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Serological Diagnosis of Ovine Enzootic Abortion by Comparative Inclusion Immunofluorescence Assay, Recombinant Lipopolysaccharide Enzyme-Linked Immunosorbent Assay, and Complement Fixation Test

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Since the 1950s, serological diagnosis of ovine enzootic abortion (OEA), caused by strains of *Chlamydia psittaci*, has been based mainly on the complement fixation test (CFT), which is neither particularly sensitive nor specific since antibodies to other chlamydial and enterobacterial pathogens may be detected. In this study, a recombinant enzyme-linked immunosorbent assay (rELISA) (medac, Hamburg, Germany), based on a unique chlamydial genus-specific epitope of *Chlamydia trachomatis* L2 lipopolysaccharide, was evaluated for sensitivity and specificity as a primary screening assay for OEA by comparison with the CFT. A comparative inclusion immunofluorescence assay (IFA), in which antibody titers to *C. psittaci* and *Chlamydia pecorum* were examined, was used as the reference test for 573 serum samples from four flocks. Reactivity to *C. pecorum* was measured since inapparent intestinal infections by *C. pecorum* are believed to be common in British flocks. In detecting positive sera from an abortion-affected flock, in which a *C. pecorum* infection was also suggested by IFA, the rELISA outperformed the CFT with significant evidence for increased sensitivity ($P = 0.003$). In two flocks in which *C. pecorum* infections alone were suggested by IFA, the rELISA and CFT were prone to detect low levels of false-positive results, but the values were not significant. The rELISA provided results in one flock in which sera that were anticomplementary could not be resolved by the CFT. In another flock in which abortion had not occurred but infection by both chlamydial species was suspected, no significant difference was found between the sensitivities of the rELISA and CFT. The rELISA could not differentiate ovine *C. psittaci* and *C. pecorum* infections but was shown to be a more sensitive primary screening test for OEA than was the CFT, particularly where abortion had occurred and even when antibodies due to additional inapparent infection(s) by *C. pecorum* were present.

Ovine enzootic abortion (OEA) is the most diagnosed cause of ovine fetopathy and reproductive failure in Great Britain (10), with an estimated economic loss of up to £20 million ($32 million) a year (1). In Britain, the current population of breeding sheep is about 20 million, with one in four flocks estimated to be affected by the disease. A resurgence of OEA in southeast Scotland in the late 1970s (34), which was probably due to a combination of factors such as intensive management, increased sheep movement, and vaccine breakdown, has been followed by the spread of the disease to most other areas of Britain.

Isolation of the causative agent, the obligate intracellular gram-negative bacterium *Chlamydia psittaci*, as identified by Stamp et al. (53), from the products of conception is generally regarded as the "gold standard" procedure for diagnosis of the disease but may not always be applicable or possible in field or laboratory situations. As a result, diagnosis of chlamydial infections in animals has been aided by the serological demonstration of either raised or rising levels of chlamydia-specific immunoglobulin G (IgG) antibodies between the acute and convalescent phases of infection. The most widely used serodiagnostic test for OEA in veterinary laboratories has been the complement fixation test (CFT), which was first developed and used in the 1950s (54). The CFT is based on the detection of antibodies to the genus-specific chlamydial lipopolysaccharide (LPS) antigen which possesses several epitopes, including a surface-exposed, immunoaccessible, genus-specific epitope. The broad specificity of the test is a major disadvantage in the diagnosis of OEA, since antibodies to other infections, such as clinically inapparent chlamydial intestinal infections, and to other serologically cross-reactive gram-negative bacteria may be detected in the test. Clinically inapparent intestinal infections, which may be caused by strains of *Chlamydia pecorum* (22), are prevalent in both OEA-affected and unaffected flocks (30). Storz (55) reported that apparently normal and healthy sheep may excrete chlamydial agents in feces and that CFT antibody levels may be typically low in these animals. It was also observed that the poor humoral antibody responses produced to some chlamydial infections may not be detected by CFT because of the low sensitivity of the test (57). Antigenic and genomic differences between *C. psittaci* and *C. pecorum* isolates have been reported by several groups. Among these reports, Spears and Storz (52) described biotype characteristics and differences in a range of mammalian isolates, which were

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supported by serum neutralization studies (48, 56, 60) and immunotyping studies with the microimmunofluorescence test (19, 41, 51). Differences have also been revealed with monoclonal antibodies (2, 17, 23, 32, 50) and in pathogenicity studies of mice (3, 8, 45). In genomic studies, variations have been shown by restriction endonuclease analysis (18, 21, 37, 39, 46, 58), by DNA-DNA homology (22), and by sequencing analysis (31). Type and/or species differences between isolates have also been demonstrated by culture, inclusion immunofluorescence, polyacrylamide gel electrophoresis, and immunoblotting profiles (25) and by PCR based on amplification of part of the *ompA* gene (28).

