Acta vet scand 1996, *37*, 31-39

A Long Term Study of Goats Naturally Infected with Caprine Arthritis-Encephalitis Virus

By *J. Hanson, E. Hydbring* and *K. Olsson*

Department of Animal Physiology, The Swedish University of Agricultural Sciences, Uppsala, Sweden

Hanson, J., E. Hydbring and K. Olsson: A long term study of goats naturally infected with caprine arthritis-encephalitis virus. Acta vet. scand. 1996, 37, 31-39. – The caprine arthritis-encephalitis virus (CAEV) is a big problem in dairy goat industry. Little is known about its characteristics in naturally infected goat herds. The aims of this study were. 1) to study how antibody expression, measured by agar gel immunodiffusion test (AGIDT), varied over time in naturally infected, seropositive goats, 2) to observe clinical signs in seropositive adult goats and 3) to follow seroconversion and gamma globulin concentration in goat kids artificially reared on cow milk replacement product only, compared to kids reared on untreated goat milk.

The antibody expression pattern to the viral proteins gp135 and p28 varied in the individual goat and intermittent negative reactions were seen in 19 adult animals followed for 30-91 weeks. Four seropositive goats developed clinical symptoms with difficulties to move However, no correlation between clinical signs and antibody expression pattern was seen During the first 27 weeks of age no kid in the milk replacement reared group (N=4) seroconverted, but 5 of the 7 kids fed goat milk occasionally showed a positive antibody reaction. The gamma globulin concentration was significantly higher in the goat milk fed group until the kids had become more than 19 weeks old

The results show that a great variation of the antibody pattern in individual goats occur, and therefore the AGIDT is only reliable as a herd screening test. Frequent sampling is necessary to get reliable information about spreading of the CAEV in a naturally infected goat herd. Removing kids from their dams immediately after birth combined with segregation and artificial rearing protected them from CAEV infection. However their gamma globulin concentration was initially low.

retrovirus; gamma globulin; seroconversion; lentivirus; artificial rearing.

Introduction

Caprine arthritis-encephalitis virus (CAEV) belongs to the genus lentivirus *(Stowring* 1979) and affects goats specifically. The virus is widespread and of great concern for the dairy goat industry. In accordance with other subspecies of this genus the animal can carry the virus for several years without showing symptoms *(Fenner et al.* 1993). The maedi-visna virus infects sheep and is closely related to CAEV *(Dahlberg et al.* 1981, *Gogelowsky et al.* 1985). Recently an eradication program was started among Swedish sheep herds *(Anon.* 1993).

Diagnosis of virus-carrying animals in Sweden is made indirectly by measuring antibodies to CAEV in blood serum using agar gel immunodiffusion test (AGIDT). Antibodies to the virus are found in animals of most goat herds *(Cornell* 1991), and several months can pass between infection and occurrence of detectable antibodies to CAEV. However, the variation of

antibody expression in naturally infected goats has not been studied.

The predominant spread of the CAEV is believed to be by milk from infected does to their offspring *(Adams et al.* 1983), but contact transmission and intrauterine infection have been reported *(East et al.* 1993). In order to diminish exposure of kids to the CAEV, it has been recommended to remove them from their dams immediately after birth and feed them heat-treated colostrum and milk or cow milk replacement products *(Adams et al.* 1983, *MacKenzie et al.* 1987). However, heating milk is a tedious procedure. Feeding kids cow milk replacement products, on the other hand, has the disadvantage that they are not given any immunoglobulins. Little is reported about the effects of colostral deprivation in goat kids.

The first aim of this study was to see how the CAEV antibody expression varied over time in naturally infected goats. Blood samples were repeatedly taken in 19 adult goats and serum was analysed by AGIDT. The second aim was to study, if there was any correlation between clinical signs and seroconversion. Thirdly, the gamma globulin concentration was measured in 2 groups of kids: one which was given cow milk replacement only and another group which was fed untreated goat colostrum and pooled goat milk. In both groups, CAEV-analysis was done repeatedly and morbidity recorded.

