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Detection of *Campylobacter* Antibodies in Sheep Sera by a Dot-ELISA Using Acid Extracts from *C. fetus* ssp. *fetus* and *C. jejuni* Strains and Comparison with a Complement Fixation Test

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With 2 figures and 3 tables

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Summary

In this study, a dot-enzyme-linked immunosorbent assay (Dot-ELISA) was evaluated in comparison with a complement fixation test (CFT) for the detection of *Campylobacter* antibodies in sheep sera. Acid glycine extracts (AGE) of both *Campylobacter fetus* ssp. *fetus* and *Campylobacter jejuni* strains that had been isolated from the gall-bladder of slaughtered sheep was used as antigen in both tests. A total of 153 sheep sera from aborted (74) and slaughtered (79) sheep were examined by both Dot-ELISA and CFT. Twenty-two sera showed anti-complementary activity were not suitable for CFT. Of the 22 sera showing anti-complementary activity, two sera were found to be positive in Dot-ELISA. Eighty-eight (67.2%) of the remaining 131 sera were negative by both Dot-ELISA and CFT using AGE of both *Campylobacter* strains whereas 43 sera (32.8%) gave different reaction patterns in Dot-ELISA and CFT with the extracts of both *Campylobacter* strains. Twelve sera were positive by both tests using AGE of *C. fetus* ssp. *fetus* but CFT failed to detect antibodies in nine of these sera when AGE of *C. jejuni* was used. Twelve sera were positive by both tests only when AGE of *C. fetus* ssp. *fetus* was used. Eleven sera were positive only by CFT. Seven of these reacted only with the AGE of C. *fetus* ssp. *fetus* and four sera were positive by using AGE of both *Campylobacter* strains. The remaining eight sera were found to be positive only by dot-immunobinding assay either with the AGE of both *Campylobacter* strains or with the AGE of one of the *Campylobacter* strains. It is concluded that Dot-ELISA using AGE from *C. fetus* ssp. *fetus* could be employed for the detection of *Campylobacter* antibodies in sheep sera and the additional use of AGE from *C. jejuni* as antigen appeared not to be profitable for this purpose.

Introduction

Campylobacteriosis is an infectious disease associated with abortion, premature births, weak lambs and enteritis in sheep, cattle and humans. *Campylobacter fetus* ssp. *fetus* are known to be a causative agent of enzootic abortion in sheep. However, *C. jejuni* and *C. coli* have also been isolated from the cases of ovine campylobacteriosis (Blobel and Schliesser, 1982). An increasing antibody response after *Campylobacter* infections can be detected by several serological tests such as agglutination, bactericidal activity of sera and complement fixation test (CFT) (Watson et al., 1979; Jones et al., 1980; Mosimann et al., 1981). Recently an enzyme-linked immunosorbent assay (ELISA) has been employed for the detection of *Campylobacter* antibodies in human (Svedhem et al., 1983; Melby, 1987), cattle (Hum et al., 1991) and sheep (Gröhn and Genigeorgies, 1985; Yardımcı et al., 1994, 1998).

In the traditional ELISA a polystyrene plate is widely used as the solid phase for the binding of antibody or antigens. Nitrocellulose and nylon membranes have also been found suitable as alternative solid phase (Blais et al., 1989). Immunoassay on such membranes was first described as the dot-immunobinding assay (DIA; Hawkes et al., 1982) and in recent years DIA using enzyme-conjugated antibody has been termed Dot-ELISA (Kumar et al., 1985; Chand et al., 1989). The assay does not require special laboratory equipment such as an ELISA reader, which is necessary for conventional ELISA, and was found to be specific, sensitive, rapid and economical. The Dot-ELISA has already been used for the diagnosis of rabies (Heberling et al., 1987), amoebiasis (Kumar et al., 1985), for the detection of antibodies to bluetongue virus (Afsar et al., 1992) and to rinderpest (Ramadass et al., 1993) in cattle and for the detection of *Brucella* antibodies in both ovine sera (Chand et al., 1989a; Gürtürk et al., 1997) and bovine sera (Chand et al., 1989b: Gürtürk et al., 1999) and milk (Gürtürk et al., 1999). The use of Dot-ELISA for discrimination between the thermophilic *Campylobacter* species has already been reported (Griffiths et al., 1992), but no reports in respect of the use of Dot-ELISA for detecting *Campylobacter* antibodies in sheep sera was available.

