

Contents:

- 1 Interrelationships of Traits Measured on Fine-wool Rams During a Central Performance Test
C.J. Lupton, D.F. Waldron, and F.A. Pfeiffer
- 8 Effects of Supplementing Polyethylene Glycol to Goat Kids Grazing *Sericea Lespedeza* and Early Post-weaning Nutritive Plane Upon Subsequent Growth
Roger C. Merkel, Arthur L. Goetsch, and Nissim Silanikove
- 14 Use of DNA Markers to Determine Paternity in a Multiple-Sire Mating Flock
A.M. Laughlin, D.F. Waldron, B.F. Craddock, G.R. Engdahl, R.K. Dusek, J.E. Huston, C.J. Lupton, D.N. Ueckert, T.L. Shay, and N.E. Cockett
- 18 Use of Lamb Vision System to Predict Carcass Value
A.S. Bradey, B.C.N. Cunha, K.E. Belk, S.B. LeValley, N.L. Dalsted, J.D. Tatum, and G.C. Smith
- 25 Potential Associative Effects of Increasing Dietary Forage in Limit-fed Ewes Fed a 6% Fat Diet
O. Kucak, B.W. Hess, P.A. Ludden, and D.C. Rule
- 34 Quebracho Tannin Influence on Nitrogen Balance in Small Ruminants and In-Vitro Parameters when Utilizing Alfalfa Forage
K.E. Turner and J.P.S. Neel
- 44 An Investigation into the Risk Factors Associated with Clinical Mastitis in Colorado Sheep
K.N. Forde, B.J. McCluskey, and K.S. Morgan
- 47 Weight Changes in Fall and Spring Lambing Ewes Grazing Fallow Wheat Fields During the Summer
W.A. Phillips, F.T. McCollum, J. Volesky, and H.S. Mayeux
- 55 Effect of Ethanol Supplementation on In Vitro Digestion and VFA Production and Growth Performance of Newly Weaned Lambs
J. Gould, E.J. Scholljegerdes, P.A. Ludden, D.C. Rule, and B.W. Hess
- 61 Growth and Reproductive Performance of Ewe Lambs Implanted with Zeranol after Weaning, but before Sexual Maturation
B.M. Alexander, B.W. Hess, R.V. Lewis, R.H. Stobart, and G.E. Moss
- 65 Consumer Evaluation of Pre-Cooked Lamb
John A. Fox, Londa S. Vander Wal, Prayong Udomvarapant, Donald H. Kropf, Elizabeth A.E. Boyle, and Curtis L. Kastner
- 69 Caprine Arthritis-Encephalitis: An Update
A. de la Concha-Bermejillo
- 79 An Evaluation of Different Energy Supplements for Lambs Consuming Endophyte-free Tall Fescue
B.W. Hess, J.E. Williams, and E.J. Scholljegerdes
- 83 Effects of the *FecB* Gene in Half-sib Families of Rambouillet-cross Ewes
K.S. Schulze, D.F. Waldron, T.D. Willingham, D.R. Shelby, G.R. Engdahl, E. Gootwine, S. Yoshefi, G.W. Montgomery, M.L. Tate, and E.A. Lord
- 89 The Effects of Energy Source and Ionophore Supplementation on Lamb Growth, Carcass Characteristics and Tenderness
M A Murphy, H N Zerby, and F L Fluharty
- 96 Effects of Supplementing Ewes with a d- α -Tocopherol on Serum and Colostrum Immunoglobulin G Titers and Preweaning Lamb Performance
C.L. Schultz, T.T. Ross, and M.W. Salisbury
- 101 Comparing Indicators of Sheep Grazing Leafy Spurge and Perennial Grasses
Bret E. Olson, and Roseann T. Wallander
- 109 *Research Note:* Monesin Poisoning in a Sheep Flock
O. Mendes, F. Mohamed, T. Gull, and A. de la Concha-Bermejillo
- 114 *Case Report:* Repeated Injections of Pregnant Mare Serum Gonadotrophin (PMSG) Failed to Induce Antibody Production in Fall Lambing Ewes
M.A. Diekman, M.K. Neary, and G.R. Kelly

Caprine Arthritis-Encephalitis: An Update

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Abstract

Caprine arthritis encephalitis (CAE) is a disease most commonly recognized in adult goats and manifested clinically as chronic degenerative polyarthritis, interstitial mastitis, and/or pneumonia. Occasionally, CAE may be characterized by acute leukoencephalomyelitis, particularly in 2 to 6 month-old kids. The disease is caused by caprine arthritis-encephalitis virus (CAEV), a lentivirus closely related to other human and animal lentiviruses, particularly ovine lentivirus (OvLV).

In the majority of industrialized countries, CAE is considered one of the most devastating diseases of dairy goats. In the United States of America, the prevalence of serum antibodies against CAEV in dairy goat herds ranges from 38 to 81%. The negative impact of this infection is associated to direct production losses as a result of clinical disease and related secondary infections, to animal deaths, and indirectly due to trading restrictions that some countries impose on infected herds.

The main route of transmission is through milk and colostrum from infected nannies to their offspring. Currently, there are no commercial treatments or vaccines available to control or prevent CAE. As a result, identification of CAEV-infected goats by serological means and elimination of positive reactors is a practice often used to achieve CAE-free status in herds. The separation of offspring from their mothers before ingestion of colostrum and their feeding with heat-inactivated colostrum and milk replacers is used for control purposes as well. However, this method is labor intensive and not always effective.