In the indirect diagnosis of OEA there have been several reports of the use of the whole-inclusion immunofluorescence assay (IFA) (11, 20, 30, 36), which has been adapted from the method of Richmond and Caul (44) for *Chlamydia trachomatis* antibody detection. The superior sensitivity of the IFA over the CFT, which may be due to the additional detection of type-specific antibodies, has been noted. In comparative IFAs, using more than one chlamydial strain or type, the test may provide effective resolution of the serological status of sheep at the flock level and to a lesser extent in individual animals (14).

In time, improved enzyme-linked immunosorbent assay (ELISA)-based tests, which may allow automation and quantitation, may replace the currently used immunofluorescence methodologies. A simple, sensitive, and reproducible serological test for OEA could be of considerable clinical and epidemiological value and also decrease the cost of screening. The recombinant ELISA (rELISA) evaluated in this study was based on a unique and immunodominant genus-specific epitope of *C. trachomatis* L2 LPS, which did not possess any cross-reactive determinants that might have been recognized by antibodies to other enterobacteria. In our investigation, sera from an OEA-affected flock, an OEA-free flock, and two flocks suspected of subclinical chlamydial intestinal infection were examined for chlamydial IgG antibodies by comparative IFA as the reference test (in which reactivities to *C. psittaci* and *C. pecorum* were determined) and by the rELISA and CFT. The aim was to determine whether the rELISA was a suitable and improved alternative to the CFT as a primary screening assay for OEA.

MATERIALS AND METHODS

Chlamydia. A *C. psittaci* isolate, designated T35, was recovered from a lowland sheep flock with a history of abortion from North Wales and was a kind gift from M. J. Clarkson and H. L. Philips of Liverpool University, Liverpool, United Kingdom. The type strain of *C. pecorum* (ATCC VR628), associated with sporadic bovine encephalomyelitis, was obtained from the American Type Culture Collection, Rockville, Md.

Serum samples. Serum samples were obtained from three flocks from England (Berkshire, Shropshire, and Surrey) and from the North Wales flock indicated above: (i) 174 serum samples were obtained from the North Wales flock, in which there was clinical evidence of abortion, several months prior to the isolation of T35; (ii) 200 serum samples were obtained from the Surrey flock, which had no previous history of chlamydial abortion or other clinically apparent chlamydial infections and was regarded as the negative control flock; (iii) 170 serum samples were obtained from the Berkshire flock, and (iv) 29 serum samples were obtained from the Shropshire flock. The latter two flocks had shown no signs of abortion, but subclinical chlamydial infection was suspected since a number of serum samples from both flocks had demonstrated low positive CFT titers. On receipt all sera were stored at −20°C. Sera were inactivated at 60°C for 30 min prior to use in serological tests, since it had been shown previously (13) that inactivation at 60°C, compared with that at 56°C, reduced the proportion of anticomplementary sheep sera in the CFT.

Indirect inclusion IFA. The comparative IFA was carried out by preparing separate multiwell slides (ICN Biomedicals Ltd., Thame, Oxfordshire, United Kingdom) of acetone-fixed inclusions of *C. psittaci* T35 and the *C. pecorum* type strain ATCC VR628 in infected McCoy cell monolayers as described previously (25). In the IFAs, 8-μl drops of sera diluted serially from 1/100 to 1/6,400 in phosphate-buffered saline (Dulbecco A) (PBSA), pH 7.2, were incubated for 45 min at 37°C. Slides were washed three times for 3 min each wash in the buffer. Fluorescein-isothiocyanate labelled donkey anti-sheep whole IgG (Sigma, Poole, Dorset, United Kingdom) was added and incubated for 30 min at 37°C, after which slides were washed as before in buffer containing 2.5 μg of Evans blue counterstain ml^{-1}. Slides were mounted in 1:1 glycerol-buffer and observed at ×252 magnification on a Leitz fluovert microscope. Sera with end-point titers equal to or greater than 1/1,600 were considered positive. This threshold value was derived from an earlier study (data not shown) in which sera from a closed, abortion-free flock produced titers of 1/800 or less by IFA compared to much higher mean titers for non-abortion-free flock sera. In the closed flock a natural *C. pecorum* infection was shown to be present by isolation of the agent (42) and subsequent sequencing of the *ompA* gene (24).