The aim of the present study was to evaluate the Dot-ELISA using acid glycine extracts (AGE) from *C. fetus* ssp. *fetus* and *C. jejuni* for the detection of *Campylobacter* antibodies in sheep sera in comparison with CFT and its usefulness for sero-epidemiological studies as an alternative screening test.

Materials and Methods

Serum samples

A total of 153 sera collected from 74 aborted sheep from seven different flocks and from 79 slaughtered sheep were examined. The samples were collected from all aborted sheep 3–6 weeks after abortion. The serum samples were stored at −35°C until used.

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Control sera

Nine sera taken from two different sheep flocks in which abortions occurred were used as positive control. *Campylobacter fetus* ssp. *fetus* strains were isolated separately from two aborted fetus from both sheep flocks that were examined bacteriologically. All positive control sera showed antibody titres of ≤ 1 : 10 – 1 : 20 in CFT. Twelve sera taken from healthy sheep served as negative control. All negative control sera had antibody titre of ≤ 1 : 5 in CFT. AGE prepared from *C. fetus* ssp. *fetus* and *C. jejun*i separately served as antigen in CFT as described below.

Bacterial strains

Two strains of *C. fetus* ssp. *fetus* 64 and *C. jejuni* 58 were chosen to extract the antigen used in this study. Both strains were isolated from the contents of gall-bladders of different slaughtered sheep.

The *C. fetus* ssp. *fetus* strain was nalidixic acid-resistant and grew at +25°C. The C. *jejuni* strain was nalidixic acid-sensitive, hydrolysed Na-hippurate and grew +43°C but not +25°C. Both strains could be cultured on the selective medium with Skirrow's selective supplement (Oxoid Ltd., Basingstoke, UK) in a micro-aerobic atmosphere but not aerobically and anaerobically. They were catalase- and oxidase-positive, Gram negative bacteria with typical S form. Both strains were identified by testing for further biochemical characteristics as described previously (Holt et al., 1994).

Extraction of antigen

The antigen was extracted separately from both *Campylobacter* strains and used in CFT and Dot-ELISA. The bacteria were cultured on blood agar base (Oxoid No.2; Oxoid) supplemented with 7% defibrinated sheep blood and Skirrow's selective supplement for 48–72 h at 37 and 42°C, respectively.

Acid glycine extraction was performed after the method described originally by McCoy et al. 1975. The cultures were harvested into distilled water, washed twice and then suspended in 0.2 M glycine-hydrochloride, pH 2.2 (1 g of cell per 25 ml). The suspension was stirred and whole cells were removed by centrifugation at ~ $4000 \times g$ for 45 min The supernatant was neutralized with NaOH. Thimerosal at a concentration of 0.01% was added to the extracts and they was stored in small portions at −35°C until used.

Complement fixation test

In the CFT, the adapted micro-technique (Kolmer method) using cold fixation was employed as described previously (Alton et al., 1988). Serum samples were diluted from 1 : 5 to 1 : 160 and a serum sample showing 50% fixation at the dilutions of 1 : 5 and 1 : 10 were considered as doubtful and positive, respectively. An anti-complementary serum control and positive and negative control sera were also used for each test.

Dot-ELISA procedure

Dot-ELISA was carried out as described previously (Kumar et al., 1985) with some modifications. The nitrocellulose membrane (NCM) of 0.45 μm average pore size (Schleicher and Schüll, Dassel, Germany) was used as a solid phase in the Dot-ELISA. Firstly the NCM was embedded into distilled water and was cut into pieces (approx. 0.6 cm × 0.7 cm) and then dried at room temperature. For the binding of the antigen on the NCM, approximately 0.5 μl antigen diluted in phosphate-buffered saline (PBS; 0.01 mol/l pH 7.2) was dropped by means of a microcapillary into the centre of each piece of NCM. They were kept at room temperature until dried and then incubated in PBS-T (PBS plus 0.05% Tween 20) containing 5% milk powder for 1 h at 37°C to block the non-specific binding.