Materials and methods

Animals and housing

All goats belonged to the Swedish domestic breed. The goats were divided into 4 groups:

Group 1: Adult female goats (2-8 years) were followed for 30 weeks (N = 13), 49 weeks (N = 10), and 91 weeks (N = 3). The latter 3 goats were pregnant from week 39 and gave labour in week 59 to some of the kids belonging to groups 3 and 4. The goats were kept in pens indoors and on pasture during the summer (weeks 17-27). During the second summer, only the non lactating goats (No 9, 11 and 12) were on pasture (weeks 64-77), while the other goats were kept in pens outdoors. Times for blood sampling in individual goats are shown in Fig. 1.

Group 2: Six female goat kids were purchased from a goat dairy farm. They were 4 weeks old at the beginning of the study. Four of these (No 2-4 and 6) were pregnant from week 41-43 and gave labour in weeks 63-64. All goats were followed until they were 95 weeks old. These goats were kept in pens indoors and on pasture during the summer (weeks 21-31). During the second summer, only the non lactating goats (No 14 and 18) were on pasture (weeks 67-81), while the others were kept in outdoor pens. Times for blood sampling in individual goats are shown in Fig. 1. In this group, week number is the same as the age of the goats.

Group 3: Seven male offspring from the goats belonging to groups 1 and 2. The males were allowed to stay with their mothers and suck colostrum for one day. They were then taken away to a pen where they were reared together and fed unpasteurized, pooled milk from all lactating goats, and hay, concentrates, and minerals. These kids were held in the same room as the adult goats. During the summer they were let out in a pen outdoors, but fed as before except for the milk. Blood samples were taken 8, 12, 19, and 27 weeks after birth. One kid had to be euthanised due to an accident after 12 weeks.

Group 4: Four female offspring from the goats in groups 1 and 2. These kids were immediately removed from their dams at birth. They were then reared together, but isolated from the other goats and fed a cow milk replacement product (Kalv 1, Lantmännen, Uppsala, Sweden), hay, concentrates, and minerals. During