Positive reactions to both the *C. psittaci* and *C. pecorum* strains with approximately similar IFA titers of 1/1,600, 1/3,200, or 1/6,400, or results in which the reaction to *C. psittaci* alone was positive (or stronger), were interpreted as being indicative of *C. psittaci* infection and may also have represented the presence of a mixed infection by strains of both chlamydial species. Higher positive reactivity to the *C. pecorum* strain (in the range 1/1,600, 1/3,200, or 1/6,400) with lesser reactivity to the *C. psittaci* strain (i.e., typically, negative at 1/800 or less or, less frequently, positive at 1/1,600) was regarded as indicating a *C. pecorum* type infection.

Enzyme immunoassay. Procedures for the *Chlamydia* IgG rELISA (medac, Hamburg, Germany) were carried out in accordance with the manufacturer's recommendations. Sera were tested at a single dilution of 1/100 in duplicate, and horseradish peroxidase-labelled donkey anti-sheep whole IgG (Sigma) was used as the conjugate. Performance time was about 3 h, excluding serum dilution preparation and ELISA plate reading. To derive a cutoff value—above which sera were considered positive—a mean optical density value for blank wells (i.e., treated with conjugate and substrate only) was subtracted from the mean optical density value for the 200 serum specimens from the abortion-free, negative-status Surrey flock. The modified negative mean plus 3 standard deviations represented the cutoff.

CFT. A standard microplate method (16) which was modified from the procedure of Stamp et al. (54) was performed. Sheep erythrocytes were obtained and washed in Veronal buffer. Hemolysin and guinea pig complement (Tissue Culture Services, Botolph Claydon, Bucks, United Kingdom) were pretitrated before use. The *C. psittaci* OEA isolate A22 (kindly supplied by I. Aitken, Moredun Research Institute, Edinburgh, United Kingdom) was propagated in McCoy cell cultures for 7 to 10 days, after which the infected cells were harvested by scraping, sonicated, heat inactivated at 56°C for 30 minutes, and used as CFT antigen. In the test, sera were titrated from 1/4 to 1/1,024 in CFT diluent (Oxoid, Basingstoke, Hants, United Kingdom), complement fixation was performed for 1 h at 37°C, and the end-point titers were scored as wells containing approximately or less than 50% lysis. A titer equal to or greater than 1/32 was considered positive, which was based on a previous assessment of the CFT with a number of selected flocks with known clinical histories (43).

Statistical analysis. Standard procedures were used to calculate the sensitivity, specificity, and positive and negative predictive values for each test (27). Significance probabilities of differences in test sensitivities were determined by the two-sided McNemar test (40).

RESULTS

Five hundred seventy-three sheep serum samples from four flocks were examined by comparative IFA (versus *C. psittaci* T35 and *C. pecorum* VR628) and by rELISA and CFT for the presence of chlamydial antibodies. A summary of the results is presented in Table 1, with details of the clinical status of each flock, and an interpretation of the serological results is offered.

It was noted that IgG reactivity to *C. pecorum*, as determined by IFA, was present to a high level in sera from the flocks from North Wales (92.5%), Berkshire (93.5%), and Shropshire (75.9%), which was likely to indicate the presence of infection(s) by that species. In contrast, in the Surrey flock, which was regarded as the *C. psittaci*-negative control flock, a lower level of sera (30%) showed reactivity to *C. pecorum*. In the North Wales OEA-affected flock, antibody reactivity to *C. psittaci* by IFA was detected in 51.7% of sera and in almost twice as many sera by rELISA (16.6%) as by CFT (8.6%).

