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Short Communication

Ovine Enzootic Abortion (OEA): Antibody Response in Vaccinated Sheep Compared to Naturally Infected Sheep

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INTRODUCTION

Ovine enzootic abortion (OEA) caused by *Chlamydophila (C.) abortus* is an economically important disease worldwide, which is characterised by late-term abortions, neonatal losses and weak lambs. In the course of an epidemic outbreak of OEA, up to 30% of ewes abort. In endemically infected flocks, the annual losses decrease to 5-10% (Stamp *et al*., 1950; Aitken, 1993). If chlamydial infection is present in a flock, an appropriate strategy of vaccination and/or use of oxytetracyclines should be employed (Buxton and Henderson, 1999). Several vaccines have been developed in the last 50 years. Since 1956, an egg-grown, formalin-inactivated, whole-organism vaccine has been used before breeding. Application of this vaccine significantly reduced the incidence of abortion, but did not prevent all abortions nor eliminate the disease (McEwen and Foggie, 1954; Foggie, 1973; Linklater and Dyson, 1979; Rodolakis and Souriau, 1979). Furthermore, application of this vaccine does not reduce shedding of chlamydiae at lambing and thus the cycle of infection is maintained. More recently, an avirulent, temperature-sensitive, live chlamydia vaccine has been developed, that seems to confer strong, long-lasting protection. The attenuated strain, named 1B, was obtained from the virulent strain AB7 by nitrosoguanidine mutagenesis (Rodolakis,

1983; Rodolakis and Souriau, 1983; Rodolakis and Bernard, 1984). This vaccine is commercially available in several countries. As it is a live vaccine, precautions must be taken to prevent human exposure, especially to pregnant women (Aitken, 2000).

The Sheep and Goat Health Schemes in England and Wales and the Premium Health Scheme in Scotland are based on identification and registration of OEA-free flocks. However, successful confirmation of negative OEA status requires a reliable serological test. False positive results in the complement fixation test (CFT) for example, are due to cross-reactive antibodies to *C. pecorum* (Rodolakis *et al.*, 1998; Aitken, 2004). Since it is conceivable that vaccination can induce seropositive reactions indistinguishable from the serological reactions of naturally infected animals, flocks that had been vaccinated in the last 3 years were excluded from the Health Scheme (Prettejohn, 1988). The question of distinguishing between serological reactors due to enzootic abortion infection and vaccinal-reactors has still to be examined. To this end, a pilot study using a small number of animals was initiated to assess the antibody response in vaccinated sheep by complement fixation test and competitive ELISA.

Two chlamydial vaccines are currently available in Switzerland, both have been licensed by the Federal Veterinary Office (FVO) in Berne. One is an inactivated, egg-grown preparation of *C. abortus* (Ovax Clamidia, Fatro, Italy), which has been in use for several years. The other product (Ovilis® Enzovax, Intervet, The Netherlands) contains the thermo-sensitive (ts) strain 1B of *C. abortus* and has only recently (December 2002) been licensed in Switzerland.

MATERIALS AND METHODS

The two vaccines were administered to the sheep according to the instructions of the manufacturers. Four groups of sheep were tested (groups 1, 2, 3 and 4).

Group 1 contained six sheep A, B, C, D, E and F, held conventionally in a closed flock under optimal hygienic conditions and confirmed free of ovine Herpesvirus type 2 and *Mycoplasma* sp. infections. Sheep A, B and C were vaccinated twice intramuscularly in the neck with an interval of six weeks using the inactivated egg-grown vaccine Ovax Clamidia. Sheep D and E and F served as unvaccinated controls. Blood samples were taken on days 0 (first blood sampling and first vaccination), 14, 28, 42 (second vaccination), 56, 70, 82 and 98. Group 2 included three sheep I, II and III, each vaccinated once intramuscularly in the neck with the attenuated live vaccine Ovilis® Enzovax. Due to the fact that at the time of the experiments the attenuated live vaccine was not yet officially licensed in Switzerland, all experiments were carried out under pathogen-free conditions at the high security laboratory of the Institute of Virology and Immunoprophylaxis, Mittelhaeusern, Switzerland. For this reason the number of animals was limited to 3, the frequency of blood sampling was kept low and the samples could not be taken on the same days as for the inactivated vaccine. Blood samples were taken on day 0 (before vaccination) and on days 7 and 21 after vaccination. In group 3, four sera from a field flock in Switzerland infected with *C. abortus* served as positive controls (sera 1, 2, 3 and 4). The diagnosis of OEA infection in these sheep was initially suspected following necropsy and routine bacteriology of