As a result of recent advances in genetic engineering and molecular virology, new approaches for the control of infectious diseases of livestock are being developed. These new technologies are likely to provide the means for more effective ways to control and eradicate CAE and other infectious diseases of livestock.

Introduction

Infection by caprine arthritis-encephalitis virus (CAEV) is a world-wide cause of slowly progressive inflammatory disease of joints, mammary gland, lung and less commonly brain of mature goats. Seldom, paralysis, as a result of leukoencephalomyelitis, is reported in young kids (Cork et al., 1974; Crawford et al., 1980 a; Crawford and Adams, 1981; Rowe and East, 1997). In a large proportion of infected animals, the infection is subclinical (Knowles, Jr., 1997). Serological testing combined with segregation of positive reactors is the most common method to achieve CAEV-free status (Rowe and East, 1997). However, some CAEV-infected goats remain seronegative for weeks or months after infection. These goats may shed CAEV in milk and other body secretions at irregular intervals and may be a source of virus for non-infected goats (Knowles, Jr., 1997). These subclinical, CAEV-infected, seronegative animals represent a major roadblock in CAE control and eradication programs.

Caprine arthritis encephalitis virus shares many genetic and pathogenic characteristics with ovine lentivirus (OvLV) and to a lesser extent with the human immunodeficiency virus (HIV), the cause of the acquired immunodeficiency syndrome

(AIDS) in humans (de la Concha-Bermejillo, 1997; Joag et al., 1996). Due to the magnitude of the Worldwide AIDS epidemic, a tremendous amount of research has been done on HIV in the last 2 decades. Some of this research has provided valuable insights into CAE pathogenesis. In addition, infection of goats with CAEV is used as a model to understand several aspects of HIV (Narayan et al., 1988). As a result, CAE is the single most studied disease of goats in recent years. In spite of this, effective prophylactic and therapeutic methods to control CAE are still lacking. Currently available tools of biotechnology, reproductive biology and knowledge of CAEV molecular virology and pathogenesis give hope that progress in controlling CAE and other lentivirus infections will be imminent (DeMartini et al., 1999). The objective of the present review is to provide an overview of CAE with emphasis on recent discoveries in the epidemiology, diagnosis and control of this disease.

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Classification, genomic organization and replication of caprine arthritis-encephalitis virus

The molecular structure and replication cycle of CAEV have been reviewed (Clements and Zink, 1996; Davis and Clements, 1988). A detailed discussion of these aspects of CAEV is beyond the objectives of this paper. However, some understanding of these concepts is necessary to properly interpret diagnostic tests and establish adequate control measures.

Lentiviruses constitute a genus of the retrovirus family. In addition to CAEV, this genus also includes HIV, simian immunodeficiency virus (SIV), feline immunodeficiency virus (FIV), bovine immunodeficiency virus (BIV), equine infectious anemia virus (EIAV), and ovine lentivirus (OvLV) (Joag et al., 1996). All lentiviruses share genetic, replicative and pathogenic characteristics. In particular, CAEV has very close similarities to OvLV, the cause of maedi-visna (MV) in sheep, also known as ovine progressive pneumonia (OPP) (de la Concha-Bermejillo, 1997; Evermann, 1990). Phylogenetic analyses of various small ruminant lentiviruses (SRLV) show that divergences between different genome regions of these viruses range from 16% to 35% (Zanoni, 1998).

Caprine arthritis encephalitis virus was first isolated in 1980 from a goat with arthritis (Crawford et al., 1980 a). Like all lentiviruses, CAEV particles are 80 to 100 nm in diameter and have an envelope that surrounds a dense conical core. During replication, CAE virions bud out through the cell membrane. During this process the envelope incorporates cell membrane lipids rendering the virions susceptible to inactivation by lipid solvents, such as ether (Haziza et al., 2001; Joag et al., 1996).

Within the core, there are two identical copies of single-stranded genomic RNA and small amounts of viral enzymes (Joag et al., 1996). The CAEV genome contains structural-, enzymatic- and regulatory-encoding genes. The structural genes, arranged from the 5' end to the 3' end of the genome, are *gag* (group associate antigens), and *env* (envelope). The *gag* gene of CAEV is for the most part highly conserved across strains and

encodes information for one major (Capsid [CA] or p28) and two minor (Matrix [MA] and nucleocapsid [NC]) core proteins (Clements and Zink, 1996). The capsid protein is highly immunogenic and antibodies against this protein can be detected by Western blot as early as 4 days after experimental infection (Ding and Xiang, 1997).

The *env* gene encodes the information for the envelope glycoprotein (gp135 or surface [SU] protein) and the transmembrane (TM) protein. SU is the main ligand for viral interaction with the cell receptor (Hullinger et al., 1993). The SU protein induces a strong antibody response by the immune system in naturally-or experimentally-infected goats (Adams et al., 1985; Adams and Gorham, 1986). During replication, *env* mutates more often than other regions of the CAEV genome (Knowles et al., 1991). In particular, sequence diversity among strains has been identified in five major regions of SU (Valas et al., 2000). Antigenic variation within the SU protein confers the biological and serological properties of different isolates. Two discrete regions of CAEV SU designated HV1 and HV2 have been shown to undergo rapid sequence variation in persistently infected goats and play an important role in virus-host interaction (Hotzel et al., 2002). On the other hand, some SU epitopes are highly conserved and monoclonal antibodies targeting these areas, which cross react with OvLV, have been used as research and diagnostic reagents for lentivirus infections of small ruminants (Knowles et al., 1991; Ozyoruk et al., 2001; Zhang et al., 2003).