In the Surrey and Shropshire flocks antibody reactivity to *C. psittaci* by IFA was considerably lower, with only 0.5 and 6.9% of sera positive, respectively. In these two flocks the rELISA detected a low number of positives, i.e., 2.5% in the Surrey flock and 3.4% in the Shropshire flock, whereas no sera were CFT positive in the Surrey flock. Of the 29 serum samples from the Shropshire flock, 2 of the 14 serum specimens for which CFT titers were obtained were CFT positive; CFT titers were

TABLE 1. Summary of the serological analyses of four sheep flocks by *C. psittaci* and *C. pecorum* IFAs, rELISA, and CFT

Flock location (breed[s])	Clinical status	No. of serum samples total	No. (%) of samples positive by:				Serological interpretation of infection
			C. psittaci T35 IFA[a]	*C. pecorum* VR628 IFA[a]	rELISA[b]	CFT[c]	
North Wales (Lleyn)	Chlamydial abortion (T35 isolate)	174	90 (51.7)	161 (92.5)	29 (16.6)	15 (8.6)	*C. psittaci* and *C. pecorum* infections
Surrey (polled Dorset and Scottish blackface)	Abortion free	200	1 (0.5)	60 (30)	5 (2.5)	0 (0)	Moderate-level *C. pecorum* infection
Berkshire (Romney cross and Cambridge)	Abortion-free, suspected subclinical infection	170	25 (14.7)	159 (93.5)	23 (13.5)	20 (11.8)	High-level *C. pecorum* and probable low-level *C. psittaci* infections
Shropshire (Texel)	Abortion-free, suspected subclinical infection	29	2 (6.9)	22 (75.9)	1 (3.4)	2/14[d]	*C. pecorum* infection

[a] Sera with titers of ≥1/1,600 were considered positive.
[b] Cutoff value determined as the mean value of 200 serum samples from the Surrey flock plus three standard deviations.
[c] Sera with titers of ≥1/32 were considered positive.
[d] Of the 29 serum samples, 15 were anticomplementary and thus no CFT titers were obtainable; of the remaining 14 samples, 2 were CFT positive.

not obtained for the remaining 15 serum samples since the sera were anticomplementary. In the Berkshire flock, 14.7% of sera had a positive reaction to *C. psittaci* by IFA, whereas 13.5% were positive by rELISA and 11.8% were positive by CFT.

Analysis of the North Wales OEA-affected flock. Further characteristics of the results of the four tests with the North Wales flock are indicated in Table 2. In the IFAs, 89 of 174 serum samples reacted positively to both *C. psittaci* and *C. pecorum* and 1 serum sample reacted positively to *C. psittaci* only. The rELISA showed 64.9% overall agreement with the *C. psittaci* IFA results, compared to 56.9% for CFT. In this flock, 72 of 174 serum samples (i.e., 41.4%) reacted positively to *C. pecorum* by IFA but were negative in all the other tests.

In the 89 serum samples that reacted to *C. psittaci* and *C. pecorum* by IFA, in each group of titer reactivity to *C. psittaci* (at 1/6,400, 1/3,200, and 1/1,600) the rELISA performed better than the CFT by detecting 28 compared to 14 positive serum samples. The majority of serum samples ($n = 55$) reacted at 1/3,200 to *C. psittaci*. One further serum sample was positive in all four tests.

Analysis of the Surrey negative control flock. As shown in Table 3, 60 of 200 serum specimens from the negative control flock gave positive reactions to *C. pecorum* by IFA—with the majority (43 samples) giving titers of 1/1,600—but showed negative reactivity to *C. psittaci*. Only 1 of the 60 serum samples showed a positive reaction to *C. psittaci* by IFA, reacting to both species at 1/1,600. However, none of the sera which reacted positively to *C. pecorum* by IFA showed a completely negative reaction to *C. psittaci* by IFA, since titers ranging between 1/100 and 1/800 were found. Of the 60 reactive serum samples, 59 (i.e., 27.5% of the total flock) reacted to *C. pecorum* alone and were negative in the other tests. The CFT showed a slightly better agreement at 99.5% with the *C. psittaci* IFAs compared with 97% for the rELISA. The rELISA showed some evidence of less specificity than the CFT under the infection conditions in this flock, in which five serum samples that were positive by rELISA were negative by both *C. psittaci* IFA and CFT ($P = 0.06$).

