HEGESH, E., GRUENER, N., COHEN, S., BOCHKOVSKY, R. & SHUVAL, H. I. (1970) *Clinica Chimica Acta* **30**, 679
JACOB, H. S., BRAIN, M. C. & DACIE, J. V. (1968) *Journal of Clinical Investigation* **47**, 2664
KOBAYASHI, K. (1981) *Feline Practice* **11**, 22
LEES, G. E., POLZIN, D. J., PERMAN, V., HAMMER, R. F. & SMITH, J. A. (1979) *Journal of the American Animal Hospital Association* **15**, 143
MAEDE, Y. (1977) *Japanese Journal of Veterinary Science* **39**, 187
MAEDE, Y., KASAI, N. & TANIGUCHI, N. (1982) *Blood* **59**, 883
OHKAWA, H., OHISHI, N. & YAGI, K. (1979) *Analytical Biochemistry* **95**, 351
PRCHAL, J. T., CASTLEBERRY, R. T., PARMLEY, R. T., CRIST, W. M. & MALLUH, A. (1982) *Pediatric Research* **16**, 484
RIFKIND, R. A. (1965) *Blood* **26**, 433
SEBRELL, W. H. (1930) *Public Health Reports* **45**, 1175
SPICE, R. N. (1976) *Canadian Veterinary Journal* **17**, 181
SRIVASTAVA, S. K. & BEUTLER, E. (1968) *Analytical Biochemistry* **25**, 70
SRIVASTAVA, S. K. & BEUTLER, E. (1970) *Biochemical Journal* **119**, 353
STEVENS, H. (1984) *Veterinary Record* **115**, 363
STOCKS, J. & DORMANDY, T. L. (1971) *British Journal of Haematology* **20**, 95
THORP, F. & HARSHFIELD, G. S. (1939) *Journal of the American Veterinary Medical Association* **94**, 52
YAMATO, O. & MAEDE, Y. (1992) *American Journal of Veterinary Research* **53**, 134
YAMATO, O., YOSHIHARA, T., ICHIHARA, A. & MAEDE, Y. (1994) *Bioscience, Biotechnology and Biochemistry* **58**, 221

Short Communications

ELISA detection of antibodies to glycoprotein E of bovine herpesvirus 1 in bulk milk samples

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A GLYCOPROTEIN E (gE) deleted strain of bovine herpesvirus 1 (BHV-1) provides the basis for efficacious and safe attenuated and inactivated marker vaccines (Kaashoek and others 1994, Bosch and others 1996). Use of these vaccines in the Dutch BHV-1 eradication programme will be compulsory commencing in 1998. The companion diagnostic test to differentiate between infected and vaccinated cattle is a blocking ELISA that detects only antibodies directed against gE of BHV-1. Hence antibodies against wild-type BHV-1 and conventional non-gE deleted whole BHV-1 vaccines are detected, whereas antibodies against the gE-deleted marker vaccine are not (Van Oirschot and others 1997). The BHV-1-gE ELISA used in this study was a commercially available product (Idexx). The principle of the test is based on the use of one monoclonal antibody (Mab 66) whose binding can be blocked by antibodies in the test sample. This ELISA has a relative sensitivity and specificity of 99 per cent as compared with a BHV-1-gE ELISA using two monoclonal antibodies (Kaashoek 1995).

The testing of bulk milk has been shown to be of value in screening herds for bovine brucellosis (Knosel and others 1991) and in estimating the prevalence of BHV-1 (Hartman and others 1997, Van Wuijckhuise and others 1998). The aim of this study was to examine whether the BHV-1-gE ELISA is suitable for detecting antibodies directed against BHV-1 in bulk milk, and whether testing of bulk milk could give an indication of the percentage of infected animals in unvaccinated herds and in herds that were vaccinated with the marker vaccine.

To evaluate the specificity of the BHV-1-gE ELISA, Danish negative bulk milk samples were collected from 500 randomly chosen BHV-1-free herds. These freeze-dried bulk milk samples were dissolved in 1·0 ml of deionised water and analysed undiluted in the BHV-1-gE ELISA and in the BHV-1-gB ELISA, which was used as the standard test (Kramps and others 1994). The quality of each BHV-1 ELISA batch was first checked and only approved batches were used in this study (Wellenberg and others 1998). All Danish bulk milk samples were negative in both ELISA systems. This indicated that for the detection of antibodies directed against BHV-1 in bulk milk samples the relative specificity of the BHV-1-gE ELISA was 100 per cent as compared with the BHV-1-gB ELISA.