For testing of the sera, the prepared NCM pieces were washed five times with PBS-T and incubated with dilutions of sera in PBS-T for 30 min at 37°C. After washing five times with PBS-T they were incubated with conjugate at a dilution of 1 : 500 in PBS for 30 min at 37°C. Horseradish peroxidase-conjugated donkey anti sheep immunoglobulin (Ig) G (whole molecule) was used as a conjugate (Sigma, St Louis, MO, USA). Finally, the NCM pieces were washed again five times with PBS-T and the binding was revealed with freshly prepared substrate. In order to prepare the substrate, 3 mg of 4-chloro-1-naphtol (Merck, Darmstadt Germany) was dissolved in 1 ml of methanol and then 5 ml of PBS and 0.02 ml 30% hydrogen peroxide was added per millilitre of the solution. After

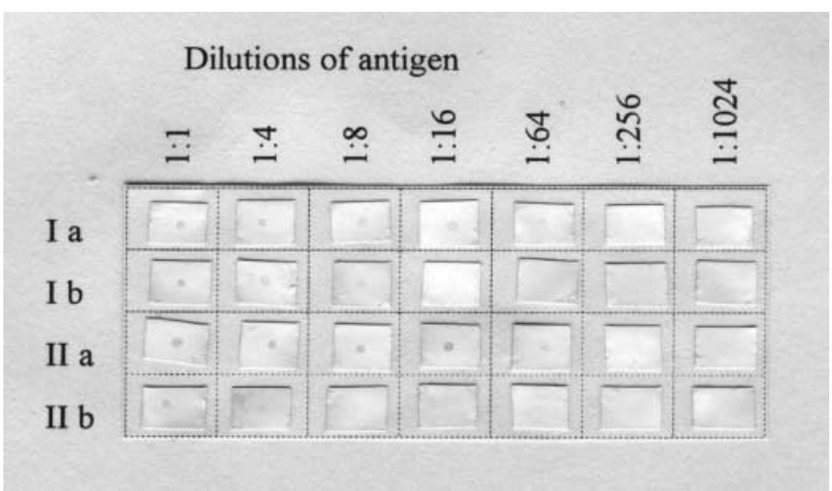

Fig. 1. The colour development in positive (a) and negative (b) control serum with varying dilutions of acid glycine extracts from *Campylobacter jejuni* (I) *and C. fetus* ssp. *fetus* (II) by dot-enzyme-linked immunosorbent assay.

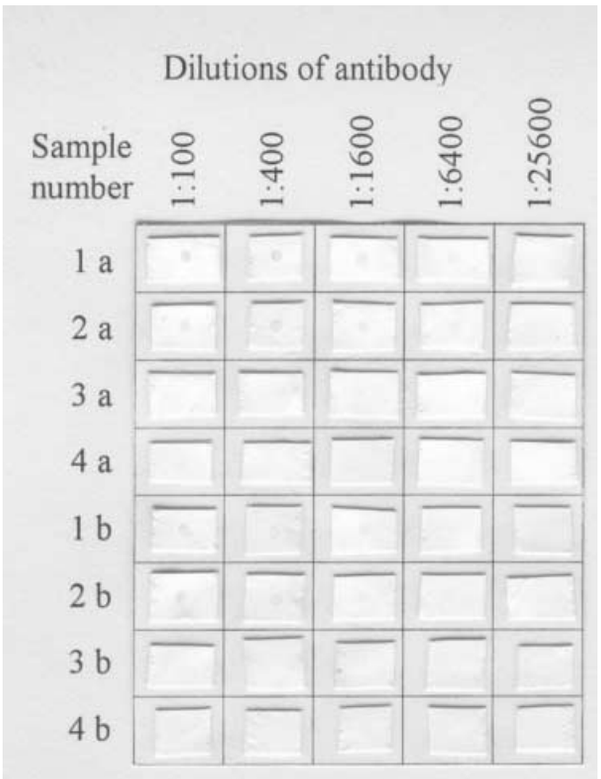

Fig. 2. The colour development in serum samples giving positive (1,2) and negative (3,4) reactions with varying dilutions of antibody by dot-enzyme-linked immunosorbent assay, using acid glycine extracts from *Campylobacter fetus* ssp. *fetus* (a) and *C. jejuni* (b).