The *pol* gene of CAEV encodes the information for the viral enzymes reverse transcriptase (RT), protease (PR), endonuclease/integrase (IN) and dUTPase. These enzymes play an important role in viral nucleic acid transcription and protein synthesis during replication (Berger et al., 2001; Clements and Zink, 1996; Payne and Elder, 2001). A genetic feature unique to all lentiviruses is the presence of small regulatory genes located between the *pol* and *env* genes and at the 3' terminus, that encode information for proteins that regulate the rate of viral replication. These genes which are critical for efficient virus replication, are *vif*, *tat* and *rev* (Harmache et al., 1996 a;

Schoborg, 2002; Schoborg and Clements, 1996; Seroude et al., 2002). Like other lentiviruses, the proviral DNA of CAEV is flanked on both sides by long terminal repeats (LTR's) that contain enhancer-promoter elements for the initiation of DNA transcription and play a role in tissue tropisms (Saltarelli et al., 1993; Sherman et al., 1989). The CAEV LTR can be activated by interferon-gamma, a cytokine that is secreted in response to viral infections. Therefore, this cytokine may play a role in the transition from the subclinical to the clinical stage of the infection (Sepp and Tong-Starksen, 1997; Tong-Starksen et al., 1996).

An important characteristic of all lentiviruses is that their replication cycle inside the cell includes the integration of a viral DNA genome intermediate (the provirus) into the host cell's chromosomes. After virus infection, the viral enzyme RT transcribes the genomic viral RNA into a double-stranded DNA copy (pro-viral DNA). Subsequently the viral enzyme IN inserts some copies of the proviral DNA into the host cell DNA. The viral genome thus becomes part of the cellular DNA and is replicated during cell division (Joag et al., 1996). As a result, once infected with CAEV, goats remain infected for life, and CAEV can be isolated from seropositive goats years after the original infection (Cheevers et al., 1988).

Cells of the monocyte/macrophage lineage are the main target for CAEV replication *in vivo* (Clements et al., 1994; Narayan, 1990). *In vitro*, goat synovial membrane (GSM), choroid plexus, microglia, granulosa epithelial, and mammary gland epithelial cells are permissive to CAEV infection (Adeyemo et al., 1996; Daltabuit et al., 1999; Mselli-Lakhali et al., 1999; Mselli-Lakhali et al., 2001). Caprine oviduct epithelial cells (COEC), which are routinely used for *in vitro* goat embryo production to stimulate early embryonic development, support CAEV replication *in vitro*. Although further studies are necessary to determine the exact role of COEC from CAEV-positive goats, their use in *in vitro* fertilization protocols could represent a risk of CAEV transmission to cultured embryos and to surrogate dams (Lamara et al., 2002 b).

Epidemiology and transmission

Goats are the natural host of CAEV infec-

tion. Experimentally, sheep can be infected with CAEV (Banks et al., 1983). Previous epidemiological evidence indicated that cross-species transmission of small ruminant lentiviruses occurred infrequently or did not occur at all in nature (Petrusson et al., 1992). However, recent genetic and phylogenetic analyses of caprine and ovine lentivirus sequences suggest that the two viruses are interspersed in the two species, supporting the theory of potential cross-species transmission (Chebloune et al., 1996; Rolland et al., 2002). Based on available sequence phylogenetic data, at least six different clades can be differentiated among SRLV, with no clear separation of SRLV strains derived from goats (caprine arthritis-encephalitis virus) or sheep (maedi-visna virus). Another study shows that U.S. and French caprine SRLVs form a clade that potentially emerged from a much more diverse group containing all SRLVs infectious for sheep (Valas et al., 1997). The epidemiological evidence showing lack of interspecies transmission of SRLV in previous reports may reflect regional differences in management practices that prevent cross-species transmission rather than lack of cross-species susceptibility. Mouflon-domestic sheep hybrids also are susceptible to experimental infection with isolates of CAEV (Guiguen et al., 2000). Currently, there is no evidence in the scientific literature that CAEV infects humans.

Serological surveys and reports of clinical disease indicate that infection of goats with CAEV is common in many countries (Adams et al., 1984; Contreras et al., 1998; Cutlip et al., 1992; Daltabuit et al., 1999; de la Concha-Bermejillo et al., 1998). In the United States, seroprevalence of CAEV ranges from 38 to 81% in dairy goat herds (Crawford et al., 1980 a; Crawford and Adams, 1981; Cutlip et al., 1992; East et al., 1987). There is practically no information on the magnitude of infection in other goat types. A very limited sero-survey conducted several years ago indicated that CAEV infection is not prevalent in meat or hair goats in west Texas, where the majority of these goat varieties are raised in the US (author's unpublished observation). Nevertheless, because production objectives, management practices and breeds of goats are changing in the US, it is important that goat enterprises take the necessary measures to prevent the introduction of CAEV into their premises.