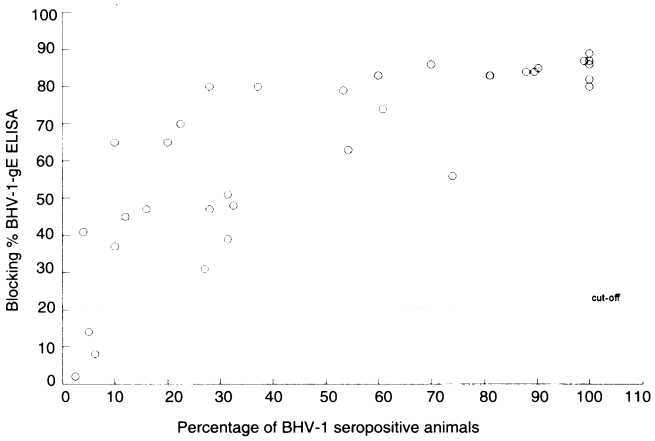

FIG 1: Blocking percentages of bulk milk samples versus the percentages of BHV-1-gE-seropositive cattle in unvaccinated herds

To evaluate the sensitivity of the BHV-1-gE ELISA, Dutch bulk milk samples were collected from unvaccinated herds with a BHV-1 history, and from herds with a BHV-1 history that had been vaccinated three times with the attenuated gE-deleted marker vaccine. The bulk milk samples obtained from vaccinated herds were collected five to six months after the third vaccination. All the herds had participated in a field trial for vaccine efficacy where they were randomly assigned to either an unvaccinated placebo group or to a vaccine group. From each unvaccinated (n = 35) and vaccinated herd (n = 34) one bulk milk sample was collected. On the same day as bulk milk samples were collected, individual serum samples (n = 4297) were collected from all animals older than two years, including those that were dry. The bulk milk samples collected from unvaccinated herds were analysed undiluted in the BHV-1-gE ELISA and the BHV-1-gB ELISA. The bulk milk samples collected from vaccinated herds were analysed undiluted in the BHV-1-gE ELISA only. All bulk milk samples were analysed without concentrating the immunoglobulin fraction as described by Von Forschner and others (1986). The concentration of bulk milk is too laborious for routine screening (Frankena and others 1997). The serum samples were analysed in a 1/2 dilution in the BHV-1-gE ELISA according to the instructions of the manufacturer. The blocking percentages of the bulk milk samples were plotted against the percentages of BHV-1-gE-seropositive cattle per unvaccinated (Fig 1) or per vaccinated herd (Fig 2).

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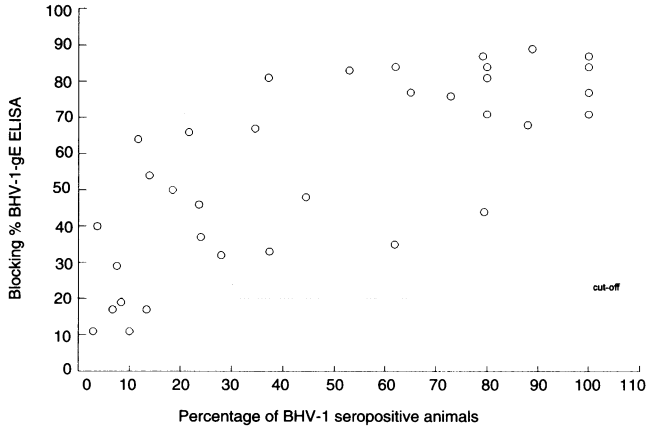

FIG 2: Blocking percentages of bulk milk samples versus the percentages of BHV-1-gE-seropositive cattle in vaccinated herds

In the unvaccinated herds, three bulk milk samples scored negative in the BHV-1-gE ELISA. In these unvaccinated herds five (6 per cent), three (5 per cent) and two (3 per cent) animals were seropositive in the BHV-1-gE ELISA (Fig 1). The same three, and two other herds from the unvaccinated group, scored negative in the BHV-1-gB ELISA. These latter two herds had eight (16 per cent) and six (12 per cent) animals with BHV-1-gE-seropositive results. Based on these results the BHV-1-gE ELISA had a relative sensitivity of 100 per cent in comparison with the BHV-1-gB ELISA.

In the vaccinated herds five bulk milk samples were negative in the BHV-1-gE ELISA. In these vaccinated herds five (10 per cent), five (7 per cent), one (3 per cent), four (13 per cent) and four (8 per cent) animals were seropositive in the BHV-1-gE ELISA (Fig 2). Overall, the BHV-1-gE ELISA detected antibodies in 88·4 per cent of the bulk milk samples (n = 69) originating from BHV-1 positive herds. There was no difference between the blocking percentages in bulk milk from unvaccinated and vaccinated herds, indicating that antibodies produced against the vaccine strain probably did not have any enhancing effect on the blocking percentages in the BHV-1-gE ELISA, as suggested earlier (Van Oirschot and others 1997).