the placenta and the fetal organs (lung, liver and kidney) according to the method of Hässig and colleagues (1995). Confirmatory tests included (a) histopathology on formalin-fixed and paraffin-embedded tissue specimens of placenta and different fetal organs (brain, lung, heart, kidney, liver, ileum, lymphatic organs, third eyelid, skeletal muscle and umbilicus), (b) immuno-histochemistry using a *Chlamydiaceae* family-specific mouse monoclonal antibody directed against the chlamydial lipopolysaccharide (cLPS; Clone ACI-P, Progen, Heidelberg, Germany) and the EnVision Kit (Dako ChemMate™, Glostrup, Denmark) and (c) polymerase chain reaction on the formalin-fixed and paraffin-embedded placenta for the 16S rRNA gene specific for the order *Chlamydiales* with sequencing of the amplified fragments (Everett *et al.*, 1999). In group 4, four serum samples (sera 5, 6, 7 and 8) from two field flocks in Switzerland with no history of abortion nor vaccination against OEA in the past were used as negative controls (group 4). The sheep sera had been characterised as negative in a previous study (Gut-Zangger *et al.*, 1999).

The blood samples were collected using Vacutainer® tubes (Becton Dickinson, Heidelberg, Germany), centrifuged at 3000*g* for 10 min and stored at –20°C until further processing. Serum samples were tested by CFT (Stamp *et al.*, 1952) using a commercial antigen and standard reagents (Virion/Serion, Würzburg, Germany), as well as by a competitive enzyme-linked immunosorbent assay (cELISA), using the monoclonal antibody mAb 188 directed against variable segment 1 of the major outer membrane protein of *C. abortus* (Salti-Montesanto *et al.*, 1997). CF titres were expressed as the highest dilution of serum with less than 50% lysis of red blood cells. A CF titre of $\geqslant$1:40 was considered positive. The results of the cELISA were expressed as 'percentage of inhibition'. Inhibition values above 55% were considered positive for OEA-infection (positive cut-off) whereas inhibition values between 30–55% were considered inconclusive, attributable to either *C. abortus* or *C. pecorum*. Inhibition values below 30% were considered negative (Salti-Montesanto *et al.*, 1997; Gut-Zangger *et al.*, 1999).

RESULTS

In group 1, unvaccinated control ewes D, E and F were negative for antibodies against *C. abortus* by CFT and by the cELISA throughout the period of examination (data not shown). All three ewes A, B and C vaccinated with the inactivated Ovax Clamidia were negative for antibodies against *C. abortus* up to day 82 when tested with CFT and cELISA (Table I). None of the animals had titres above 30% (negative cut-off value). On days 82 and 98, sheep C was positive by cELISA, but negative by CFT.

The results of the sheep in group 2 are shown in Table II. The titres on day 0 were negative for all three animals in the CFT as well as in the cELISA. On day 7, ewe III had an inconclusive titre in the cELISA, but was negative in the CFT. Sheep I and II were negative on day 7. On day 21, all three ewes showed positive antibody titres by both CFT and cELISA.

The results of the positive and negative control groups (groups 3 and 4, respectively) are shown in Table III. All four positive controls in group 3 (sera 1, 2, 3 and 4) tested

TABLE I
CFT and cELISA results of the three sheep A, B and C (group 1) vaccinated with the inactivated vaccine (Ovax Clamidia, Fatro). Days p.v. are counted after the first vaccination

Day p.v.	Animal number	CFT (titre)	cELISA (%)
0	A, B, C	0, 0, 0	13.1, 8.8, 10.3
14, 28, 42	A, B, C	0, 0, 0	3.3 to 10.3
56	A, B, C	0, 0, 1:5	5.2, 10.5, 16.2
70	A, B, C	0, 0, 0	4.0, 17.8, 11.8
82	A, B, C	0, 0, 0	15.6, 14.8, 74.7
98	A, B, C	0, 0, 0	22.3, 17.2, 70.6

p.v. = post vaccination

TABLE II
CFT and cELISA results of animals I, II and III (group 2) vaccinated with the attenuated vaccine (Ovilis® Enzovax, Intervet)