Transmission of CAEV occurs mainly through ingestion of colostrum and milk by newborns from their infected dams. Up to 78% of kids born to CAEV-infected nannies eventually become infected (Adams et al., 1983). The practice of feeding pooled colostrum or unpasteurized milk increase the risk for transmission (Dawson and Wilesmith, 1985). Poor management and bad hygiene practices during milking are believed to play a role in CAEV transmission through the transfer of infected mammary cells to non-infected goats (Greenwood et al., 1995).

Lentiviruses are quite fragile outside the host. Although close contact transmission between infected and non-infected goats, particularly during lactation and time of parturition, also may occur, this route is not considered efficient (Adams et al., 1983). The shedding of CAEV in the semen of one buck has been reported, but there is no clear evidence that venereal transmission of CAEV plays an important role in the epidemiology of CAE (Travassos et al., 1998). CAEV has not been isolated from fetuses of seropositive dams, nor anti-CAEV antibodies have been demonstrated in precolostrum sera of newborn kids (East et al., 1987). In sheep infected with OvLV, intrauterine transmission has been shown to occur infrequently (Brodie et al., 1994). In one study, CAEV was not isolated from uterine flushing of seropositive donors, nor from fetuses, placentas or colostrum from CAEV-negative goats used as recipients for embryos collected from seropositive goats (Wolfe et al., 1987). However, embryonic goat cells can be infected with CAEV *in vitro* (Lamara et al., 2002 a). Furthermore, a recent publication found the presence of polymerase chain reaction (PCR) CAEV-positive cells in 11 oviductal flushing media samples from 25 CAEV infected donor does (Fieni et al., 2002). Also using PCR, CAEV-infected cells were detected in the uterus and oviducts of CAEV-infected goats, suggesting that vertical transmission from doe to embryo or to the fetus may occur (Fieni, 2003). For practical purposes, the venereal and transplacental routes should be considered as potential sources of CAEV transmission. Consequently, infected bucks and the offspring of infected dams, even if kids are separated from their mothers before ingestion of colostrum, should be segregated from the herd.

Clinical disease and pathology

Like other lentiviruses, CAEV replicates in the presence of anti-viral immune responses and persists for the life of the host (Cheevers et al., 1988). The majority of lentivirus-infected individuals go through an acute phase of virus replication during the first few weeks after primary infection. Then, the host's immune response partially controls the infection, but with time, new virus mutants emerge leading to repeated peaks of viremia followed by immune responses to the new virus variants. This constant fight between the immune system of the animal and the virus causes the development of chronic inflammatory lesions in joints, mammary gland and other tissues (Cheevers et al., 1988; de la Concha-Bermejillo, 1997; Juste et al., 1998; Narayan, 1990).

In most cases, clinical disease develops only after several years after primary CAEV infection (clinical latency). Even so, only less than 25% of infected goats ever develop signs of the disease (Hanson et al., 1996; Narayan and Cork, 1990). Several factors, including differences in virus strain and host genetic predisposition, are likely to influence the outcome of lentivirus-infected small ruminants (de la Concha-Bermejillo et al., 1995; Dolf and Ruff, 1994; Rowe and East, 1997). The role of host genetic factors in the susceptibility to SRLV-induced disease has been previously demonstrated in isogenic twin lambs inoculated with OvLV (de la Concha-Bermejillo et al., 1995). Susceptibility to CAEV-induced arthritis has been associated with differences in the frequency of certain caprine leukocyte antigens (CLA). Goats of the Saanen breed that carry the CLA Be7 specificity are less likely to develop arthritis after CAEV infection than goats missing this specificity (Ruff and Lazary, 1988).

Chronic, proliferative arthritis in mature goats is the predominant clinical disease manifestation of CAE (Fig. 1) (Cheevers et al., 1988). Affected goats, show swelling of the joints particularly the carpi and less frequently the stifle, hock, hip and atlantooccipital joints. The swelling also affects associated tendons, bursa and adjacent periarticular tissues (Adams et al., 1980; Crawford et al., 1980 b). The onset of arthritis is insidious, in which short periods of

swelling are followed by periods of remission. As the disease progresses, swelling of the joints becomes permanent and affected animals show pain, decreased range of articular motion, stiff gate and weight loss. Severely affected goats walk with their front legs flexed, using their carpi to put the weight down ("knee walking") and eventually become recumbent. Affected goats are usually thin to emaciated and have long, coarse, dull hair coats (Crawford and Adams, 1981). Particularly in the early stages of the disease, the volume of synovial fluid is increased. Death of affected animals months or years after primary infection is often the end result of the disease, usually as a consequence of secondary infections (Narayan and Cork, 1990). Microscopically, joint swelling is characterized by villi proliferation and severe infiltration of lymphocytes, plasma cells and macrophages in the synovial lining. Fibrosis and mineralization are common in later stages of the disease (Von Bodungen et al., 1998).