treatment of NCM pieces with substrate for 10 min at room temperature, NCM pieces were washed in distilled water and the colour development on the site of the antigen was assessed. A positive reaction occurred as the development of a blue colour against a white background in a few minutes. Colour development appearing as a circular dot did not change for a long time at room temperature. The test was performed in a polystyrene 96-well microtitre plate and the reagents were taken in a volume of 0.3 ml.

The optimal dilutions of antigen and conjugate were determined after preliminary experiments employing different dilutions of pooled negative and pooled positive sera in a chequer-board analysis. Colour development at 1 : 100 dilution of the test sera was considered as positive, and positive and negative controls were used for each test.

Table 2. Results from 131 serum samples examined by dot-ELISA and CFT using AGE from *Campylobacter fetus* ssp. *fetus* and *C. jejuni* strains

Antigen extract from	Tests	Positive sera from aborted sheep	Positive sera from slaughtered sheep	Total
C. fetus ssp. *fetus*	Dot-ELISA	16	15	31
	CFT	19	16	35
C. jejuni	Dot-ELISA	14	2	16
	CFT	5	2	7

Dot-ELISA, dot-enzyme-linked immunosorbent assay; CFT, complement fixation test; AGE, acid glycine extracts.

Statistical evaluation

The chi–square test for association was used for the statistical evaluation of results.

Results

In the standardization of Dot-ELISA by checker-board analysis, colour development at the site of the antigen on NCM with positive control sera was decreased at high dilutions of antigen from both *C. fetus* ssp. *fetus* and *C. jejuni* (Fig. 1). No colour was observed in the absence of antigen or antibody. However, colour appeared at the low dilutions (1 : 4 to 1 : 8) of antigen of both *C. fetus* ssp. *fetus* and *C. jejuni* and also at low dilutions (1 : 25 to 1 : 50) of some negative control sera (Fig. 1). Use of the higher dilutions of antigens from both strains decreased the sensitivity of Dot-ELISA but on the other hand the sensitivity of the test was not increased when low dilutions of antigens were used. Thus a serum dilution of 1 : 100 and 1 : 10 dilution of antigen of both *Campylobacter* species were used in the Dot-ELISA test. Under these conditions all the positive control sera were also positive and all the negative control sera were negative in Dot-ELISA. Colour development with serial dilutions of positive and negative serum samples in Dot-ELISA are shown in Fig. 2.

Table 1. Comparison of the results obtained by Dot-ELISA and CFT using AGE from *Campylobacter fetus* ssp. *fetus* and *C. jejuni* in 153 sheep sera

Dot-ELISA $_{fetus}$	CFT_{fetus}	Dot-ELISA $_{jejuni}$	CFT_{jejuni}	Aborted sheep	Slaughtered sheep	Total
+	+	+	+	2	1	3
+	+	+	–	7	2	9
+	+	–	–	3	9	12
–	+	–	–	4	3	7
–	+	–	+	3	1	4
–	–	+	–	1	0	1
+	–	–	–	0	3	3
+	–	+	–	4	0	4
–	–	–	–	37	51	88
+	AC	+	AC	1	0	1
–	AC	+	AC	0	1	1
–	AC	–	AC	12	8	20
Total				74	79	153

Dot-ELISA, dot-enzyme-linked immunosorbent assay; CFT, complement fixation test; AGE, acid glycine extracts; AC, anti-complementary sera.