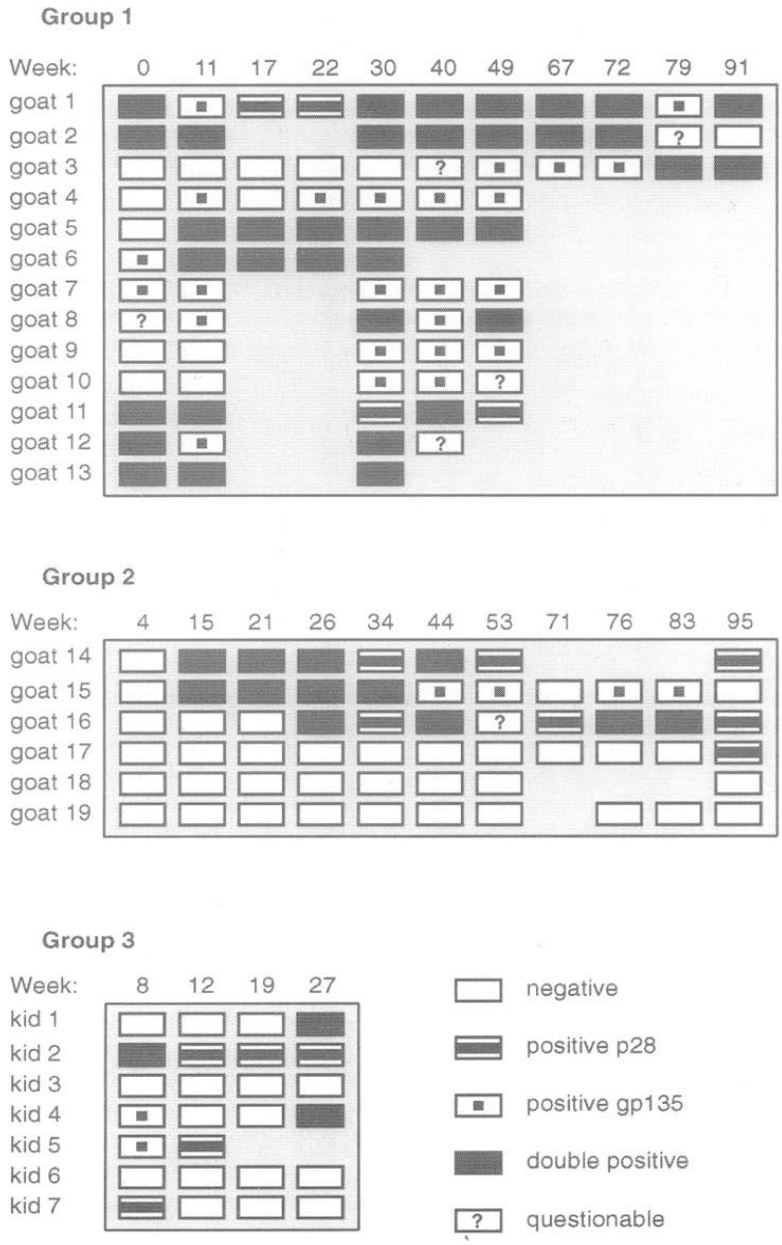

Figure 1. Serological reactions in individual goats measured by Agar Gel Immunodiffusion test. Group 1: Thirteen adult female goats age 2-8 years when blood sampling started (week = 0). Group 2: Six female kids 4 weeks of age at the onset of blood sampling (week = 4). Group 3. Seven male kids at the age of 8 weeks at the onset of blood sampling. Note that several goats changed reaction pattern during the course of the study.

the summer they were let out in a pen outdoors, separated from those of the other goats, but fed as before except for the milk. Blood samples were taken on the same occasions as in the male kids.

The selection of female kids for artificial rearing was due to the ultimate goal of this investigation, which was to clear our herd from the CAEV.

Blood samples

Jugular blood samples were collected by venipuncture from the adult goats. Before blood sampling in the kids, lidocain was injected subcutaneously (Xylocain®, Astra, Södertälje, Sweden) and a cannula (Secalon, Viggo Products, Helsingborg, Sweden) was inserted into one of the jugular veins. Ten ml of blood was then collected into glass tubes and left at room temperature (21°C) to clot. Serum was separated by centrifugation (1500 g; 10 min) and stored at –20°C until analysed.

Serology

Serum antibodies to CAEV were detected by AGIDT using a commercial kit (Central Veterinary Laboratory, MAFF, Addlestone, United Kingdom). The kit consists of ovine strain WLC-1, which contains 2 of the main reactive antigens, surface glycoprotein 135 (gp135) and core protein 28 (p28) to which infected goats can produce detectable precipitating antibodies. The goat serum was tested in triplicate, using reference sera against both gp135 and p28. The reaction was divided into 4 groups: double positive (positive reaction to both gp135 and p28), positive to gp135, positive to p28 and negative. If the results were unclear, the test was repeated, but with only one type of antisera (p28 or gp135) in the wells, respectively. If the reaction was still doubtful, the result was referred to as "questionable".

Gamma globulin concentration

The composition of serum proteins was determined by gel electrophoresis. The samples were run on agarose gels (Paragon Serum Proteins Electrophoresis (SPE) Kit, Beckman, Brea, 92621-6209, USA) for 25 min, pH 8.6, 10 volts/cm. Proteins were visualised using Paragon Blue Stain and quantified with laser densitometry (Ultroscan XL laser densitometer, Pharmacia, Uppsala, Sweden).

Clinical observations

The carpal circumference was measured with a measuring-tape in connection to the blood sampling. Swellings of the atlanto-occipital bursa was estimated (Yes/No). The general behaviour and movements of each goat were noted.

Statistics

The globulin values are presented as means ± SE. The calculations were done using the Statistical Analysis System *(SAS Institute Inc.* 1987). For testing significances within treatments in the same animal paired t-test was used. Student's t-test was used to analyse if there was any correlation between different groups of animals in gamma globulin concentration, carpal joint circumference, and seroreaction.

Results

Serology

Group 1: At the onset of the study, 7 goats were seropositive, 5 were negative and one had questionable results (Fig. 1). During the observation period all goats became positive, but the pattern of antibody expression changed over time in all but 2 goats (Fig. 1). Goats No 6, 12 and 13 were killed; goat No 13 because it had difficulties in moving. It is noteworthy that 2 goats, No 2 and No 4, were negative after periods of positive reaction.