Analysis of the Berkshire flock. The majority of sera in the Berkshire flock (i.e., 64.1%) reacted positively to *C. pecorum* by IFA but negatively in the other tests (Table 4). In the group of 25 serum samples that reacted positively to both *C. psittaci* and *C. pecorum* by IFA, 14 showed equal or greater reaction to *C. pecorum* whereas 11 reacted more strongly to *C. psittaci*. In the same group of sera, the rELISA detected 7 positives compared with 11 by CFT. Of the 145 serum samples that were *C. pecorum* positive but *C. psittaci* negative by IFA, the rELISA and CFT gave 16 and 9 potentially false-positive results for OEA, respectively. Isolation of *C. pecorum* intestine-type chlamydia, or *C. psittaci* for that matter, was attempted from 14 fecal samples obtained from the Berkshire flock but was not successful. The CFT gave better overall agreement with the *C. psittaci* IFA (at 86.5%) than the rELISA (at 80%), but differences in sensitivity and specificity were not significant.

Analysis of the Shropshire flock. The main reactivity in the Shropshire flock was in 22 serum samples to *C. pecorum* by IFA (Table 5). Only two serum samples reacted to *C. psittaci* by IFA. One serum sample was positive by rELISA and *C. pecorum* IFA but negative in the other two tests. CFT titers were not obtainable for 15 of 29 serum samples because of anticomplementary effects. This phenomenon was not observed in sera from the other flocks. The rELISA showed 89.7% agreement with the *C. psittaci* IFA results, whereas an accurate value for the CFT was not calculable because of the number of anticomplementary sera.

Characteristics of rELISA, CFT, and *C. psittaci* IFA with sera from the North Wales flock. The comparative IFAs were used to determine antibody reactivity to both chlamydial species as a means to facilitate interpretation of the serological status of each flock. However, the sensitivities, specificities, and positive and negative predictive values for detecting antibodies to chlamydial abortion strains by rELISA and CFT were derived by comparison to the *C. psittaci* IFA alone (Table 6). Sensitivities of the two tests were considerably lower than that of the IFA, but the rELISA was almost twice as sensitive in detecting positives as the CFT (32.2 compared to 16.7%). This

TABLE 2. Analysis of the North Wales OEA-affected flock

Result of:				No. of samples[a]
C. psittaci IFA	*C. pecorum* IFA	rELISA	CFT	
+	+	+	+	13
+	+	+	−	15
+	+	−	+	2
+	+	−	−	59
+	−	+	−	1
−	+	−	−	72
−	−	−	−	12

[a] Agreement when the *C. psittaci* IFA was positive or negative: *C. pecorum* IFA, 101 of 174 = 58%; rELISA, 113 of 174 = 64.9%; CFT, 99 of 174 = 56.9%.

TABLE 3. Analysis of the Surrey negative control flock

Result of:				No. of samples[a]
C. psittaci IFA	*C. pecorum* IFA	rELISA	CFT	
+	+	−	−	1
−	+	+	−	4
−	−	+	−	1
−	+	−	−	55
−	−	−	−	139

[a] Agreement when the *C. psittaci* IFA was positive or negative: *C. pecorum* IFA, 141 of 200 = 70.5%; rELISA, 194 of 200 = 97%; CFT, 199 of 200 = 99.5%.

result provided significant evidence for an increased sensitivity for the rELISA ($P = 0.003$). The specificities and positive predictive values of the rELISA and CFT were similar by comparison with the IFA, but a higher negative predictive value was demonstrated by the rELISA at 57.9% compared with the CFT at 52.8%.

Characteristics of the rELISA, CFT, and *C. psittaci* IFA with sera from the Berkshire flock. In the Berkshire flock, with no abortion, the CFT reacted more frequently (at 44%) than the rELISA (at 28%) to sera that reacted positively to *C. psittaci* by IFA (Table 7). But this was not significant evidence of a difference in sensitivity ($P = 0.26$). In addition, the specificities of rELISA and CFT with sera that were negative by *C. psittaci* IFA did not show a significant difference ($P = 0.24$). Specificities of 89 and 93.8% for the rELISA and CFT, respectively, and negative predictive values of 87.7 and 90.7%, respectively, were obtained, whereas the positive predictive values differed at 55% for the CFT and 30.4% for the rELISA.

