

cent Tween + 2 per cent bovine serum albumin and bound antibody was detected using a biotinylated anti-sheep immunoglobulin G followed by horseradish peroxidase labelled protein G. Serial dilutions of reference sera (negative, weakly positive and strongly positive) were assayed to define the sensitivity of the ELISA. In the blind trial all sera were tested in duplicate on three separate occasions at dilutions of 1/10 and 1/50. Control wells in the ELISA were coated with control antigens (irrelevant GST fusion protein) protein to avoid detection of any antibodies which may have been cross reactive with other components in the rec-gag fusion protein preparation. ELISA readings were recorded by optical density (OD) 405 on an automated ELISA reader. The average netto extinctions (NE_{405}) of controls and samples were calculated from the OD readings. A weak positive sera was used as a reference positive and results compared to this. Readings of less than 60 per cent of the weak positive were regarded as negative. Many negative sera gave values of 20 per cent of the weak reference positive or less.

The results of titrations with sets of reference negative sera, reference weak positive sera and strongly positive sera are shown in Fig 1. These results show that none of the negative sera gave OD readings above background at any dilution. Both weak positives and strong positives were clearly positive in the ELISA and both groups of sera could be diluted to 1/100 before losing activity. AGID strong positives had a greater titre than AGID weak sera but at low dilutions it was not possible to discriminate between these AGID groups.

Having established the sensitivity of the ELISA 100 unknown sera were then tested at two dilutions and in triplicate on both rec-gag and control antigen. The results were scored as +/- based on the OD readings compared to control antigen and with known positives and negatives included in the assay. These results were then reported to CVL and the results decoded and compared to the CVL AGID result. Of the 100 sera tested, 98 were correctly identified as either positive or negative. The two sera giving uncertain negative or doubtful readings were retested and confirmed negative. This was in agreement with the CVL AGID test. Thus from this blind control assay with a randomised collection of positive and negative sera the ELISA gave 100 per cent concordance with the AGID test (Table 1).

These results confirm two important points on MVV serodiagnosis. First, the MVV recombinant ELISA using rec-gag as antigen can be used with confidence to detect MVV status with field sera drawn from naturally infected sheep. Importantly, the rec-gag ELISA did not give any false positive readings with the reference negative sera at any dilution. Secondly, the ELISA can be used at serum dilutions of up to 1/100 thus establishing that it has excellent sensitivity as well as specificity with field sera. This has been previously established for field situations in the USA (Keen and others 1995).

MVV is an endemic disease which could be eradicated with rapid and sensitive serological testing such as that offered by this ELISA. The sensitivity of the assay implies that for flock monitoring the ELISA may allow the detection of recent infections (for example in screening young lambs from 40 to 60 days after infection). Furthermore, the detection of antibody at high dilutions suggests that sera could be pooled at least in batches of five to 10 for testing. This would produce considerable savings in test fees thus making the ELISA a viable option for whole flock monitoring or large commercial or breeding flocks. In the commercial sheep sector especially, the level of MVV infection is thought to be considerable and far higher than in pedigree flocks. Eradication of MVV remains an urgent priority since, in 1994, the incidence of flocks having 1 per cent of MVV infected animals was estimated to be about 3 per cent (Gibson and Dawson 1994). UK sheep flocks used to be MVV free before the importation of sheep from the continent in the mid-1970s. Use of the ELISA and extensive flock monitoring and culling of seropositive animals may regain this position, putting MVV free sheep in the UK at a competitive advantage. This ELISA provides an alternative assay to the AGID test which has been validated with reference sera and suggests that it could be used as an alternative test for MVV serodiagnosis. The next stage would be full validation in the field.