Table 3. Agreement between the results obtained by dot-enzyme-linked immunosorbent assay and complement fixation test using acid glycine extracts from *C. fetus* ssp. *fetus* and *C. jejuni*

Results	Tests	
	Dot-ELISA $_{jejuni}$	CFT_{fetus}
$Dot\text{-}ELISA_{fetus}$		
Agreement		
Pos/pos	16	24
Neg/neg	99	89
Disagreement		
Pos/neg	15	11
Neg/pos	1	7
Total	131	131
% agreement	87.8*	86.3*
	Tests	
	CFT_{fetus}	$Dot\text{-}ELISA_{jejuni}$
CFT_{jejuni}		
Agreement		
Pos/pos	7	3
Neg/neg	96	110
Disagreement		
Pos/neg	0	4
Neg/pos	28	14
Total	131	131
% agreement	78.6*	86.3**

Difference between the results from both extracts are found statistically significant: *$P < 0.001$; **$P < 0.05$.

A total of 153 sheep sera from aborted (74) and slaughtered (79) sheep were examined by CFT and Dot-ELISA using AGE from both *C. fetus* ssp. *fetus* and *C. jejuni* strains. Twenty-two sera showed anti-complementary activity was not suitable for CFT. Of the 22 sera showing anti-complementary activity, one serum was positive in Dot-ELISA with AGE from both *Campylobacter* strains and another was positive in Dot-ELISA with AGE from *C. jejuni*. (Table 1). Of the remaining 131 sera, 31 and 35 sera were found to be positive in Dot-ELISA and CFT, respectively using AGE from *C. fetus* ssp. *fetus*. When AGE from *C. jejuni* was used, 16 and seven sera gave positive reactions in Dot-ELISA and CFT, respectively (Table 2).

When the results from both tests were compared, 88 (67.2%) sera were negative by both Dot-ELISA and CFT using AGE from both *Campylobacter* strains. The remaining 43 sera (32.8%) gave different reaction pattern in both tests with the extracts of both *Campylobacter* strains. Twelve sera were positive by both tests using AGE from *C. fetus* ssp. *fetus,* but CFT failed to detect antibodies in nine of these sera when AGE from *C. jejuni* was used. Twelve sera were positive by both tests only when AGE from *C. fetus* ssp. *fetus* was used. Eleven sera were positive only by CFT. Seven of these reacted only with the AGE from C. *fetus* ssp. *fetus* and four sera were positive with AGE of both *Campylobacter* strains. The remaining eight sera were found to be positive only by Dot-ELISA either with the AGE from both *Campylobacter* strains or with the AGE from one of the *Campylobacter* strains (Table 1).

Comparisons between the results obtained by Dot-ELISA and CFT using AGE from *C. fetus* ssp. *fetus* and *C. jejuni* are given in Table 3.

Discussion

The use of serological tests to detect antibodies after natural infection are helpful for the diagnosis of infectious disease. Agglutination and complement fixation tests are the serological methods widely used for detecting *Campylobacter* antibodies in sheep, cattle and human (Watson et al., 1979; Jones et al., 1980; Mosimann et al., 1981; Blobel and Schliesser, 1982; Oosterom et al., 1985). However, the use of both tests are limited due to low sensitivity or specificity for sero-epidemiological studies such as screening tests. Recently, ELISA has been developed by several workers for the diagnosis of *Campylobacter* infection caused by *Campylobacter jejuni/coli* in human (Svedhem et al., 1983; Oosterom et al., 1985; Melby, 1987) and for the detection of *Campylobacter* antibodies in naturally infected (Yardımcı et al., 1994) or immunized (Gröhn and Genigeorgis, 1985) sheep. A Dot-ELISA that discriminates between the thermophilic *Campylobacter* species has already been reported (Griffiths et al., 1992). In the present study, *Campylobacter* antibodies in sheep sera were detected by a Dot-ELISA and complement fixation tests using AGE prepared separately from a single strain of *C. fetus* ssp. *fetus* and *C. jejuni*.