Characteristics of the rELISA, CFT, and *C. psittaci* IFA with sera from all four flocks. If the 15 anticomplementary serum samples are disregarded, among the remaining 558 samples the rELISA and CFT produced different results in 56; 38 were rELISA positive and 18 were CFT positive, implying a greater sensitivity for the rELISA ($P = 0.012$). However, when the *C. psittaci* IFA results were adduced, the rELISA agreed with the IFA in 27 cases while the CFT showed agreement for the other 29 serum samples, so that no overall difference in test accuracy was apparent.

DISCUSSION

In this study, comparative inclusion IFA, rELISA, and CFT were evaluated for the serological diagnosis of OEA in sera obtained from three sheep flocks from England and one flock from North Wales. The aim of the study was to determine whether the rELISA, based on a genus-specific LPS epitope of *C. trachomatis* L2, would perform as an improved primary screening assay for OEA compared with the traditionally used CFT. The comparative IFA based on antibody reactivity to strains of *C. psittaci* and *C. pecorum* was used as the reference method and because of its sensitivity is used in this laboratory as a secondary test to aid in the serodiagnosis of chlamydial infections. The test detects IgG antibodies to chlamydial inclusions containing both protein and LPS antigenic determinants.

For the purpose of this study, the T35 ovine abortion-associated strain of *C. psittaci* and the type strain of *C. pecorum*, i.e., ATCC VR628 (22), were used in the IFAs. It could be argued, in view of the apparent heterogeneity within the *C. pecorum* group, that the sporadic bovine encephalomyelitis-associated strain may not be particularly antigenically representative of less invasive chlamydial intestinal strain agents found in sheep. Nevertheless, in the Berkshire, Shropshire, and North Wales flocks a major proportion of sera reacted to *C. pecorum* ATCC VR628 by IFA and were negative in all other tests.

Since the homologous abortion-affected flock isolate T35 was used as the *C. psittaci* IFA antigen in this study, it could be expected that type-specific, abortion-associated strain antibodies as well as those to species- and genus-specific epitopes would be detected, thereby increasing the sensitivity of the test. Despite this sensitivity, application of the comparative IFA is not particularly feasible for large-scale routine use because of the labor-intensive antigen preparation and a degree of sub-

TABLE 4. Analysis of the Berkshire flock

Result of:				No. of samples[a]
C. psittaci IFA	*C. pecorum* IFA	rELISA	CFT	
+	+	+	+	6
+	+	+	−	1
+	+	−	+	5
+	+	−	−	13
−	+	+	−	16
−	+	−	+	9
−	+	−	−	109
−	−	−	−	11

[a] Agreement when *C. psittaci* IFA was positive or negative: *C. pecorum* IFA, 36 of 170 = 21.2%; rELISA, 136 of 170 = 80%; CFT, 147 of 170 = 86.5%.

TABLE 5. Analysis of the Shropshire flock

Result of:				No. of samples[a]
C. psittaci IFA	*C. pecorum* IFA	rELISA	CFT	
+	+	−	−	1
+	−	−	+	1
−	+	−	+	1
−	+	−	−	8
−	−	−	−	3
−	+	+	AC[b]	1
−	+	−	AC	11
−	−	−	AC	3

[a] Agreement when *C. psittaci* IFA was positive or negative: *C. pecorum* IFA, 7 of 29 = 24.1%; rELISA, 26 of 29 = 89.7%; CFT (when possible), 12 of 14 = 85.7%.

[b] AC, anticomplementary.

TABLE 6. Characteristics of the rELISA and CFT compared with *C. psittaci* IFA with sera from the North Wales OEA-affected flock

Test vs IFA and result	No. of samples with *C. psittaci* IFA result	
	+	−
rELISA[a]		
+	29	0
−	61	84
CFT[b]		
+	15	0
−	75	84

[a] rELISA sensitivity, 29 of 90 = 32.2%. rELISA specificity, 84 of 84 = 100%. Positive predictive value, 29 of 29 = 100%. Negative predictive value, 84 of 145 = 57.9%.

[b] CFT sensitivity, 15 of 90 = 16.7%. CFT specificity, 84 of 84 = 100%. Positive predictive value, 15 of 15 = 100%. Negative predictive value, 84 of 159 = 52.8%.