Acknowledgements. - This work was supported by a Wellcome Trust Programme Grant Award to Professor I. McConnell and Dr

D. R. Sargan, reference number 035157/Z/1.27. The technical assistance of Ms D. Stock is gratefully acknowledged.

References

- CUTLIP, R. C., JACKSON, T. A. & LEHMKUHL, H. D. (1972) *American Journal of Veterinary Research* **38**, 1081
- GIBSON, L. & DAWSON, M. (1994) Report of the Maedi-Visna Working Group, Sheep and Goat Health Scheme Advisory Group. MAFF, UK
- KEEN, J., KWANG, J. & ROSATI, S. (1995) *Veterinary Immunology and Immunopathology* **47**, 295
- KUANG, J. & CUTLIP, R. S. (1992) *Biochemistry and Biophysics Research Community* **188**, 20
- PRITCHARD, G. C. & DAWSON, M. (1987) *Veterinary Record* **120**, 208
- REYBURN, H. T., ROY, D. J., BLACKLAWS, B. A., SARGAN, D. R. & McCONNELL, I. (1991) *Journal of Virological Methods* **37**, 305
- SARGAN, D. R., BENNET, I. D., COUSENS, C., ROY, D. J., BLACKLAWS, B. A., DALZIEL, R. G., WATT, N. J. & McCONNELL, I. (1991) *Journal of General Virology* **72**, 1983
- SIGURDSSON, B. (1954) *British Veterinary Journal* **110**, 254
- WATT, N. J., KING, T. J., COLLIE, D., McINTYRE, N., SARGAN, D. R. & McCONNELL, I. (1992) *Veterinary Record* **131**, 455
- ZANONI, R. G., NANTA, I. M., PAULI, V. & PETERHANS, E. (1991) *Journal of Clinical Microbiology* **29**, 1290

Concurrent paratuberculosis and parasitism in a five-month-old lamb in the Netherlands

D. P. Dercksen, J. Muskens, J. Vos, D. Bakker

Veterinary Record (1998) **142**, 433-434

OVINE paratuberculosis caused by the bacterium *Mycobacterium paratuberculosis* is a chronic wasting disease of adult sheep. Reliable data on the prevalence of paratuberculosis in the Netherlands are not available, although it is known from the records of the Animal Health Service that paratuberculosis is present in cattle, sheep and goats (D. P. Dercksen, unpublished observations). Unlike in cattle, profuse diarrhoea is not a feature of clinical disease and diagnosis may therefore be particularly difficult (Scott and others 1995). Natural Johne's disease occurs in sheep at any age over about one year, although the disease may occur in younger sheep after experimental infection (Gilmour and Angus 1983). The weight loss which occurs during the clinical stage of paratuberculosis is associated with protein malabsorption and protein-losing enteropathy (Stehman 1990). Postmortem examination in sheep shows thickening of the intestinal wall, particularly of the ileum and caecum and less frequently of the jejunum. However, gross lesions are often mild. Microscopically, proliferative enteritis is present with the most severe lesions in the terminal ileum (Carrigan and Shaman 1990). *M. paratuberculosis* is a fastidious, slow-growing organism in vitro. Positive cultures are rarely identifiable before six weeks and cultures should be incubated for at least 24 weeks. The interspecies infectivity of *M. paratuberculosis* was established in 1913 when clinical paratuberculosis was demonstrated in a goat, using a bovine-derived inoculum (Twort and Ingram 1913). In utero infection of the fetus has been documented in cattle (McQueen and Russell 1979) and in sheep (Tamarin and Landau 1961).

D. P. Dercksen, Department of Small Ruminants Health, J. Muskens, Department of Ruminants Health, J. Vos, Department of Pathology, Animal Health Service, PO Box 4, 5280 AA Boxtel, The Netherlands
D. Bakker, Institute for Animal Science and Health, (ID-DLO), PO Box 65, 8200 AB Lelystad, The Netherlands



Cattle and sheep are most likely to be infected at an early age by ingestion of *M paratuberculosis*. Generally, in infected ruminants the infection persists in a dormant state in the lamina propria of the intestines and the mesenteric lymph nodes for a variable period into adulthood. The onset of the clinical stage is triggered by stress or other factors that are not fully understood. Symptoms include diarrhoea and weight loss in cattle, and progressive weight loss, mostly without diarrhoea, in sheep and goats. It is not known why diarrhoea is a characteristic sign in bovine paratuberculosis but an infrequent finding in affected small ruminants. The different ways water is resorbed in the colon of small ruminants could be an explanation for this phenomenon (Smith and Sherman 1994). Bacterial culture of faeces is still the most widely accepted laboratory technique for antemortem diagnosis.