Group 2: At 4 weeks of age all 6 goats in this

group were negative (Fig. 1). Three of them had seroconverted at an age of 26 weeks, one at the age of 95 weeks, while 2 of them remained negative (Fig. 1). Some interchange between different positive reactions was seen. One goat was negative on 2 occasions more than one year after seroconversion.

Group 3: Two of the 7 milk fed male kids were negative during the whole study, whereas the other 5 showed great variation in antibody expression (Fig. 1). Eight weeks after birth the antibody expression pattern in kids No 2, 4, 5, and 7 corresponded to that of their does (goats No 1, 3 (twins), and 16, respectively) during pregnancy or after parturition. At 12 weeks 2 of these kids were negative, and at 19 weeks after birth only one of the kids was positive. At 27 weeks 3 kids were positive.

Group 4: All 4 females, artificially reared kids were negative on all occasions for 27 weeks after birth.

Clinical findings

The carpal joint circumference of the adult goats varied between 12.5 cm and 14 cm. Only one goat (No 7) had enlarged carpal joints, swollen atlanto-occipital bursa and thin appearance. In that goat the carpal swelling increased during pasture time (16 cm left leg and 17 cm right leg) and the goat obviously had difficulties in moving. The joint circumference decreased again when she had been back from pasture for 3 months (15 cm left leg and 16,5 cm right leg). In the other goats no significant increase of the carpal joint circumferences was seen during or after pasture. However, as mentioned above, goat No 13 had to be killed because of difficulties in moving. In the spring of the second year of observation, 2 of the goats from group 1 (No 4 and 11) suddenly got stiff in their hindquarters and had difficulties to get up and to move. Both had been positive for CAEV for a long time but had never showed any symptoms. All kids grew well without signs of arthritis or stiffness.

Gamma globulin concentration in kids

The goat milk fed kids in group 3 had higher concentrations of gamma globulin compared to the artificially reared kids in group 4 at the age of 8-19 weeks (Fig. 2). The gamma globulin concentration increased significantly in the male kids at 19 weeks and in the female kids at 27 weeks. The difference between the groups persisted until the kids were 27 weeks old, when the gamma globulin concentration had reached the same level in both groups. There was no difference in gamma globulin concentration between seropositive and seronegative milk fed kids.

Discussion

The main findings in this investigation were that the pattern of antibody expression varied over time in individual CAEV-infected goats, and that intermittent seronegative reactions were observed. The gamma globulin levels in artificially reared kids were significantly lower than those in the milk fed kids until an age of more than 19 weeks.

Retroviral infections are characterised by incorporation of the viral genome into the host cell genome, where the virus can stay silent for a long time *(Yaniv et al.* 1985). The extent of expression of the viral genome varies as well as the presence of free extra cellular virions *(Evermann* 1990). In this study, the antibody response fluctuated markedly in individual goats. Intermittent seroreaction using AGIDT was mentioned by *Rowe et al.* (1992), but no details were given. Our goats predominantly responded both to gp135 and p28. When positive reaction to only one of the viral proteins occurred, it was most often to gp135 in the older goats. In group 2, comprising younger goats,

