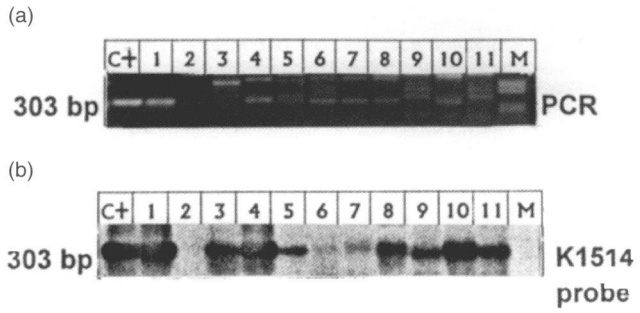

FIG 1: (a) PCR detection of CAEV DNA in lactoserum cells (lanes 1 to 11). M Molecular weight marker pBR 322/Hae III, C+ Control positive DNA. (b) Autoradiographic detection after southern blot hybridisation of PCR products with a fluorescein labelled probe (chemiluminescent reaction)

transferred on to nylon membranes (Appligene, Oncor). The membranes were hybridised with a probe generated from the reference maedi-visna strain K1514, which recognises *pol* amplified sequences of CAEV. The probe was labelled by fluorescein using the Genes Images random prime labelling module (Amersham). Amplified products were revealed by chemiluminescence using the Genes Images CDP-Star detection module (Amersham).

Attempts to isolate the virus on cell cultures proved negative in every case (Table 1). Although the total cell numbers in lactosera were low, DNA was extracted from cell samples and controlled by amplification of the 332 bp fragment of the gene of ovine glyceraldehyde 3 phosphate dehydrogenase (data not shown). PCR performed with the *pol* primers gave six positive results after visualisation by ethidium bromide staining. Two samples were difficult to interpret (Fig 1a). After southern hybridisation, 10 samples were found to be positive, including one from a seronegative flock (Fig 1b and Table 1). Attempts to detect cellular RNA by an RT-PCR protocol (Leroux and others 1995) did not give accurate results, partly because of the low levels and the poor quality of RNA extracts.

Proviral DNA was detected in the pellet of lactoserum, which comprised mononuclear cells and granulocytes. These cells endure unfavourable conditions: very low pH (Table 1), proteolysis of the media by lactic bacteria and hypertonicity of the environmental conditions. In spite of these drastic conditions, a significant percentage of cells remained viable (Russo and Vitu 1989; Table 1) and proviral CAEV DNA was detected. It is possible that these proviral DNAs are infectious to goats and capable of spreading the virus. The potential risk is, however, minimised, because the lactoserum, with low levels of proviral DNA, is shared out between adult goats and it could also be very difficult for the cells of the monocyte/macrophage lineage to cross the intestinal barrier in adults (Perrin 1989). Nevertheless, the presence of CAEV DNA in lactoserum could increase the viral pressure in a dairy flock with a low level of infection, and an intranasal passage during feeding could not be excluded. Therefore, further studies in which seronegative goats are fed with contaminated lactoserum are necessary to demonstrate the exact role of lactoserum in spreading lentiviral infection in goats.

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Within-herd BHV-1 prevalence prediction from an ELISA on bulk milk

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SOME European countries endeavour to obtain an infectious bovine rhinotracheitis (IBR)-free status. In the Netherlands the herd prevalence of bovine herpesvirus 1 (BHV-1) infections is very high (over 70 per cent [van Wuijckhuise and others 1993]), raising technical and financial problems in the control of the disease. As the IBR status may influence the export position in the near future, the Netherlands has initiated a programme to eradicate BHV-1 from the Dutch cattle population. Bulk milk testing might be a valuable tool in an eradication and/or monitoring programme because it offers the possibility of quick and cheap screening. The purpose of this study was to assess the correlation between the bulk milk test result and the within-herd prevalence, that is, the percentage of seropositive animals that can be expected on a farm with a defined blocking percentage of the bulk milk.

Data collected from 295 dairy farms throughout the Netherlands were used. Herd sizes varied between 10 and 174 cows. Bulk milk and individual blood or milk samples were taken on the same day. Bulk milk and individual samples were then tested using a BHV-1 gB-blocking ELISA (ID-DLO) for the presence of antibodies.

Serum or defatted milk was pipetted into BHV-1 coated wells in a microtitre plate. Unbound material was then removed by washing. A perioxidase conjugated monoclonal antibody directed against the glycoprotein gB of BHV-1 was added. After washing, bound conjugate was detected by reaction with a substrate solution. Colour development was measured by spectrophotometry (Kramps and others 1994).

Bulk milk was considered to be 'negative' if the blocking percentage was less than 10; 'weak positive' between 10 and 50 per cent; and 'positive' when over 50 per cent. The correlation between the blocking percentage in the bulk milk and the percentage of seropositive or milk positive animals was calculated.

Based on 295 bulk milk samples, 223 herds (75·6 per cent) were positive, 40 (13·6 per cent) weak positive and 32 (10·8 per cent) negative. The relation between the bulk milk blocking percentage and the within-herd prevalence is shown in Fig 1.