Most CAEV-infected goats affected with arthritis also have various degrees of mammary gland inflammation. In these cases, the mammary gland is symmetrically enlarged and firm on palpation ("hard bag"). Milk may be normal in appearance but scant, and in severe cases there is agalactia that occurs at the time of parturition (Bulgin, 1990). In one study, the production of milk, protein, fat and lactose and the milk somatic cell counts were found to be similar in CAEV-seronegative and -seropositive goats (Nord and Adnoy, 1997). However in this study, the comparison was made in goats one year of age or younger. Clinical signs of CAEV-induced mastitis are not manifested in young goats. In older goats, a significant increase in reproductive failure, reduced kid birth weights, decreased growth rate, lower milk yields and reduced lactation lengths has been associated to CAEV seropositivity (Greenwood, 1995). In a separate study, a positive CAE test result was associated with poor production and predisposition to nonhemolytic staphylococcal infections of the mammary gland (Smith and Cutlip, 1988). Microscopically, there is accumulation of lymphocytes, plasma cells and macrophages in the interstitium of the mammary gland (Fig. 2). This non-suppurative inflammation distinguish mastitis caused by CAEV from bacterial mastitis which is characterized by infiltration of neutrophils mostly in the lumen of mammary

ducts and alveoli. Chronic non-suppurative pneumonia with signs of dyspnea and weight loss occurs in some affected goats. CAEV antigen can be demonstrated by immunohistochemistry in macrophages near areas of inflammation (Storset et al., 1997).

A different clinical presentation of CAE, recognized in kids 2 to 6 months of age, is characterized by afebrile progressive paralysis that follows a rapid clinical course (Cork et al., 1974). The paralysis starts in the rear legs and progresses towards the front of the animal. A few affected kids appear blind or show signs of circling. These kids are alert, and continue to eat if hand-fed, but may develop bloating and urinary retention as a result of persistent recumbency. During the acute stages of the disease, affected kids develop severe pleocytosis with up to 100,000 mononuclear inflammatory cells per cubic millimeter in the cerebrospinal fluid (Narayan and Cork, 1990). Microscopically, pathologic changes in brain of neurologically-affected goats are characterized by chronic, non-suppurative inflammation accompanied by destruction of myelin and proliferation of glial cells (Narayan and Cork, 1990). Neurological involvement in adult goats has been reported, but it is unusual. In these cases, affected goats show slight aberration in gait which progresses over the course of several months to complete paralysis (Norman and Smith, 1983).

Host Immune Response

Although not effective in eliminating CAEV from the infected animal, humoral and cellular immune responses do occur in CAEV-infected goats. The immunologic and virologic profiles of clinically healthy, CAEV-persistently infected goats differs from those of goats showing clinical signs of CAE and typically consist of low virus loads and a type 1 immune response (Beyer et al., 2001). The latter is characterized by low antibody titers to SU and TM CAEV proteins, predominant IgG2 responses to SU and a dominant subset of CD4+ T lymphocytes that secrete interferon-gamma (IFN- γ) (Cheevers et al., 1997).

On the other hand, in goats with clinical CAEV-induced arthritis, virus loads are often high, and the dominant immune response is of type 2, which is characterized by a strong antibody response that targets the SU protein (Johnson et al., 1983). These antibodies, which are of the IgG1

subclass, are inconsistently non-neutralizing *in vivo* (Narayan et al., 1984), but are thought to play a role in the pathogenesis of CAE (Narayan et al., 1984). Previous studies have shown that serum and/or synovial fluid antibody titers against SU and TM are elevated in goats with CAEV-induced chronic arthritis (Bertoni et al., 1994; Knowles, Jr. et al., 1990; McGuire et al., 1992). Antibody titers in these studies had a positive correlation with the severity of carpal arthritis. Epitope mimicry by envelope proteins of CAEV, once thought to be mechanism of joint inflammation, now seems not to be associated to CAEV-induced arthritis (Davies et al., 1997).

Cell mediated immunity in goats with chronic arthritis is characterized by the proliferation of T lymphocytes that secrete interleukin (IL)-4 (Cheevers et al., 1997). Therefore, the difference between CAEV infection that leads to unapparent infection or to clinical disease depends on the type of immune response of the host (type 1 versus type 2), as well as the pathogenicity of individual CAEV isolates (Cheevers et al., 1988). The immune responses against CAEV in turn may be controlled by genetic factors in the host (Dolf and Ruff, 1994; Ruff and Lazary, 1988).

Recent experiments have shown that vaccination of goats with genetically engineered plasmids (naked circular DNA) expressing the CAEV *env* gene induces a stable type 1 immune response (Beyer et al., 2001). Although poorly characterized in goats, other aspects of the host immune response, such as cytokines, chemokines and chemokine receptors, are likely to play an important role on CAEV pathogenesis and progression to disease (Adeyemo et al., 1997; Gangur et al., 2002). The expression of IFN- γ , IL-2, monocyte chemoattractant protein (MCP)-1, IL-16 and tumor necrosis factor (TNF) in joints of CAEV experimentally infected, and the dysregulation of some of them has been documented (Lechner et al., 1997 a; Lechner et al., 1997 b; Sharmila, 2002). A clear understanding of the cellular and molecular aspects of the host immune responses against CAEV is of paramount importance if we hope to develop effective prophylactic measures to control CAE.

