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Control of Caprine Arthritis-Encephalitis Virus and Corynebacterium Pseudotuberculosis Infection in a Norwegian Goat Herd

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Nord K, Holstad G, Eik LO, Grønstøl H: Control of caprine arthritis-encephalitis virus and Corynebacterium pseudotuberculosis infection in a Norwegian goat herd. Acta vet. scand. 1998, 39, 109-117. – A control programme for caprine arthritis-encephalitis virus (CAEV) and *Corynebacterium pseudotuberculosis* (C. pseudotuberculosis) infection was established in a Norwegian goat herd comprising approximately 100 milking goats. The herd seroprevalences of antibodies against CAEV and C. *pseudotuberculosis* were 97% and 94%, respectively.

Kids were removed from the infected flock at birth, avoiding any contact between dam and kid. The kids were kept completely segregated from the seropositive flock and fed cow's colostrum and milk. A seronegative flock was established, based on the removed kids and their offspring. Goats belonging to the seronegative flock were allowed to kid naturally and to mother their kids. The seropositive flock was slaughtered during the second year of the control programme. After washing and disinfection, housing systems and nearby outdoor premises were left empty for 3 months.

Of 230 goats examined for antibodies against CAEV with ELISA regularly during 3 years of the control program, altogether 6 were found to be seropositive, while for 10 the result was indeterminate. All 16 animals were immediately culled. During the third year of the control programme, all goats were examined and proved negative for antibodies against *C. pseudotuberculosis* by a haemolysis inhibition test. Clinical examination revealed no signs of CAE or caseous lymphadenitis.

control programme; ELISA; haemolysis inhibition test.

Introduction

Caprine arthritis-encephalitis (CAE) and caseous lymphadenitis (CLA) are prevalent and important diseases in intensive dairy goat industries (*Adams et al.* 1984, *Brown & Olander* 1989, *Dawson* 1987). Both infections are common in Norwegian goat herds (*Nord et al.* to be published, *Holstad* 1986a).

The lentivirus disease CAE is characterized by a long incubation period, protracted clinical course and persistent infection (*MacDiarmid* 1984). The most common signs are arthritis, encephalitis, pneumonia, mastitis, and chronic wasting (*Adams et al.* 1983, *Dawson* 1987, *Robinson & Ellis* 1986, *Woodard et al.* 1982, *Zink & Narayan* 1989, *Zwahlen et al.* 1983). The majority of CAEV-infected goats are subclinical carriers. Natural secretions and excretions such as milk, saliva, urine, and faeces may

contaminate feed and drink. In dairy goat enterprises, the infection is easily spread as kids are reared mostly on pooled unpasteurized goat's milk. Transfer of virus also occurs during licking and milking (*Adams et al.* 1983, *East et al.* 1993). Lateral transmission has been registered as a result of close contact, even at pasture (*Adams et al.* 1983, *Rowe et al.* 1992b). Virus may be transferred from dam to caesarean derived kids (*East et al.* 1993), and from male to female during mating (*Rowe et al.* 1992b). CAEV might be transferred with semen via infected leucocytes. Infection by artificial insemination has not yet been demonstrated.

Caseous lymphadenitis, which is caused by *Corynebacterium pseudotuberculosis (C. pseudotuberculosis),* is characterized by suppurative inflammation in one or more lymph nodes (*Brown et al.* 1989). Other organs might also be affected. The clinical signs are abscesses in superficial lymph nodes, most commonly in the head and neck region (*Ashfaq & Campbell* 1979, *Holstad* 1986a). The bacterium is shed from ruptured abscesses, being transmitted to other goats directly or through contaminated materials. Control of caseous lymphadenitis has been a major concern to the goat industry, and several vaccination trials against the infection have been carried out (*Anderson & Nairn* 1984, *Holstad et al.* 1989, *Holstad* 1989). The protection achieved by vaccination has not been complete. Environmental sanitation measures based on washing and disinfection of animal housing may eliminate the organism from the fomites. However, the environment soon becomes recontaminated when infected animals are introduced.