It has been reported that antibodies against *C. fetus* ssp. *fetus* are widely distributed in sheep sera, being more common than *C. jejuni/coli* (Gröhn and Genigeorgis, 1985; Yardımcı et al., 1994). In the present study, antibodies in sheep sera detected by Dot-ELISA and CFT also seemed to be directed against AGE from *C. fetus* ssp. *fetus* rather than *C. jejuni*. Whereas 46.5% of the sera found to be positive in Dot-ELISA and/or CFT reacted with the AGE from both *Campylobacter* strains, almost all the positive sera were detected by AGE from C. *fetus* ssp. *fetus*. The difference between results obtained with AGE from both *Campylobacter* strains was found to be statistically significant. This may indicate that the antigenic cross-reactivity between AGE from both *Campylobacter* strains was not significant. It is also consistent with the finding that with one exception, no positive serum reacted only with AGE from *C. jejuni,* although 60.4% of the sera found to be positive in Dot-ELISA and/or CFT gave a positive reaction only with AGE from *C. fetus* ssp. *fetus* and 46.5% of the positive sera reacted with AGE from both *Campylobacter* strains. This could be attributed to the fact that the both extracts might contain common as well as species-specific antigens reacting with heterologous or homologous antibodies. Yardımcı et al. (1998) reported that AGE from *C. jejuni* was able to detect nearly all the positive sera that were also found to be positive with AGE of *C. fetus* ssp. *fetus* in ELISA. In contrast to these findings, it has been reported that AGE from *C. fetus* ssp. *fetus* and *veneralis* were antigenically similar to each other but different from *C. jejuni* and *C. coli* strains (Gröhn and Genigeorgis, 1985; Rautelin, 1987). In agreement with the present findings, further studies have indicated that AGE from *C. jejuni* was found to be a mixture of different proteins, including a flagellar antigen and acid-dissociable surface proteins which were responsible for antigenic cross-reactivity between AGE from C. *fetus* ssp. *fetus* and *C. jejuni*. However, the acid-dissociable protein with low molecular weight appeared to be an antigenic determinant that is common to the thermophilic *Campylobacter* strains but not in *C. fetus* ssp. *fetus*. On the other hand, glycine-extractable protein at high molecular weight was found mostly in *C. fetus*

ssp. *fetus* but not in C. *jejuni*. (Logan and Trust, 1983; Cawthraw et al., 2000).

The preparation of the antigen plays a major role for the specificity and sensitivity of the serological tests. Alkaline extracts and acid extracts are widely used in ELISA and CFT to detect *Campylobacter* antibodies in sheep (Gröhn and Genigeorgis, 1985; Yardımcı et al., 1994) and human (Mosimann et al., 1981; Kosunen et al., 1983; Rautelin and Kosunen, 1983). It has been suggested that acid extracts from *C. fetus* ssp. *fetus* and *C. jejuni* provided more suitable antigens than alkaline extracts in ELISA (Rautelin, 1987). Rautelin and Kosunen (1987) reported that the use of the acid extract from *C. jejuni* in ELISA gave a sensitivity of 78% and a specificity of 99%. In another study, Oosterom et al. (1985) also found that the sensitivity and specificity of ELISA using antigen from the disintegrated cells of *C. jejuni* were 77 and 95%, respectively. The Dot-ELISA employed in this study appeared to be specific but more sensitive when AGE from *C. fetus* ssp. *fetus* was used as antigen. In the present study 68.6% of the serum samples found to be positive in CFT were also positive with Dot-ELISA and 92.7% of the sera found to be negative in CFT were also negative with Dot-ELISA using AGE from *C. fetus* ssp. *fetus*. On the other hand, only 42.9% of the sera found to be positive in CFT were also positive with Dot-ELISA and 88.7% of the sera found to be negative in CFT were also negative with Dot ELISA when AGE from *C. jejuni* was used as antigen.

It has been suggested that the detection of *Campylobacter* antibodies in paired sera affected the sensitivity of ELISA and that a single serum sample decreased the sensitivity of ELISA (Kosunen et al., 1983; Oosterom et al., 1985). In addition, the use of a mixture of acid extracts from *C. fetus* ssp. *fetus* and *C. jejuni* strains raised the sensitivity of ELISA (Rautelin and Kosunen, 1987). The sensitivity of Dot-ELISA employed in this study might also be affected by the use of AGE from a single strain of *C. fetus* ssp. *fetus* and *C. jejuni* as well as by using a single serum sample.