An organic sheep farmer with a flock of 56 Texel x Zwartbles crossbred ewes, with a long history of wasting and diarrhoea in both adult sheep and lambs, presented a five-month-old lamb for necropsy at the Animal Health Service. At postmortem examination the lamb was emaciated with a chronic catarrhal pneumonia of the right apical lobes. The consistency of the ruminal contents was reduced. The small intestine was distended with reduced consistency of the contents. The mesenteric lymph nodes were moderately enlarged. Microscopically, lymphoplasmacellular enteritis was diagnosed with focal accumulation of macrophages in the jejunum and ileum and small granulomas in the colonic mucosa. In addition, microphages were present in the mesenteric lymph nodes. Samples from the ileum tested negative by Ziehl-Neelsen staining. At parasitological examination more than 5000 (tricho)strongylides eggs per gram of faeces (epg) were found. From a pooled sample of jejunum, ileum and ileocaecal lymph nodes *M paratuberculosis* was cultured and found to be positive in five weeks using a conventional culture technique (Smith and Williams 1953). Ten colony forming units (cfu) were found in 3 g of this pooled sample. This culture was sent to the Institute for Animal Science and Health (ID-DLO) in Lelystad for reconfirmation. The strain identity was further confirmed by its mycobactin dependence, classical biochemistry and by *M paratuberculosis*-specific polymerase chain reaction, using IS900 specific oligonucleotides (Moss and others 1992).

Five months later, another two lambs were presented to the Animal Health Service for necropsy. These lambs were one year old, but appeared much younger. They showed signs of diarrhoea and were in a very poor condition. Before they were euthanased two faecal samples were taken from each animal at a week's interval for bacteriological culture. *M paratuberculosis* was cultured again from one of these samples but only after 34 weeks incubation and only one cfu was found in 3 g of faeces, indicating an early stage of infection.

At necropsy both animals were in a poor condition. One animal showed focal catarrhal pneumonia and had a slight chronic abomasitis. Moreover, this animal also had footrot in both front legs and in one hindleg. The other animal had slightly enlarged mesenteric lymph nodes and mesenterial oedema. Samples of ileum from both animals were negative by Ziehl-Neelsen staining. Microscopically, slight chronic enteritis was found in both animals. Both had more than 5000 (tricho)strongylides epg. One animal also had to 50 to 100 *Nematodirus* epg. *M paratuberculosis* could not be cultured from pooled samples of jejunum, ileum and ileocaecal lymph nodes from either of these animals.

Although the Ziehl-Neelsen staining of samples of the ileum was negative in the five-month-old lamb, the lymphoplasmacellular enteritis with focal accumulation of macrophages in the jejunum and ileum and small granulomas in the colonic mucosa is indicative of Johne's disease. The chronic enteritis in the two one-year-old lambs is not indicative of Johne's disease although *M paratuberculosis* was cultured from the faeces of one of these lambs. The profuse diarrhoea in all three lambs, which is not a feature of clinical disease in sheep, was probably caused by the heavy infestation with endoparasites. This sheep farmer was not using anthelmintic treatment regularly because of his organic farming principals. The farmer's non-deworming management could be one of the triggers for clinical Johne's disease in this lamb at such a young age. Although in recent years there had been

only sheep and no other ruminants (cattle or goats) on this farm it is possible that the lambs were passive carriers and picked up *M paratuberculosis* from the environment. It is also possible that these lambs were dying from pneumonia and parasitism and coincidentally harbouring small numbers of *M paratuberculosis* either passively or actually incubating the disease.