Control programmes for CAEV have been implemented in Switzerland, France, U.S.A., Australia and New Zealand, and are reported to successfully reduce the number of infected animals (*Adams et al.* 1983, *Ellis* 1988, *Ellis et al.* 1983, *MacDiarmid* 1984, *MacKenzie et al.* 1987, *Rowe et al.* 1992a, *Rowe et al.* 1992b). A successful eradication program for *C. pseudotuberculosis* in goat and sheep herds have been described from the Netherlands (*Dercksen et al.* 1996, *Schreuder et al.* 1994). Strict segregation and isolation strategies are employed to eliminate both CAEV infection and caseous lymphadenitis. The object of the present study was to establish a programme to control both these diseases simultaneously, which has not been described previously.

Materials and methods

Animals and herd management

The selected goat herd belonging to the Agricultural University of Norway (herd A) comprised 113 goats, including about 100 milking animals, of the goat breed Norwegian. Parturition occured from December to June, the main kidding seasons being January/February and April/May. Kids had been fed pooled, unpasteurized goat milk over the last 2 or 3 decades.

In order to obtain a sufficient number of kids to establish a herd free from CAEV and *C. pseudotuberculosis* infection in the course of only 2 kidding seasons, 50 additional pregnant females from 2 other herds, B and C, were temporarily transferred to herd A in the second year of the control programme.

Before starting the control programme, all goats were blood-sampled once or twice for examination for antibodies against CAEV and *C. pseudotuberculosis,* and for isolation of virus.

All goats in the original infected flock (herd A) were slaughtered during spring and early summer in the second year of the control programme.

Washing and disinfection

After slaughter of the infected flock, the housing systems were washed, using high pressure, disinfected with 2% caustic soda and slaked

lime, and left empty for about 3 months. Milking equipment was washed and disinfected using routine procedures (0.15% sulphonamidic acid and 4.1% sodium chloride). In addition to removal of the top 10 cm of soil in the paddock used during winter and spring, slaked lime was spread evenly on nearby outdoor premises 3 times at weekly intervals.

Rearing procedures and herd management in the control programme

The control programme was established prior to the kidding season of 1993, and continued during 1994 and 1995. Kids were removed from goats in the infected flock at birth, avoiding any contact, including sucking. Separate staff using separate clothing looked after the seronegative animals. After removal from their dams, the kids were kept in separate pens in another building until blood samples taken before 14 days of age were analysed for antibodies against CAEV. On testing negatively, the kids were mixed, 2 to a pen. About 58 of the 121 kids entering the control programme in the second year were delivered by caesarian section. These kids were immediately mixed, 2 or 3 to a pen, without prior serological testing. All kids were raised on cow's colostrum and cow's sour milk or sour milk replacers.

At the age of about 2 or 3 months, the kids were mixed into groups of 10, and later on of 20 or 30, on different isolated pastures. All pastures were more than 10 metres apart, secured with double fences, and had not been grazed by goats during the previous year. Feeding and drinking places were raised above the ground to minimize faecal contamination. The goats born in the first year were kept together as 1 flock during the first half of the second year (1994), isolated from the others. Their kids were born naturally, and were kept with and allowed to suck their dams until weaning, as were the kids of all the goats in the seronegative flock. In June of the second year (1994), all the CAEV-antibody negative animals were grazed together on mountain pastures.

After washing and disinfection of housing systems and milking equipment, the CAEV – antibody negative flock was moved into the goat sheds during the autumn of the second year.

CAEV-antibody negative females were mated naturally by CAEV-antibody negative males, or by artificial insemination. Semen used for artificial insemination originated mostly from untested males.

All kids born naturally during the first 2 years of the control programme were tested for antibodies against CAEV before their 14th day of life. Subsequently, all animals were examined serologically for CAEV every sixth month. All goats which tested positive for antibodies against CAEV were immediately slaughtered. Goats which had been in close contact in small pens with test-positive animals were also slaughtered. Animals for which test results were indeterminate were mostly culled, although some were kept in isolation for about 3 months, and retested. On retesting negatively, some were allowed back into the negative herd. Serological testing for antibodies against *C. pseudotuberculosis* was performed 3 times at intervals of 7 (1st to 2nd test) and 2 months (2nd to 3rd test) during the third year of the control programme.

Clinical examination

All goats above 6 months of age were examined clinically for CAE, including general inspection of condition and gait, inspection and palpation of joints and udder, and auscultation of the chest.