Diagnosis

The diagnosis of CAE is first made by the

observation of typical clinical signs. Confirmation of CAE is done by a variety of ways. Infected goats that develop specific serum antibodies for CAEV remain seropositive for life. Therefore, detection of anti-CAEV antibodies in sera is the method most commonly used to identify infected goats (Knowles, Jr., 1997). Time of seroconversion in naturally and experimentally infected kids ranges from 3 to 6 weeks after exposure, but delayed seroconversion has been described (Cheevers et al., 1988; Rimstad et al., 1993). Reports indicate that some infected goats may remain seronegative for 8 months in spite of being virus and PCR positive (Rimstad et al., 1993). Some of these discordant results may be the consequence of differences in tests sensitivities (Knowles, Jr., 1997). In any case, repeated testing of all animals of a herd followed by elimination of positive reactors is necessary to successfully eradicate CAE.

The two most commonly used serological assays for CAE are the agar gel immunodiffusion (AGID) test and the enzyme-linked immunosorbent assay (ELISA). Often, these tests are not standardized across diagnostic laboratories, and they are run under dissimilar conditions and using different formats (Archambault et al., 1988; Knowles et al., 1994; Knowles, 2001; Kwang et al., 1995; Ozyoruk et al., 2001). This represents a major problem because in many cases, the sensitivities and specificities of these tests have not been well established. As a result, unnecessary culling of false positive goats from, or the retention of false negative goats in herds during eradication programs may occur.

The AGID test is relatively simple to run and does not require sophisticated equipment. In general, this test is considered to have low sensitivity (gives false negative results), but the specificity (ability to detect a true positive [infected] animal as positive) is often 100% (Knowles et al., 1994). In this test, wells are made in a layer of solid agar in a Petri dish creating a characteristic pattern that contains a central well and six peripheral wells (Fig. 3). The central well is filled with CAEV antigen and alternative peripheral wells are filled with undiluted serum samples from goats to be tested. The other three peripheral wells are filled with a positive control serum. After incubating the plates at room temperature in a humidified

chamber (tupperware) for 24 to 48 hrs, results are read by looking at the dishes against a light source. During incubation, CAEV antigen and CAEV antibody (the latter present in sera of infected animals) diffuse out of the wells into the agar forming antigen-antibody complexes. At the optimal concentration between antigen and antibody, the immune complexes form a line of precipitation between the antigen well and the test well. This line is called a precipitin line. Two types of precipitin lines can be observed in positive sera. In the first, the precipitin line between the antigen well and the test well bends towards and joins the adjacent precipitin line formed between the antigen well and the positive control well (this is referred to as a line of identity). In the second, a non-identity precipitin line that does not join the positive control line and extends towards the positive control well is formed closer to the test serum well. A common mistake when preparing agar plates for the AGID test consists of lifting the agar layer from the plate while removing the agar plugs to create the wells. When this happens, sera or antigen may leak out underneath the agar resulting in false negative tests (Brodie et al., 1998).

In one study, the CAEV-based AGID test had a 91% sensitivity and 100% specificity when compared to immunoprecipitation, a highly sensitive but laborious test (Knowles et al., 1994). Some laboratories still run the AGID test for CAE using OvLV antigen due to the cross-reactivity between these two viruses. However, the sensitivity of the test is 35% greater when CAEV antigen is used (Knowles et al., 1994). Furthermore, the use of gp135 CAEV antigen provides higher sensitivity to the AGID test than the p28 antigen (Adams and Gorham, 1986). The lower the sensitivity of a test, the higher the risk that more true infected goats remain unnoticed in flocks. These goats pose a hazard of infection to other goats and jeopardize control programs.

The use of ELISAs for the diagnosis of CAE is popular. Many varieties of the test have been developed including direct and indirect assays, assays that use whole CAEV or individual CAEV recombinant proteins as antigen, and competitive ELISAs. Sensitivities and specificities for all these ELISAs vary (Castro et al., 1999; Knowles, Jr., 1997; Ozyoruk et al., 2001; Saman et

al., 1999; Simard et al., 2001). In some of these cases, the comparison of the sensitivity and specificity of a particular CAE test has been made using other CAE tests that are not optimal. In order to determine the specificity and sensitivity of a test using clinical samples, the test needs to be compared against the gold standard (immunoprecipitation) (Knowles, Jr., 1997). Alternatively, a test can be optimized using known serum samples from goats experimental inoculated with CAEV or mock inoculated (Juste et al., 1995).

Probably the best ELISA format is the one called competitive ELISA (cELISA) (Ozyoruk et al., 2001). In this format, a well-characterized anti-CAEV monoclonal antibody (Mab) is mixed with undiluted serum from the animal to be tested, and the mixture is applied to wells of 96-well plates coated with whole CAEV or recombinant CAEV proteins. Thus, the Mab and anti-CAEV antibodies (the latter present in the test serum from infected goats) compete for binding to the same virus epitope. As a result, the specificity of cELISA depends only on the monoclonal antibody used. Because serum samples do not have to be diluted, the sensitivity of the test is greater (Knowles, Jr., 1997).

An advantage of the AGID test is that its cost may be half of some ELISAs. For large herds that are suspected to have a high incidence of CAE and wish to eradicate the disease, the AGID test can be used the first time the herd is tested. All positive reactors resulting from this test round should be segregated from the herd. Then, a more sensitive ELISA could be used for subsequent rounds of testing at 3 to 6 month intervals until all animals give negative results in two consecutive tests. Once this is accomplished, annual retesting and the purchase of replacement animals from CAEV-free flocks are highly recommended (de la Concha-Bermejillo, 1997). ELISA has also been used to test bulk milk for the presence of antibodies to CAEV and predict the prevalence of CAE in goat flocks (Motha and Ralston, 1994).