TABLE 7. Characteristics of the rELISA and CFT compared with *C. psittaci* IFA with sera from the Berkshire flock

Test vs IFA and result	No. of samples with *C. psittaci* IFA result	
	+	−
rELISA[a]		
+	7	16
−	18	129
CFT[b]		
+	11	9
−	14	136

[a] rELISA sensitivity, 7 of 25 = 28%. rELISA specificity, 129 of 145 = 89%. Positive predictive value, 7 of 23 = 30.4%. Negative predictive value, 129 of 147 = 87.7%.

[b] CFT sensitivity, 11 of 25 = 44%. CFT specificity, 136 of 145 = 93.8%. Positive predictive value, 11 of 20 = 55%. Negative predictive value, 136 of 150 = 90.7%.

jectivity in the reading of the assay and therefore in the interpretation of the results.

The rELISA used in this study is based on a unique and immunodominant chlamydial genus-specific epitope (9) not containing any cross-reactive epitopes recognized by antibodies arising from exposure to other enterobacterial LPSs (5). Furthermore, the epitope has been reported as a chemotaxonomic marker for *Chlamydia* spp. since it has not been found in the LPSs of other bacteria (6). The rELISA might be expected to perform with less sensitivity than the whole-inclusion chlamydial IFA since it is based on a single recombinant antigen. In the absence of other, perhaps more suitable tests based on *C. psittaci* antigen(s), we decided to evaluate the rELISA based on L2.

In contrast to the rELISA, the CFT detects genus-specific antibodies to chlamydial LPS, as well as antibodies to other enterobacterial species which cross-react with epitopes on the chlamydial LPS. It is also possible that the IFA, like the microimmunofluorescence test, is subject to false-positive results due to interbacterial antigenic cross-reactivity as reported for *C. trachomatis* serodiagnosis in humans (47).

In the current study, in the examination of the North Wales abortion-affected flock, from which *C. psittaci* T35 was obtained from a placenta, there was evidence that the sensitivity for detecting *C. psittaci*-positive results was significantly greater in the rELISA compared with the CFT. In addition, because of the use of a defined chlamydia-specific antigen, the rELISA might be expected to perform with greater specificity on the abortion-affected flock sera than the CFT. But this was not the case, since both tests showed 100% specificity of positive reaction. The high level of antibody reactivity to *C. pecorum* by IFA, particularly in sera which were negative in the other tests, led to the interpretation that a mixed infection by both *C. psittaci* and *C. pecorum* may have been present in this flock. It was noted in the abortion-affected flock sera that in each category of IFA-positive reactivity to *C. psittaci* the rELISA outperformed the CFT in sensitivity.

In the Surrey flock, which was regarded as the abortion-negative control flock, the majority of the 200 serum samples were negative in all four tests. However, 27.5% of the sera were positive by *C. pecorum* IFA alone, suggesting the presence of a moderate-level, clinically inapparent infection. Five serum samples that were negative by *C. psittaci* IFA apparently gave false-positive results by rELISA. In four of the samples this was probably due to cross-reactive antibodies to *C. pecorum*. There was some evidence that the rELISA showed a lower specificity than the CFT in this flock.

Where clinical abortion had not occurred, e.g., in the Berkshire flock, and where the presence of CFT-positive sera gave the suspicion of a *C. psittaci* infection, the rELISA performed with less sensitivity and specificity than the CFT with sera positive by *C. psittaci* IFA (Table 7), but not to a significant extent in either case. This suggests, however, that the rELISA could not be used as a predictive abortion assay; it appears to be more effective in flock serodiagnosis for cases in which abortion has already occurred. The IFA reactivity pattern in the Berkshire flock implied a *C. pecorum* infection with a probable *C. psittaci* infection.

Notwithstanding this, the rELISA performed more reliably with sera from the Shropshire flock, in which many sera were anticomplementary by CFT. Abortion had also not occurred in this flock, but the IFA reactivity pattern was more similar to that of the negative Surrey flock than to those of the likely mixed-infection flocks from Berkshire and North Wales. It is likely that performance characteristics of the rELISA could be improved by the use of conjugates with greater specificity for sheep IgG subclasses. So far, the dissection of the chlamydial antigen-specific immune response in sheep has been reported only in limited antibody studies (33).