Both the rapid growth of *M paratuberculosis*, within five weeks, and the young age at which clinical and histopathological lesions consistent with Johne's disease appeared in the five-month-old lamb under natural circumstances do not appear to have been reported before. The relevance of the infection with *M paratuberculosis* in the five-month-old lamb, with respect to the clinical symptoms is, as discussed before, not obvious due to the concurrent (tricho)strongylides infection. Therefore, a challenge experiment in lambs, calves and goat kids to find out if this sheep strain can induce clinical symptoms in these species is underway. Since a national control programme for Johne's disease in the Netherlands is being planned, the authors are interested to know if this kind of sheep strain can cause clinical Johne's disease in ruminants other than sheep and whether the control programme should focus on cattle or on both cattle and sheep.

References

- CARRIGAN, M. J. & SHAMAN, I. T. (1990) *Australian Veterinary Journal* **67**, 47
 GILMOUR, N. J. L. & ANGUS, K. W. (1983) *Disease of Sheep*. Ed W. B. Martin. Oxford, Blackwell Scientific Publications, p 52
 McQUEEN, D. S. & RUSSELL, E. G. (1979) *Australian Veterinary Journal* **55**, 203
 MOSS, M. T., SANDERSON, J. D., TIZARD, M. L. V., HERMON-TAYLOR, J., EL-ZAATARI, F. A. K., MARKESICH, D. C. & GRAHAM, D. Y. (1992) *Gut* **33**, 1209
 SCOTT, P. R., CLARKE, C. J. & KING, T. J. (1995) *Veterinary Record* **137**, 173
 SMITH, M. C. & SHERMAN, D. M. (1994) *Goat Medicine*. Philadelphia, Lea & Febiger, p 307
 SMITH, H. & WILLIAMS, J. (1953) *Journal of Pathology and Bacteriology* **66**, 375
 STEHMAN, S. M. (1990) *Large Animal Medicine*. Ed B. P. Smith. St Louis, C. V. Mosby, p 823
 TAMARIN, R. & LANDAU, M. (1961) *Refuah Veterinarith* **18**, 43
 TWORT, F. W. & INGRAM, G. L. (1913) *A Monograph on Johne's Disease*. London, Baillière Tindall & Cox

Acute disseminated candidiasis in a puppy associated with parvoviral infection

F. Rodríguez, A. Fernández, A. Espinosa de los Monteros, P. Wohlsein, H. E. Jensen

Veterinary Record (1998) **142**, 434-436

CANDIDA species normally inhabit the mucosa of the alimentary, upper respiratory, and genital tracts of mammals (Greene and Chandler 1990). Candidiasis in animals is mainly produced by *Candida albicans* and *C tropicalis* (Barker and others 1993) which form blastospores, pseudohyphae and true hyphae when growing in tissues (Anderson and Pidgeon 1987). Systemic spread of *Candida* species, which is recognised as an opportunistic infec-

F. Rodríguez, A. Fernández, A. Espinosa de los Monteros, Department of Histology and Pathological Anatomy, Veterinary Faculty, University of Las Palmas de Gran Canaria, C/Francisco Inglott Ariles 12A, 35016 Las Palmas de Gran Canaria, Spain

P. Wohlsein, Institute of Pathology, Faculty of Veterinary Medicine, W-3000 Hannover, Germany

H. E. Jensen, Department of Pharmacology and Pathobiology, The Royal Veterinary and Agricultural University of Copenhagen, Frederiksberg C, Denmark





Concurrent paratuberculosis and parasitism in a five-month-old lamb in the Netherlands

D. P. Dercksen, J. Muskens, J. Vos, et al.

Veterinary Record 1998 142: 433-434

doi: 10.1136/vr.142.16.433

Updated information and services can be found at:

<http://veterinaryrecord.bmj.com/content/142/16/433.citation>

Email alerting service

These include:

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:

<http://group.bmj.com/group/rights-licensing/permissions>

To order reprints go to:

<http://journals.bmj.com/cgi/reprintform>

To subscribe to BMJ go to:

<http://group.bmj.com/subscribe/>