Virus isolation in cell culture is the most definite way to confirm a CAE diagnosis. The isolation is more commonly done by co-cultivating blood or milk mononuclear cells from the animals to be tested with an

indicator cell line, such as GSM cells. The typical cytopathic effect consists on the formation of giant, multi-nucleated cells (syncytia) (Fig. 4) (Adeyemo et al., 1996). In the majority of cases virus isolation is unsuccessful. In addition, this test is expensive and final confirmation of the results may take several weeks. For these reasons, virus isolation is not recommended as a routine diagnostic test for CAE.

The polymerase chain reaction (PCR) is a technique that has been used to detect genes of a great variety of microorganisms in clinical samples (Mullis et al., 1986; Wagter et al., 1998). This test is based on the amplification (copying) of specific genes or gene sequences using primers (short DNA sequences that are complementary to the ends of the gene sequence to be amplified) and *Taq* polymerase, an enzyme that repairs single stranded DNA by taking nucleotides from the surrounding medium and extends the complementary DNA strand. Standard, nested and reverse transcriptase (RT)-PCR (the latter a variant of the PCR technique that amplifies viral RNA rather than DNA) have been used to detect CAEV in blood and milk of naturally and experimentally infected goats (Barlough et al., 1994; Leroux et al., 1997; Reddy et al., 1993). Although PCR is a highly sensitive technique, its usefulness for the detection of SRLV in clinical samples from naturally infected animals still needs to be corroborated. In one study, the reliability of PCR to detect CAEV in blood and milk samples was much lower than that of antibody detection using ELISA (Zanoni et al., 1996). This study also found positive PCR results in 50% of the samples of seronegative animals, but it is not clear if these cases were true infections or false positives. A major problem with PCR is that as a result of its great sensitivity, occasional cross contamination among different samples is possible, even when very stringent quality control procedures are implemented in the laboratory. This may result in false positives and the consequent elimination of valuable animals from the herd. CAEV-PCR results of clinical samples need to be interpreted with caution at this time.

Prevention and Control

Traditionally, prevention and control methods of infectious diseases of livestock have

relied on one or more of the following: use of chemotherapy, implementation of management changes, vaccination and/or identification and culling of infected animals (Brodie et al., 1998). Identification of CAEV-infected goats by serological means and elimination of positive reactors is the method most often used to achieve CAE-free status in herds (Rowe and East, 1997). The separation of offspring from their mothers before ingestion of colostrum, and their feeding with heat-inactivated colostrum and milk replacers is used also (Hanson et al., 1996). Treating colostrum for 60 minutes at 56° C (133° F) appears to reduce the possibility of CAEV transmission, but CAEV can be isolated sometimes from this colostrum after two passages in cell culture, indicating that small amounts of virus remain viable (Adams et al., 1983). In addition, overheating of colostrum may result in denaturation of immunoglobulins with loss of its protective characteristics and in the induction of osmotic diarrhea (Rowe and East, 1997). Therefore, segregation of kids from their infected dams and feeding them with goat or cow heat-inactivated colostrum and milk replacer or pasteurized milk is labor intensive and not always effective in eradicating CAEV from a herd (Nord et al., 1998; Peretz et al., 1994).

Currently, there are no commercial treatments or vaccines available to control or prevent CAE. Although recombinant ovine interferon-tau (roIFN- τ) has been shown to have potent *in vitro* and *in vivo* antiviral activity against OvLV, its use to treat SRLV infections is deterred by its high cost (Juste et al., 1996; Juste et al., 2000). Some experimental inactivated vaccines do not prevent CAEV infection after CAEV challenge and vaccinated-challenged kids show more severe arthritic lesions than controls (McGuire et al., 1986; Russo et al., 1993). There is some indication that genetically engineered gene-deletion SRLV mutants that are attenuated for pathogenicity could be used as potential vaccine candidates (Harmache et al., 1996 b; Turelli et al., 1996; Turelli et al., 1997; Zhang et al., 2003). In most cases, vaccination with these viral mutants has resulted in the development of a specific immune response against the virus and reduced viral loads after chal-

lenge with pathogenic virus. However, these vaccines do not prevent infection or shedding of the challenge virus (Harmache et al., 1998; Zhang et al., 2003).

The immunization with naked DNA that contains critical genome sequences of the microorganism of interest is a promising approach for the development of effective vaccines. DNA constructs encoding CAEV SU sequences have been developed and shown to promote stable type 1 biased immune responses in vaccinated goats (Beyer et al., 2001). These results suggest that this approach could be an effective way to prevent CAE.

Conclusions

Although diagnostic tests are not 100% effective, the practice of repeated serological testing followed by segregation of CAEV-infected kids from the herd is considered a good CAE control method. Nevertheless, this approach may be expensive and impractical, particularly in large herds. Therapeutic and prophylactic commercial products to treat and prevent CAE are non-existent.

In this review, the manner in which CAEV replicates and interacts with infected cells and the host immune system were outlined in a very simple way. In reality, these processes are complex, and many details are unknown. Only through a deep understanding of the molecular interactions between the host and the parasite, effective solutions to these very complex problems will be provided. The production of genetically engineered vaccines or the genetic engineering of farm animals resistant to viral infections are among the most promising approaches to reduce economic losses due to infectious disease of livestock (DeMartini et al., 1999). Basic research in the biology and pathogenesis of CAEV using modern techniques in biotechnology will provide the means for the effective control and eradication of this important disease of goats.