A number of antibody ELISAs for OEA based on the use of whole chlamydia with or without chemical pretreatment have been previously described (4, 11, 15, 33, 36). Where comparisons were made, CFTs were found to be less sensitive than the ELISAs and the IFA or microimmunofluorescence test (4, 11, 15, 36). The preparation of antigen, the presence of cross-reactive epitopes, and the damaging effects of chemical treatment may have affected the sensitivities and specificities of the ELISAs. It should be stated that the development of standardized serological tests is not easily achievable when the tendency has been for individual laboratories to produce ELISAs for in-house serology based on chlamydial antigens of varying purity and ill-defined cross-reactivity.

In addition to the rELISA, three other commercially available whole-chlamydia ELISAs were examined (data not presented). The tests, based on *C. trachomatis* L2, all produced considerably higher proportions of false-positive results, as indicated by IFA, compared with the rELISA. A premise of reduced sensitivity and increased false positivity could be argued for flock-screening purposes; however, our requirement was for a serological test that would not produce a high level of false results needing secondary examination. Recently, one report suggested that flock screening for OEA by CFT alone could result in a high level of false-positive sera that were negative by ELISA and MIF and that such results would be indicative of chlamydial intestinal strain infection (4).

In the currently used serological tests for OEA, considerable cross-reactivity between different chlamydial subtypes can make interpretation of results difficult. LPS, given its genus specificity, might not represent the most appropriate serodiagnostic antigen for OEA. Protein antigens or epitopes which may be highly conserved and type specific in abortion isolates may be more suited for use in differential tests in the future. Interestingly, evidence of antigenic heterogeneity among LPS epitopes of different chlamydial species has been reported in chemical (7, 35) and monoclonal antibody (23, 38) studies. The strong antibody response to LPS shortly after chlamydial abortion in sheep appears to be serodiagnostically significant in enzootic abortion. Sheep infected with clinically inapparent chlamydial intestinal strains may have low to moderate levels of antibodies that cross-react with protein antigens in ovine abortion isolates, which may be due to continual protein antigen presentation during infection, but may produce lower or lowered antibody responses to LPS.

In view of the issues associated with the use of a genus-specific serological test for the primary detection of a disease such as OEA and in particular the interference of cross-reactive antibodies due to clinically inapparent chlamydial infections in sheep, there is a requirement for screening assays of greater sensitivity and specificity than the traditional CFT. The rELISA goes some way to fulfilling these criteria. The advantages of the rELISA are its reproducibility, ease and speed of performance, and use of a chemically defined, chlamydia-specific antigen. The main disadvantage is that it is not suitable for distinguishing between infections due to different chlamydial species. However, where abortion due to *C. psittaci* has occurred, as in the North Wales flock, even though antibodies due to a subclinical *C. pecorum* intestinal infection were also present, the rELISA showed favorable sensitivity compared with the CFT. More problematic is the interpretation of results where abortion has not (yet) occurred in flocks with mixed infections, such as in the Berkshire flock, where results were resolved by IFA.

An IFA similar to the one reported here, but using ovine abortion and intestinal isolates, has been used in this laboratory since about 1987 as part of the Sheep and Goat Health Scheme in England and Wales as a secondary test to aid resolution of diagnosis. The pattern of reactivity is used to assess the infection status of flocks (14). The general observations agree in principle with those reported recently where a similar confirmation test for OEA antibodies was described (36). In a wider context and because of the apparent homogeneity of ruminant serotype I *C. psittaci* strains, improved primary screening tests such as the rELISA may also be suited to screening for diseases such as bovine chlamydial abortion, in which because of the predominantly IgG2 bovine subclass response to infection (49) the CFT is not a useful test (57). This potential is currently being investigated, particularly in view of several recent diagnoses and characterizations of bovine chlamydial infections, including abortion, in Great Britain (12, 26, 29).

As emphasized by Ward (59), there is a prerequisite in chlamydial immunochemistry for the development and use of improved antigens of defined specificity and seroepidemiological significance. Ultimately, the improved serodiagnosis of OEA, and other veterinary chlamydial infections, is likely to be based on the use of defined antigens and the detection of specific Ig isotypes to overcome the problems of poor specificity and to improve the sensitivity of indirect diagnosis.

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