Day p.v.	Animal number	CFT (titre)	cELISA (%)
0	I, II, III	0, 0, 0	11.5, 3.1, 9.2
7	I, II, III	1:2.5, 0, 0	15.8, 21.2, 40.0
21	I, II, III	1:320, 1:80, 1:40	57.8, 64.0, 61.3

p.v. = post vaccination

TABLE III
CFT and cELISA results of animals 1, 2, 3 and 4 (group 3, positive controls) and animals 5, 6, 7 and 8 (group 4, negative controls)

OEA	Animal number	CFT (titre)	cELISA (%)
Yes	1	1:5	91.6
Yes	2, 3	1:80, 1:160	95.4, 95.8
Yes	4	0	96.8
No	5	1:10	9.9
No	6, 7, 8	0, 0, 0	15.1, 10.1, 14.0

positive by the cELISA. Sheep 2 and 3 were also positive in the CFT, while the sera of sheep 1 and 4 were negative. All four serum samples (sheep 5, 6, 7 and 8) from group 4 (negative control) tested negative by cELISA and CFT.

DISCUSSION

This pilot study was undertaken to determine if administration of vaccines to protect flocks from OEA would result in antibody titres in the CFT and cELISA tests that were indistinguishable from serum values due to natural infection. Vaccination with the inactivated vaccine Ovax Clamidia raised a response in only one animal out of three at 82 and 98 days post first vaccination, which was detected by only one method, the cELISA. In contrast, the attenuated live vaccine Ovilis® Enzovax induced a titre in three out of three animals on day 21, which was detected by both serological methods. In this case, vaccinated and naturally infected animals could not be distinguished on a serological basis. Discrepancies between results of CFT and cELISA were also observed in the past. They have been attributed to the different antigens measured by the two methods (Gut-Zangger *et al.*, 1999) and to cross-reactivity in the CFT with antibodies to *C. pecorum* (Rodolakis *et al.*, 1998; Aitken, 2004). Moreover, the sensitivity of the CFT has been reported to be low with either naturally or experimentally infected sheep (Markey *et al.*, 1993; Buendia *et al.*, 2001). The usefulness of this test for diagnosing infections in flocks rather than in individual animals has been discussed (Longbottom *et al.*, 2002). Though the induction of an antibody response by vaccination with Ovax Chlamidia was variable, the unequivocal results in both tests following vaccination with Ovilis® Enzovax leave no doubt about the induction of specific antibodies using this vaccine. Although it was anticipated that after vaccination sheep would seroconvert to some extent, it is clear from this study that the vaccination status of flocks under investigation must be taken in account when interpreting serological survey results. Further investigation on a larger number of animals and a follow-up study of the induced immune response are in progress.

REFERENCES

Aitken, I.D., 1993. Ovine chlamydial abortion. In: Z. Woldehiwet and M. Ristic (eds), *Rickettsial and Chlamydial Diseases of Domestic Animals*, (Pergamon Press Ltd., Oxford), 349–360

Aitken, I.D., 2000. Chlamydial abortion. In: *Diseases of Sheep*, 3rd edn, (Blackwell Science, Oxford), 810–86

Aitken, I.D., 2004. Enzootic abortion of ewes (ovine chlamydiosis). In: *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, 5th edn, (Office International des Epizooties, Paris, France)

Buendia, A.J., Cuello, F., Del Rio, L., Gallego, M.C., Caro, M.R. and Salinas, J., 2001. Field evaluation of a new commercially available ELISA based on recombinant antigen for diagnosing *Chlamydophila abortus* (*Chlamydia psittaci* serotype 1) infection. *Veterinary Microbiology*, **78**, 229–239

Buxton, D. and Henderson, D., 1999. Infectious abortion in sheep. *In Practice*, **21**, 360–368