Examination for caseous lymphadenitis was carried out by inspection and palpation for subcutaneous abscesses or enlargements of lymph nodes. Swellings localized to the shoulder and

chest were excluded, as lesions at these sites were considered to be granulomas arising after vaccination against *Mycobacterium paratuberculosis* (*Holstad* 1986b).

Serological examinations and virus isolation
Sampled sera were stored at –20 °C. The sera were examined by an ELISA for antibodies against CAEV as described by *Rimstad et al.* (1994), and for antibodies against *C. pseudotuberculosis* by a haemolysis inhibition test (HIT) as described by *Holstad* (1986c).
Virus isolations were made in goat synovial membrane cells cocultivated with peripheral mononuclear blood leucocytes as described by *Narayan et al.* (1980).

Results

Prior to the control programme
Clinical findings. During 1992, 11 goats showed severe clinical signs of CAE. On closer examination, a further 35 animals had moderate clinical signs which might have been due to CAEV (Table 1). Clinical findings are described in more detail elsewhere (*Nord,* to be published).
Subcutaneous abscesses and/or enlargement of lymph nodes indicating caseous lymphadenitis were found in 37 of the 113 goats examined (Table I).

Serological findings and virus isolation. Altogether 110 goats had antibodies against CAEV in Herd A (Table I). Three goats, all below 18 months of age, were seronegative. The latter were slaughtered or died without being retested. Virus was isolated from 83 goats.
The prevalence of antibodies against CAEV in Herds B and C was 64% and 46%, respectively (Table 1).
In Herd A, 94% of animals had antibodies against *C. pseudotuberculosis,* the corresponding figures for Herds B and C being 88% and 79%, respectively (Table 1).

During the control programme
Clinical findings. No signs of CAE were seen in any of the goats belonging to the flock being established according to the control programme (Table 2). With regard to caseous lymphadenitis, one goat had an enlarged submandibular lymph node. Bacteriological examination of this node was, however, not carried out.

Serological findings. Of 94 kids tested for CAEV-antibodies before 14 days of age, 2 were found to be positive (Table 2). One was born by a positive goat and removed at birth. The other was mothered, as its dam belonged to the negative flock and was found to be ELISA negative in late gestation. Retesting of this dam 2 months after parturition, at the age of 24 months, revealed that she had seroconverted.
Of 229 six-month-olds, 1 positive goat and 2 with indeterminate results (Table 2) had all been born naturally by dams found to be seronegative, in late gestation at latest, and had been nursed and suckled. The dam of one of the kids with an indeterminate result had tested indeterminately twice at an interval of 6 months. This kid was slaughtered. The other was the offspring of an 18-month-old seronegative female. Mother and kid were isolated and retested about 3 months later, both then negative.
At 12 months of age, 2 out of 122 goats had indeterminate test results (Table 2). One of these was immediately slaughtered. The other was isolated. On testing negatively 3 months later, it was allowed to re-enter the seronegative flock.
Two out of 120 18-month-olds were seropositive (Table 2). Seven had indeterminate test results, including the female which also tested indeterminately at the age of 12 months. All seroreactors were offspring of females in the

Table 1. Goats, 1-9-year-old, from 3 herds examined clinically and serologically for caprine arthritis-encephalitis virus (CAEV) and Corynebacterium pseudotuberculosis (C. p) before the introduction of a control programme.

	No examined	No. positive			
		CAEV		C. p	
		Clinically[1]	Serologically (ELISA)	Clinically[2]	Serologically (haemolysis inhibition test)
Herd A	113	46	110	37	106
Herd B	42	n.p.	27	n.p.	37
Herd C	28	n.p.	13	n.p.	22
Total	183		150		165

n.p. = not performed.
[1] Goats with one or more clinical signs of CAEV, including enlargements of joints.
[2] Subcutaneous abscesses or enlargements of lymph nodes. Granulomas in the area around the vaccination site not included.

Table 2. Goats examined clinically (clin.) and serologically (sero) for caprine arthritis-encephalitis virus (CAEV) and Corynebacterium pseudotuberculosis (C. p) after the introduction of a herd control programme.