In addition, Ig M and Ig G antibody fixing complement has been detected in CFT, but Ig A does not fix complement and remains undetected in CFT (Halliwell and Gorman, 1989). It has been reported that Ig M and Ig A are the most prevalent immunoglobulin subclasses in the acute phase of *Campylobacter* infection in humans and methods such as DIG-ELISA also detect Ig A antibody (Svedhem et al., 1983; Melby, 1987). Failure of the Dot-ELISA to detect antibodies in sera which were positive in CFT could be attributed to the fact that Dot-ELISA using anti Ig G conjugate detects only Ig G antibody but not Ig M and Ig A.

It has also been reported that elevated *Campylobacter* antibody titres in sera can also exist in healthy individuals (Oosterom et al., 1985; Rautelin and Kosunen, 1987). In Dot-ELISA slight colour development was also observed with some negative control sera at low dilutions but only when low dilutions of AGE from *Campylobacter* strains, especially *C. jejuni* were used.

The Dot-ELISA employed in this study appeared to be simple and quick to use in comparison with CFT. In Dot-ELISA, the use of a small amount of extract (0.5 μl per dot) is enough to detect antibodies in sera. CFT is complex and requires the standardization of many reagents and serum samples showing anti-complementary activity and poor quality sera are not suitable for CFT. Dot-ELISA does not require heat inactivation of serum samples. However, only two sera showing anti-complementary activity were found to be positive by Dot-ELISA. Moreover, antibody in sera can rise after both natural infection or vaccination. As vaccination against campylobacteriosis was not being performed in the sheep populations from which the serum samples used in the present study were taken, almost all the sera found to be positive with Dot-ELISA and/or CFT should be associated with *Campylobacter* infection.

The results of this study indicated that specificities of the Dot-ELISA were comparable with those of CFT using AGE from both *Campylobacter* species for detecting *Campylobacter* antibodies in sheep sera. Although both tests appeared to be more sensitive when AGE from *C. fetus* ssp. *fetus* was used as antigen, Dot-ELISA would be more suitable for epidemiological studies. It is concluded that Dot-ELISA using AGE from *C. fetus* ssp. *fetus* could be employed for the detection of *Campylobacter* antibodies in sheep sera and the additional use of AGE from *C. jejuni* as antigen appeared not to be profitable for this purpose. Further studies are necessary to analyse the antigenic cross-reactivity between AGE from *C. fetus* ssp. *fetus* and *C. jejuni* strains by immunoblotting to improve the sensitivity and specificity of serological tests.

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References

Afsar, A., G. C. Dulac, and J. Riva, 1992: Comparison of blocking Dot ELISA, using a monoclonal antibody for detection of bluetongue virus antibodies in cattle. Vet. Microbiol. **31,** 33–39.

Alton, G. G., M. L. Jones, D. D. Angus, and J. M. Verger, 1988: Techniques for the Brucellosis Laboratory. INRA Ed, Paris, France.

Blais, B. W., H. Yamazaki, and C. E. Rigby, 1989: Use of hydrophobic cloths as antibody absorbents for enzyme immunoassay: detection of *Brucella* antigens. Vet. Microbiol. **20,** 155–163.

Blobel, H. and T. Schliesser, 1982: Handbuch der bakteriellen Infektionen bei Tieren. VEB Gustav. Fisher Verlag, Jena, Germany.

Cawthraw, S. A., L. Lind, B. Kaijser, and D. G. Newell, 2000: Antibodies directed towards *Campylobacter jejuni* antigens in sera from poultry abattoir worker. Clin. Exp. Immunol. **122,** 55–60.

Chand, P., J. R. Sadana, H. V. Batra, and R. S. Chauan, 1989a: Comparison of the dot-immunobinding assay with the complement fixation test for the detection of *Brucella* antibodies in sheep. Vet Microbiol. **20,** 281–286.

Chand, P., J. R. Sadana, H. V. Batra, and R. N. S. Khanna, 1989b: Comparison of dot enzyme-linked immunosorbent assay (Dot-ELISA) with other serological tests for the detection of *Brucella* antibodies in bovine sera. J. Vet. Med. B **36,** 346–352.