Everett K.D., Bush, R.M. and Andersen, A.A., 1999. Emended description of the order Chlamydiales, proposal of *Parachlamydiaceae* fam.nov. and *Simkaniaceae* fam.nov., each containing one monotypic genus, revised taxonomy of the family *Chlamydiaceae*, including a new genus and five new species, and

standards for the identification of organisms. *International Journal of Systematic Bacteriology*, **49**, 415–440

Foggie, A., 1973. Preparation of vaccines against enzootic abortion of ewes. A review of the research work at the Moredun Institute. *Veterinary Bulletin*, **43**, 587–590

Gut-Zangger, P., Vretou, E., Psarrou, E., Pospischil, A. and Thoma, R., 1999. Chlamydienabort beim Schaf: Möglichkeiten der serologischen Diagnostik mit einem kompetitiven ELISA und Einblick in die epidemiologische Situation in der Schweiz. *Schweizer Archiv für Tierheilkunde*, **141**, 361–366

Hässig, M., Waldvogel, A., Corboz, L., Strickler, L., Zanoni, R., Weiss, M., Regi, G., Peterhans, E., Zerobin, K. and Rüsch, P., 1995. Untersuchungen in Betrieben mit gehäuftem Verwerfen beim Rind. *Schweizer Archiv für Tierheilkunde*, **137**, 445–453

Linklater, K.A. and Dyson, D.A., 1979. Field studies on enzootic abortion of ewes in south east Scotland. *Veterinary Record*, **105**, 387–389

Longbottom, D., Fairley, S., Chapman, S., Psarrou, E., Vretou, E. and Livingstone, M., 2002. Serological diagnosis of ovine enzootic abortion by enzyme-linked immunosorbent assay with a recombinant protein fragment of the polymorphic outer membrane protein POMP90 of *Chlamydophila abortus*. *Journal of Clinical Microbiology*, **40**, 4235–4243

Markey, B.K., McNulty, M.S. and Todd, D., 1993. Comparison of serological tests for the diagnosis of *Chlamydia psittaci* infection of sheep. *Veterinary Microbiology*, **36**, 233–252

McEwen, A.D. and Foggie, A., 1954. Enzootic abortion of ewes. Comparative studies of different vaccines. *Veterinary Record*, **66**, 393–397

Prettejohn, M.W.H., 1988. Screening of ewes for enzootic abortion in the sheep and goat health scheme. *State Veterinary Journal*, **42**, 115–118

Rodolakis, A., 1983. *In vitro* and *in vivo* properties of chemically induced temperature-sensitive mutants of *Chlamydia psittaci* var. *ovis*: screening in a murine model. *Infection and Immunity*, **42**, 525–530

Rodolakis, A. and Bernhard, F., 1984. Vaccination with temperature-sensitive mutant of *Chlamydia psittaci* against enzootic abortion of ewes. *Veterinary Record*, **114**, 193–194

Rodolakis, A., Salinas, J. and Papp, P., 1998. Recent advances on ovine chlamydial abortion. *Veterinary Research*, **29**, 275–288

Rodolakis, A. and Souriau, A., 1979. Clinical evaluation of a commercial vaccine against chlamydial abortion of ewes. *Annales de Recherche Vetérinaire*, **10**, 41–48

Rodolakis, A. and Souriau, A., 1983. Response of ewes to temperature-sensitive mutants of *Chlamydia psittaci (*var. *ovis)* obtained by NTG mutagenesis. *Annales de Recherche Vetérinaire*. **14**, 155–161

Salti-Montesanto, V., Tsoli, E., Papavassiliou, P., Psarrou, E., Markey, B.K., Jones, G.E., and Vretou, E., 1997. Diagnosis of ovine enzootic abortion, using a competitive ELISA based on monoclonal antibodies against variable segments 1 and 2 of the major outer membrane protein of *Chlamydia psittaci* serotype 1. *American Journal of Veterinary Research*, **58**, 228–235

Stamp, J.T., McEwen, A.D., Watt, J.A.A. and Nisbet, D.I., 1950. Enzootic abortion in ewes. I. Transmission of the disease. *Veterinary Record*, **62**, 251–254

Stamp, J.T., Watt, J.A.A. and Cockburn, R.B., 1952. Enzootic abortion in ewes. Complement fixation test. *Journal of Comparative Pathology*. **62**, 93–101