Goats born	Age	No. of goats					
		Examined	CAEV			C. p	
			Clin pos.[1]	Seropos.	Indeterm.	Clin. pos.	Seropos.
First year 1993	< 14 days	31	0	1	0	n.p.[2]	n.p.
	6 months	30	0	0	0	0	n.p.
	12 months	26	0	0	1	0	n.p.
	18 months	25	0	0	1	0	n.p.
	24 months	24	0	1	0	0	0
	30 months	20	0	0	0	0	0
Second year 1994	< 14 days	63	0	1	0	n.p.	n.p.
	6 months	121	0	1	0	0	n.p.
	12 months	96	0	0	1	0	0
	18 months	95	0	2	5	0	0
Third year 1995	< 14 days	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.
	6 months	78	0	0	2	0	0

[1] pos. = positive.
[2] n.p. = not performed.

original positive flock. Of the females with indeterminate results, 2 were slaughtered immediately, including the one which tested indeterminately twice, while one was isolated, retested about 3 months later with negative result and allowed back into the flock. This goat has tested negative ever since, up to 30 months of age. The remaining 4 dams along with their 3 kids were culled in spite of negative results on retesting about 3 months later.

C. peudotuberculosis – antibodies. All goats examined were found to be negative with regard to antibodies against *C. pseudotuberculosis* (Table 2).

Discussion

No signs of caseous lymphadenitis were recorded during the investigation period. Clinical and serological findings indicated that this infection had been eradicated. One goat had an enlarged lymph node, but was, nevertheless, found to be seronegative when tested on 2 separate occasions.

C. pseudotuberculosis shed from abscesses can survive on barnyard fomites for several weeks (*Augustine & Renshaw* 1986), and any contact with infected surroundings would therefore be associated with a risk of infection. The disinfection procedures used, including the application of 2% caustic soda and slaked lime, were carried out mainly to clear the premises of *C. pseudotuberculosis*. This procedure appeared to be satisfactory in getting rid of environmental microbes.

Intrauterine transmission of *C. pseudotuberculosis* has been described following experimental infection in sheep (*Addo* 1979), but has not been reported in goats. In the present study, most of the dams in the original herds were infected. Even though horizontal transmission of the bacterium should not be entirely excluded, such an infection pathway seems uncommon in goats.

Caseous lymphadenitis has a long incubation period, and the development of abscesses in kids infected from their dams or surroundings immediately after birth, may not occur until several months later (*Holstad* 1986d). In the present study, all contact betweeen dams and offspring after birth including sucking and licking, was avoided, as was contact between the kids and the stall environment. As both caseous lymphadenitis and CAEV may be transmitted on pastures (*Adams et al.* 1983, *Holstad* 1986a, *Rowe et al.* 1992b), contact between clean and infected animals was avoided during the grazing season.

If strict hygienic procedures are followed as in the present study, transmission of *C. pseudotuberculosis* to kids can be prevented. Such a programme might eradicate caseous lymphadenitis completely from highly infected herds in the course of a couple of years.

The CAEV does not survive for any amount of time outside the host, but may survive for some time protected in cells, i.e. in faeces and milk (*Fenner et al.* 1987, *Narayan et al.* 1982). In the present study, thorough mechanical cleaning of the premises would probably have been sufficient to get rid of the virus.

Antibodies against CAEV in newborn kids are most likely derived from maternal colostrum, as kids are seronegative when born, and their own antibody production is negligible before 1 to 3 months of age (*Bulgin* 1990). Testing for antibodies against CAEV in newborn kids of dams in the seropositive flock was performed to ensure that the kids had been succesfully removed at birth without being infected. Only 1 kid was antibody positive, indicating that it had sucked and simultaneously been infected with virus. Some other kids might not have been removed before being infected through licking. This kind of virus transmission would be impossible to detect before the 14th day of life, but might give positive results later on. The kids were kept in small groups for at least the first 6 months of their lives, restricting the possibilities of further transmission.

The immediate removal of kids by caesarian section was easier to supervise, and early serological testing of these animals was considered unnecessary. However, during the study, 6 of 58 caesarian derived animals tested positively or indeterminately for antibodies against CAEV

compared to 9 of 172 naturally born animals. Even though the number of seroreactors was low in both groups, these results may indicate that a risk exists of transmission of CAEV through blood and genital secretions with both forms of delivery. Although not yet demonstrated, intrauterine infection should be considered a possibility (*Adams et al.* 1983, East et al. 1993).

