conjugate was prepared by diluting 100X antibody-peroxidase conjugate with 99 parts of conjugate diluting buffer.
- Then, IX wash solution was prepared by diluting one part of the 10X wash solution concentrate with 9 parts of distilled water.
- Finally, undiluted serum samples were tested.

2. Test procedure

- 100 µl of each serum sample as well as positive and negative controls (2 wells each) were placed in the transferred plate containing 96 wells.
- Afterwards, 50 µl of each serum sample and control were transferred to CAEV antigen-coated plate by using multichannel micropipette.
- The plate was covered with tape and then incubated for 1 hour at room temperature (21-25°C).
- After 1 hour of incubation, the plate was struck against a clean paper towel to make sure the remaining sera and controls were removed from the inverted plate. Then the plate was washed with washing solution and struck again onto a clean paper towel. This washing procedure was repeated for three times.

- And then, 50 µl of diluted antibody-peroxidase conjugate was added to each well and the plate was incubated again for 30 min at room temperature (21-25°C).
- After 30 min incubation, the plate was washed for three times using the same washing procedure described above.
- Following that, 50 µl of substrate solution was added to each well and the plate was incubated at room temperature (21-25°C) for another 20 min.
- After incubation, 50 µl of stop solution was added to each well, and the plate was tapped several times to make the well contents properly mix.
- Immediately after the adding of stop solution, the plate was read on microplate reader, set at the optical density (O.D.) reading wavelength of 650 nm.

VITAE

Mr. Thant Nyi Lin, a graduate student from Myanmar, was born in Upper Myanmar on October 17 of 1980. He got his Bachelor degree in Veterinary Science from the University of Veterinary Science, Yezin, Myanmar in the year 2005 December. On the following year, he became an assistant lecturer in that university. He studied one year in Israel from October 2007 to September 2008 on poultry production management. In 2009, he was selected by the Ministry of Livestock Breeding and Veterinary Development to attend a Master degree program in veterinary science in Chulalongkorn University. He studied the nature and effects of an infectious viral disease in goats in the field of theriogenology with an epidemiological point of view in the Department of Obstetrics, Gynaecology and Reproduction, at the Faculty of Veterinary Science, Chulalongkorn University.