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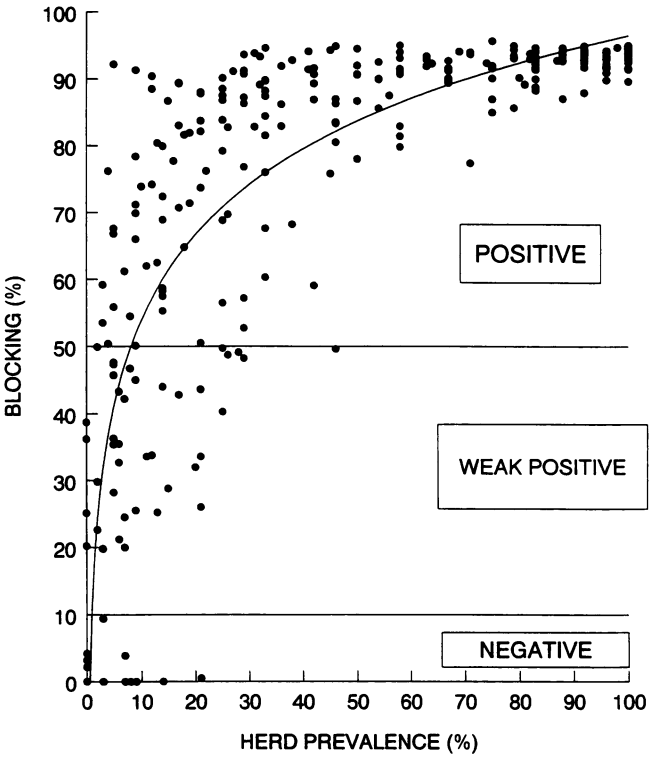

FIG 1: Relation between the bulk milk blocking percentage and the within-herd prevalence (n = 295); —— Bulk milk blocking percentage and ln(within-herd prevalence + 1)

In the herds that tested negative for bulk milk, the within-herd prevalence was less than 10 per cent; 18 (56 per cent) herds showed a prevalence of zero. Ninety per cent of herds with a weak positive bulk milk result contained seropositive animals but these seldom constituted more than 30 per cent of such herds.

Seventy-nine per cent of farms with a positive bulk milk result had a blocking percentage of more than 80 per cent. Of these herds, 76 per cent had a prevalence of IBR-positive animals of more than 60 per cent. Blocking percentages in bulk milk samples of between 51 and 80 per cent correlated with a lower prevalence in the herd, usually below 50 per cent.

The correlation between the bulk milk blocking percentage and the within-herd prevalence was 0·86. The best fit model (R^2 = 0·74) to estimate the within-herd prevalence from the blocking percentage was:

$$y = a + b \ln(\text{within-herd prevalence} + 1)$$

where y = bulk milk blocking percentage, a = 5·43 and b = 19·87. Given the bulk milk blocking percentage, the within-herd prevalence can be calculated as:

$$\text{Within-herd prevalence} = -1 + e^{([\text{blocking}\% - 5{\cdot}43]/19{\cdot}87)}$$

Van Wuijckhuise and others (1993) took a representative sample of Dutch dairy herds (2·4 per cent of 37,874 herds) and found an IBR bulk milk prevalence of 74·8 per cent (95 per cent confidence interval, 72·0 to 77·6; Bommeli and Svanovir tests). Two years later, a national bulk milk investigation of all Dutch dairy herds (n = 33,636) using gB-blocking ELISA revealed 69·6 per cent positive herds, 14·6 per cent weak positive herds and 15·8 per cent negative herds (van Wuijckhuise and Bosch 1996).

The participation of the 295 dairy farms in this study was on a voluntary basis, since testing herds for BHV-1 is not yet compulsory in the Netherlands. Based on the herd size, as well as the bulk milk prevalence, the farms in this study can be considered to be a representative sample of the Dutch cattle herds. Van Wuijckhuise and others (1993) indicated that the BHV-1 bulk milk test may not be sensitive enough to identify BHV-1-free herds (Bommeli and Svanovir tests). In this study, the herd prevalence based on individual samples was 92 per cent and on bulk milk samples 89 per cent. Therefore, it is concluded that the ELISA used was not sensitive enough. This may be due to herds containing only a few reactors. Frankena and others (1997) showed that the probability of detecting such herds using a bulk milk test is small.

In the Netherlands, the prevalence of IBR is very high, both on a herd and on an individual basis. Bulk milk testing can be very useful at the start of an eradication programme. For positive farms – due to the high within-herd prevalence – control should start with a vaccination campaign using marker-vaccines. Only in herds with a negative or weak positive bulk milk result is it economically feasible to test the herd individually. These farms may be able to control infection by removing seropositive animals and by applying management techniques.

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Abstracts

Continuous measurement of heart rate in working dogs

THE heart rates of two groups of labrador retriever x golden retriever dogs which were being trained as guides for the blind, were measured continuously by means of the Polar Sport Tester heart rate monitor during five minute walks from their training centre. The two groups had been classified by their trainers as either 'calm/non stress prone' or 'excitable/stress prone'. The heart rates of the 'calm' dogs remained in the range of 80 to 150 beats/min as they encountered other dogs, kerbs or other obstacles, whereas those of the 'excitable' dogs were far more erratic and ranged up to 240 beats/min. These patterns were consistent when the dogs were tested again two months later.

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Plasma keratan sulphate in horses with joint disease

KERATAN sulphate is a product of the breakdown of cartilage. Its concentration was measured in the plasma and synovial fluid of 67 clinically normal horses and 160 horses with various joint diseases. The horses with osteochondral (chip) fractures, other closed intra-articular fractures, inflammatory arthritis (synovitis), infectious arthritis, or osteochondrosis had significantly higher concentrations of keratan sulphate in plasma than the normal horses, but the horses with osteoarthritis did not. The breed and gender of the horse, and the type of joint disease, affected the concentration of keratan sulphate in synovial fluid. The concentration of keratan sulphate is thus affected by various joint diseases, but is not on its own a specific marker for all joint diseases.

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