When mothering was allowed, offspring or dams of animals with positive or indeterminate CAEV-antibody test results were likely to be infected, and these were therefore culled. The females which had been born during the study were found to be negative for antibodies against CAEV during late gestation. Some of their newborn kids, which were suckled, were positive for such antibodies. Serolevels of antibodies against CAEV have been reported to diminish near the time of parturition, while CAEV-antibody levels in milk increase. Passage of antibodies to the colostrum is one possible explanation of the depletion of serum antibodies (*Ellis* 1985, *Smith & Cutlip* 1988). The dams of the seropositive kids were seropositive on resampling about 1 to 2 months after parturition, indicating that serotesting of pregnant females for CAEV should not be performed near the time of parturition. On the other hand, seroconversion due to the stress of late pregnancy and parturition should not be ruled out. Testing kids before their 14th day of life allows carriers of the CAEV, dams as well as their kids, to be identified and culled at an early stage.

Animals which remain seronegative for CAEV in spite of being virus positive will definitely confound attempts to control CAEV infection, and may represent a major problem (*Adams et al.* 1983, *East et al.* 1993, *Ellis et al.* 1983, *McGuire et al.* 1990, *Rimstad et al.* 1993). On the other hand, the results obtained on the repeat testing of some of the goats using other methods such as Western blotting, Agar immunodiffusion test and virus isolation (data not shown), indicated that false positive results may also occur in the ELISA employed. An attempt to isolate virus from an ELISA-positive 18-month-old goat was unsuccessful. Such findings further complicate the evaluation of the ELISA results.

The present study showed that a programme involving strict segregation and isolation of animals might easily be adapted to control both CAEV and *C. pseudotuberculosis* infection simultaneously, although the complete eradication of caseous lymphadenitis seems achievable in less time than is the case for CAEV infection. Even with as strict a control scheme as described, a large reduction in the prevalence of virus-positive animals is probably the most realistic aim for the first 2 or 3 years. To be declared free of CAEV infection, a previously infected herd should probably test negatively on at least 3 or 4 occasions at intervals of 6 to 12 months (*Ellis* 1988, *Fenner et al.* 1987, *Halgaard* 1988, *McGuire et al.* 1990, *Regli et al.* 1985, *Rimstad et al.* 1993). Although further testing is needed, the strict segregation and isolation programme implemented seems effective in preventing both infections.

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Sammendrag

Bekjempelse av caprint artritt-encefalitt virus og Corynebacterium pseudotuberculosis infeksjon i en norsk geitebesetning.

Et kontrollprogram for caprint artritt-encefalitt virus (CAEV) og *Corynebacterium pseudotuberculosis (C. pseudotuberculsois)* infeksjon ble etablert i en norsk geitebesetning med ca. 100 melkegeiter. Forekomsten av antistoffer mot CAEV og *C. pseudotuberculosis* i besetningen var 97% og 94%. Kjeene ble fjernet fra den infiserte flokken umiddelbart etter fødsel. All kontakt mellom mor og avkom ble forhindret. Kjeene ble holdt helt atskilt fra den infiserte flokken og alet opp på kumelk. En seronegativ flokk ble etablert, basert på disse kjeene og deres avkom. Geiter som tilhørte den seronegative flokken fikk kjee naturlig og amme kjeene sine. Den seropositive flokken ble slaktet i løpet av det andre året av kontrollprogrammet. Etter vasking og desinfeksjon, ble husdyrrom og nærliggende utearealer holdt fri for dyr i tre måneder.

Av 230 geiter som ble undersøkt for antistoffer mot CAEV med ELISA i løpet av 3 års kontrollprogram, ble 6 funnet seropositive, og 10 hadde ubestemmelig resultat. Alle 16 dyr ble tatt ut av flokken umiddelbart. Det tredje året ble alle geitene i kontrollprogrammet undersøkt og funnet negative for antistoffer mot *C. pseudotuberculosis* i synergistisk antihemolysin test. Det ble ikke funnet tegn til CAE eller byllesjuke ved klinisk undersøkelse.

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