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Fig. 1. Photograph of a goat showing severe swelling of the carpal (“knee”) joints as a result of infection with caprine arthritis encephalitis virus (CAEV).

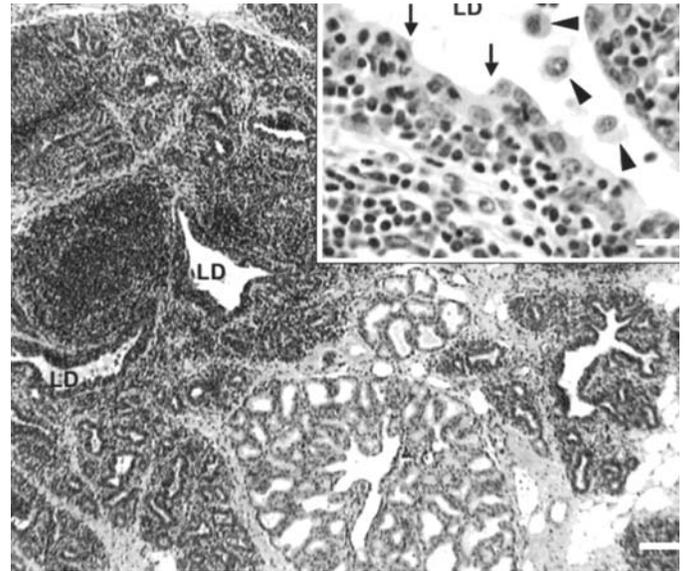


Fig. 2. Microphotograph of mammary tissue from a goat infected with caprine arthritis encephalitis virus (CAEV). Severe mononuclear inflammatory cell infiltration, characteristic of CAEV-induced mastitis, is present in the tissue surrounding lobular ducts (LD) (Bar = 200 μ). Close up view of a LD showing three mammary macrophages in the lumen (arrow heads) and severe lymphocytic infiltration under the ductal epithelium (arrows) (Bar = 30 μ).

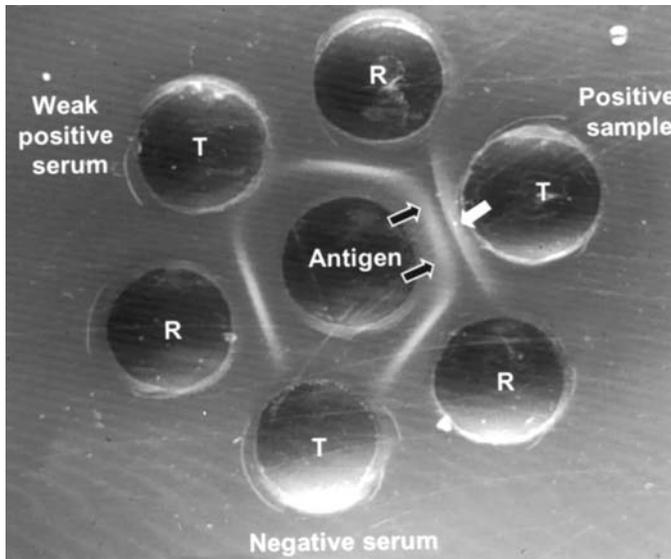


Fig. 3. Agar gel immunodiffusion (AGID) test. Caprine arthritis encephalitis virus (CAEV) antigen is placed in the central well. Test sera (T) and reference positive control sera (R) are placed in alternate peripheral wells. Precipitin reactions that result in a distinct line of identity (black arrows) with the reference sera represent antibodies specific for the CAEV surface (SU) protein. The line of non-identity (white arrow) represent antibody specific for CAEV capsid (CA) antigen.

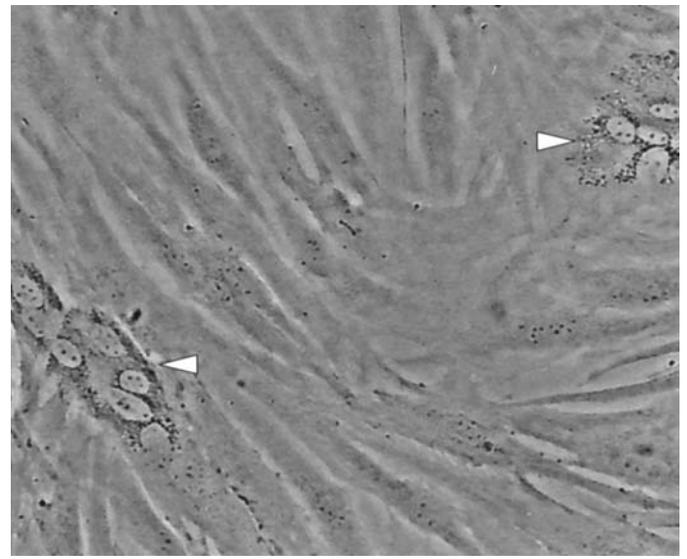


Fig. 4. Microphotograph of a monolayer of goat synovial membrane (GSM) cells infected with caprine arthritis encephalitis virus (CAEV) and showing typical cytopathic effect (CPE) characterized by the formation of multinucleated giant cells (arrow heads).