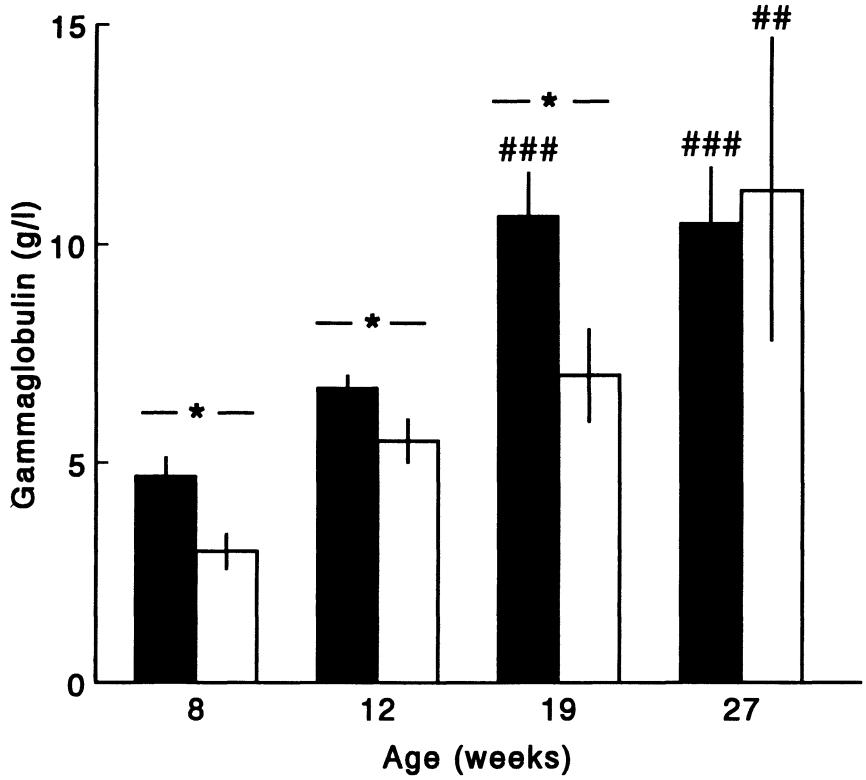

Figure 2. Gamma globulin levels in male and female goat kids 8-27 weeks old. ■ = Male kids; N = 7 at 8 and 12 weeks and N = 6 at 19 and 27 weeks □ = Female kids; N = 4 Means ± SE. *p<0.05 between male and female kids ## p<0 01 and ### p<0 001 compared to week 8, within the same group

there was no significant difference. In goats variability in the concentration of antibodies to gp135 and p28 has been reported with a response to gp135 being more common than that to p28 *(Gogolewski et al.* 1985, *Johnson et al.* 1983, *Knowles et al.* 1990). Our results in group 1 are in agreement with these findings. This is the first study that clearly shows that seronegativity may occur intermittently with seropositivity in goats. Neither has this been reported in maedi-visna infected sheep.

Seronegativity in this study means that the amount of antibodies to the viral proteins was below the detection level of the AGIDT. This test is known to have a moderate sensitivity compared to the Enzyme Linked Immuno Sorbent Assay (ELISA) *(Adams et al.* 1980, *Sundquist et al.* 1981, *Rimstad et al* 1993). However ELISA-diagnosing techniques to CAEV are presently not available in Swedish laboratories. As understood from this study, better methods for diagnosis are much needed in order to prevent infected goats to get into virus-free herds.

A positive relationship between carpal and metacarpal circumferences and virus expression in synovial fluid has been reported *(Klevjer-Anderson* 1984), but only one goat in the present study had visible enlargements of the joints. We

could not find any correlation between antibody expression pattern and clinical symptoms.

The actions taken to prevent virus transmission from mother to kid seemed to be successful. None of the artificially reared kids seroconverted during the first 27 weeks of their lives, but some of the milk fed kids did. There is still a possibility that some of the female kids have been infected intrauterinely, as has been reported to occur at a frequency of up to 5% *(East et al.* 1993, *Adams et al* 1983). However, one would expect that at least one had seroconverted within 27 weeks. Thus, it has been reported that the highest antibody titres in experimentally infected new-born goat kids occur at 8 to 16 weeks post-infection after which the titre decreased, but remained detectable *(Adams et al* 1980, *East et al* 1993).

Four of the milk fed kids had a positive reaction in the AGIDT at the age of 8 weeks. The patterns of antibody response was the same as that during or after pregnancy in their respective mothers. This suggests colostral antibody transfer. *MacKenzie et al* (1987) reported presence of maternal antibodies in goat kids at 8 to 12 weeks of age, which agrees well with the observations in our kids, in which the pattern of antibody response had changed when they had become 12 weeks old. The degradation time of maternal antibodies in goat kids thus seems to correspond more to that of calves, in which 97% of immunoglogulin G (IgG) is metabolised in 14 weeks, than to that of lambs in which the metabolisation was complete in 5 weeks *(Halliwell & Gorman* 1989). However, the clinical material in this study was small and we could only measure changes in antibody pattern for one type of immunoglobulin.