In conclusion, the data in this study demonstrate that the BHV-1-gE ELISA is suitable to detect BHV-1-gE antibodies in bulk milk samples, and that the transition from a 'negative' to a 'positive' result for bulk milk samples takes place when 10 to 15 per cent of the animals within a herd become BHV-1-gE seropositive. This means that in a BHV-1-eradication programme, the BHV-1-gE ELISA, which detects gE-specific antibodies against wild-type BHV-1 and non-gE deleted whole BHV-1 vaccines, can be used for the screening of bulk milk samples to estimate the prevalence of BHV-1-infected cattle in unvaccinated and vaccinated herds. In the situation that the bulk milk sample is strongly positive in the BHV-1-gE ELISA, vaccination of the herd with a marker vaccine should be recommended. In case the bulk milk sample becomes negative in the BHV-1-gE ELISA, all individual animals should be screened to remove the BHV-1-gE seropositive animals and cessation of vaccination can be considered. Monitoring the bulk milk of BHV-1 free (vaccinated) herds with the BHV-1-gE ELISA with the aim of detecting the introduction of a single or a few BHV-1-infected cattle is, according to these data, not feasible. The same conclusion has been drawn for the BHV-1-gB ELISA (Frankena and others 1997).

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References

BOSCH, J. C., KAASHOEK, M. J., KROEZE, A. H. & VAN OIRSCHOT, J. T. (1996) *Veterinary Microbiology* **52**, 223
FRANKENA, K., FRANKEN, P., VANDEHOEK, J., VOSKAMP, G. & KRAMPS, J. A. (1997) *Veterinary Record* **140**, 90
HARTMAN, A., VAN WUIJCKHUISE, L., FRANKENA, K., FRANKEN, P., WEVER, P., DE WIT, J. & KRAMPS, J. (1997) *Veterinary Record* **140**, 484
KAASHOEK, M. J. (1995) PhD Thesis, Utrecht University. p 110
KAASHOEK, M. J., MOERMAN, A., MADIC, J., RIJSEWIJK, F. A. M., QUAK, J., GIELKENS, A. L. J. & VAN OIRSCHOT, J. T. (1994) *Vaccine* **12**, 439
KNOSEL, H., HEMPEL, E. & VON FORSCHNER, E. (1991) *Deutsche Tierarztliche Wochenschrift* **98**, 356
KRAMPS, J. A., MAGDALENA, M., QUAK, J., WEERDMEESTER, K., KAASHOEK, M. J., MARIS-VELDHUIS, M. A., RIJSEWIJK, F. A. M., KEIL, G. & VAN OIRSCHOT, J. T. (1994) *Journal of Clinical Microbiology* **32**, 2175
VAN OIRSCHOT, J. T., KAASHOEK, M. J., MARIS-VELDHUIS, M. A., WEERDMEESTER, K. & RIJSEWIJK, F. A. M. (1997) *Journal of Virological Methods* **67**, 23
VAN WUIJCKHUISE, L., BOSCH, J. C., FRANKEN, P. & ELBERS, A. R. W. (1998) *Veterinary Record*
VON FORSCHNER, E., BÜNGER, I., KÜTTLER, E. & MEHRKENS, L. (1986) *Deutsches Tierärztliche Wochenschrift* **93**, 281
WELLENBERG, G. J., VERSTRATEN, E. R. A. M., MARS, M. H. & VAN OIRSCHOT, J. T. (1998) *Journal of Clinical Microbiology* (in press)

Isolation of *Mycoplasma fermentans* from a sheep

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MYCOPLASMAS usually show a rather strict host specificity which reflects their nutritionally fastidious nature. However the list of mycoplasmas isolated from non-typical hosts is increasing with reports of *Mycoplasma canis* in cattle (Nicholas and others 1995), *M salivarium*, a common human buccal mycoplasma, in pigs and *Acholeplasma oculi*, a goat mycoplasma, in the human amniotic fluid (Razin 1992). This lack of host specificity creates major difficulties for the diagnostic laboratory because the search for the identity of an isolate shown to be unrelated to mycoplasmas of the usual host flora must then be extended to mycoplasmas of all other animals including man. The isolate then requires testing using laborious growth inhibition and/or immunofluorescent tests with typing antisera to the whole range of animal mycoplasmas which today totals some 100 species. This report describes the isolation and identification of a human mycoplasma, *M fermentans,* from the reproductive tract of a sheep using an unconventional approach based on 16S ribosomal (r) RNA typing to aid the identification.

The mycoplasma was isolated at the St Boswells centre of the SAC Veterinary Services Division, from a swab of a vaginal cleft ulcer of a single ewe in a flock of approximately 650 Scottish mule breed sheep. The affected animal was one of three ewes showing similar lesions of the vulva. Three rams, introduced into the herd two months before, were also showing ulcers on the glans penis and were similar to those previously described (Greig 1991). *Histophilus ovis* and *Actinomyces pyogenes*, both of which have been associated with reproductive disease in ewes and rams, were isolated from all the affected ewes and rams. The swabs were also cultured for mycoplasmas in Chanock's broth and then transferred to Eaton's medium (Jones and others 1978) for identification. Cultures were maintained at 37°C in an atmosphere of 5 per cent carbon dioxide. An isolate, recovered from one of the ewes,

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