Initially the milk fed kids had significantly higher gamma globulin concentrations than the artificially reared kids. It took more than 19 weeks before the concentration was the same in both groups. The gamma globulins of the goat milk fed kids include antibodies of both maternal- and endogenous origin. Maternal antibodies are known to suppress the immune response both non-specifically and specifically in calves *(Halliwell & Gorman* 1989), therefore one can assume that the production of endogenous antibodies are slower in the milk fed group than in the group fed cow milk replacement product. *Rowe et al.* (1992) compared the effectiveness of feeding kids pasteurised milk with or without segregation. Non segregated goats were 3.37 times more likely to seroconvert by 104 weeks of age. Sex was not significantly associated with age of seroconversion. Therefore, we assume that the selection of only female kids for artificial rearing and segregation and only male kids for conventional rearing did not interfere with the serology results in the present study.

In conclusion, when diagnosing lentiviral infection by means of presence of antibodies, it is important to consider the delay period between infection and seroconversion as well as possible intermittent seroreaction. The method should therefore be used only as a herd diagnosis, as is presently done for the Maedi-Visna virus eradication program in Sweden *(Anon* 1993)

Acknowledgements

We thank the department of Clinical Chemistry, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala for the analyses of the gamma globulins The valuable help and constructive criticism by professor Berndt Klingeborn is gratefully acknowledged

References

Adams DS, Crawford TB, Banks KL, McGuire TC, Perryman LE Immune responses of goats persistently infected with caprine arthritis-encephalitis virus Infect Immun 1980, *28,* 421-427

Adams DS, Klevjer-Anderson P, Carlson JL, McGuire TC, Gorham JR Transmission and control of caprine arthritis-encephalitis virus Amer J Vet Res 1983, *44,* 1670-1675

Anonymous Svenska Djurhälsovårdens bestämmelser för hälsokontroll avseende maedi-visna hos får. (The Swedish Animal Health Service's regulations of health control of maedi-visna in sheep). Sveriges Slakteriförbund; Svenska Djurhälsovården 1993.

Cornell B · CAE hos get. Nu måste åtgärder sättas in! (CAE-in the goat. It is time to act). Fårskötsel. 1991, *7-8,* 29.

Dahlberg JE, Gaskin JM, Perk K Morphological and immunological comparison of caprine arthritis-encephalitis and ovine progressive pneumonia viruses. J Virol. 1981, *39,* 914-919

East NE, Rowe JD, Dahlberg JE, Theilen GH, Pedersen NC Modes of transmission of caprine arthritis-encephalitis virus infection. Small Rumin. Res. 1993, *10,* 251-262.

Evermann JF · Comparative features of retroviral infections of livestock. Comp. Immun Microbiol. infect. Dis 1990, *13,* 127-136.

Fenner FJ, Gibbs EPJ, Murphy FA, Rott R, Studdert MJ, White DO Veterinary Virology. Academic Press , London 1993. pp 561-594.

Gogolewski RP, Adams DS, McGuire TC, Banks KL, Cheevers WP Antigenic cross-reactivity between caprine arthritis-encephalitis, visna and progressive pneumonia viruses involves all virion-associated proteins and glycoproteins J gen Virol 1985, *66,* 1233-1240.

Halliwell REW, Gorman NT Veterinary Clinical Immunology. WB Saunders company, Philadelphia 1989. pp. 195-204

Johnson GC, Barbet AF, Klevjer-Anderson P, McGuire TC Preferential immune response to virion surface glycoproteins by caprine arthritis-encephalitis virus-infected goats. Infect Immun 1983, *41,* 657-665

Klevjer-Anderson P, Adams DS, Anderson LW, Banks KL, McGuire TC. A sequential study of virus expression in retrovirus-induced arthritis of goats. J. gen Virol. 1984, *65,* 1519-1525.

Knowles D JR, Cheevers W, McGuire T, Stem T, Gorham J Severity of arthritis is predicted by antibody response to gp135 in chronic infection with caprine arthritis-encephalitis virus J. Virol. 1990, *64,* 2396-2398.

MacKenzie RW, Oliver RE, Rooney JP, Kagei H A successful attempt to raise goat kids free of infection with caprine arthritis-encephalitis virus in an endemically infected goat herd. N. Z. vet. J. 1987, *35,* 184-186

Rimstad E, East NE, Torten M, Higgins J, DeRock E, Pedersen NC Delayed seroconversion following naturally acquired caprine arthritis-encephalitis virus infection in goats Amer. J. Vet. Res 1993, *54,* 1858-1862.

Rowe JD, East NE, Thurmond MC, Franti CE, Pedersen NC Cohort study of natural transmission and two methods for control of caprine arthritis-encephalitis virus infection in goats on a California dairy. Amer. J. Vet. Res. 1992, *53,* 2386-2395.

SAS Institute Inc SAS User's Guide Statistics, version 6.04, SAS Institute Inc., Cary, NC, USA 1987.

Stowring L, Haase AT, Charman HP Serological definition of the lentivirus group of retroviruses. J Virol. 1979, *29,* 523-528.

Sundquist B, Jonsson L, Jacobsson SO, Hammarberg KE Visna virus meningo-encephalomyelitis in goats Acta vet. scand 1981, *22,* 315-330

Yaniv A, Dahlberg JE, Tronick SR, Chiu IM, Aaronson SA Molecular cloning of integrated caprine arthritis-encephalitis virus. Virology. 1985, *145,* 340-345.

Sammanfattning

En långtidsstudie av getter naturligt infekterade med caprint artrit-encefalitvirus

Virussjukdomen caprin artrit-encefalit är ett stort problem för getnäringen. Sjukdomens särdrag och förlopp är emellertid dåligt utredda i naturligt infekterade besättningar Därför var vår målsättning med föreliggande studie trefaldig. 1) att undersöka hur antikroppssvaret, mätt med agar gel immuno-diffusionstest (AGIDT) varierade med tiden hos naturligt infekterade, seropositiva getter, 2) att undersöka sambandet mellan kliniska symtom och serokonvertering hos vuxna getter och 3) att följa serokonvertering och koncentrationen av gamma globulin hos getkillingar som antingen fått obehandlad getmjölk eller kalvnäring

Antikroppssvaret med avseende på förekomst av antikroppar mot virusproteinerna gp135 och p28 varierade hos 19 vuxna djur som följdes med upprepad provtagning under 30-91 veckor Helt negativ reaktion erhölls dessutom vid några provtillfällen hos annars serokonverterade individer. Fyra serokonverterade getter fick rörelsesvårigheter. Någon korrelation mellan symtom och antikroppsreaktion fanns emellertid inte Inte någon av de getkillingar (N = 4) som fått kalvnäring hade serokonverterat inom 27 veckor efter födseln. Fem av de 7 killingar som fötts upp på

getmjölk visade däremot positiva svar med olika antikroppsuttryck vid varje provtillfälle. Plasmans koncentration av gamma globulin var signifikant högre hos mjolkuppfödda killingar jämfört med dem som fått kalvnäring tills de var mer än 19 veckor gamla Resultaten visar att en stor variation i antikroppsvar förekommer hos den enskilda geten. AGIDT bör därför endast användas som en test av besättningens smittostatus och upprepad provtagning på alla individer är nodvändig för att säkra slutsatser skall kunna dras När killingar togs från sina mödrar omedelbart efter födseln, föddes upp på kalvnäring och hölls separerade från besättningen skedde ingen serokonvertering de första 27 veckorna till skillnad mot en grupp som fått obehandlad getmjölk. Deras koncentration av gamma globuliner i blodplasma var emellertid lägre än hos killingarna vilka fått getmjölk

(Received May 2, 1995, accepted October 2, 1995)

Reprints may be obtained from: K. Olsson, Department of Animal Physiology, Swedish University of Agricultural Sciences, Box 7045, S-750 07 Uppsala, Sweden Telephone (46) 18 672125 Telefax. (46) 18 672852 E-mail Kerstin.Olsson@djfys slu se