Griffiths, P. L., G. S. Moreno, and R. W. Park, 1992: Differentiation between thermophilic *Campylobacter* species by species-specific antibodies. J. Appl. Bacteriol. **72,** 467–474.

Gröhn, K., and C. Genigeorgies, 1985: Detection of *Campylobacter* antibodies and its application in seroepidemiological studies in sheep and cattle herds. Acta Vet. Scand. **26,** 30–48.

Gürtürk, K., B. Boynukara, Z. İlhan, İ. H. Ekin, and T. Gülhan, 1999: Comparison of the dot-immunobinding assay with the serum agglutination test, the Rose Bengal plate test and the milk ring test for the detection of *Brucella* antibodies in bovine sera and milk. J. Vet. Med. B **46,** 279–285.

Gürtürk, K., Z. İlhan, and O. Erganis, 1997: Detection of *Brucella* antibodies in sheep sera using dot-immunobinding assay and Rose

Bengal plate agglutination test. Tr. J. Vet. Anim Sci., TÜBİTAK **21,** 341–344.

Halliwell, R. E. W., and N. T. Gorman, 1989: Veterinary Clinical Immunology. W.B. Saunders Company, Philadelphia, PA, USA.

Hawkes, R., E. Niday, and J. A. Gordon, 1982: Dot-immunobinding assay for monoclonal and other antibodies. Anal. Biochem. **119,** 142–147.

Heberling, R. L., S. S. Kalter, J. S. Smith, and D. G. Hildebrand, 1987: Serodiagnosis of rabies by dot-immunobinding assay. J. Clin. Microbiol. **25,** 1262–1264.

Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams, 1994: Bergey's Manuel of Determinative Bacteriology, Ninth Edition. 1994. Williams & Wilkins, Baltimore, MD, USA

Hum, S., L. R. Stephens, and C. Quinn, 1991: Diagnosis by ELISA of bovine abortion due to *Campylobacter* fetus. Aust. Vet. J. **68,** 272–275.

Jones, D. M., J. Eldrige, and B. Dale, 1980: Serological response to *Campylobacter jejuni/coli* infection. J.Clin. Pathol. **33,** 767–769.

Kosunen, T. U., H. Rautelin, T. Pitkänen, A. Pönkä, and T. Petterson, 1983: Antibodies against an acid extract from a single *Campylobacter* strain hospitalized campylobacter patients. Infection **11,** 189–191.

Kumar, S., A. H. Band, J. C. Samantaray, N. Dang, and G. P. Talwar, 1985:: a dot-enzyme-linked immunosorbent assay for detection of antibodies against *Entemoaba histolytica*. J. Immunol. Meth **83,** 125–133.

Logan, S. M., and T. J. Trust, 1983: Molecular identification of surface protein antigens of *Campylobacter jejuni*. Infect. Immun. **42,** 675–682.

McCoy, E. C., D. Doyle, K. Burda, L. B. Corbeil, and A. J. Winter, 1975: Superficial antigens of *Campylobacter* (Vibrio) fetus: Characterization of an antiphagocytic component. Infect. Immun. **11,** 517–525.

Melby, K., 1987: Antibody response in patients infected with *Campylobacter jejuni/coli* assayed with a complement fixation test and a DIG-ELISA method. NIPH Ann. **10,** 29–31.

Mosimann, J., M. Jung, G. Schär, U. Bonifas, I. Heinzer, S. Brunner, G. Hermann, and R. A. Lambert, 1981: Serologische diagnose menschlicher *Campylobacter* Infektionen. Schweiz. Med. Wochenschr. **III,** 846–853,.

Oosterom, J. C. H. Den Uyl, J. R. J. Bänffer, S. Lauwers, J. Husiman, A. E. Busschbach, F. G. J. Poelma, and R. Bellemans, 1985: Evaluation of an enzyme-linked immunosorbent assay (ELISA) for the detection of *Campylobacter jejuni* antibodies and comparison with a Complement fixation test (CFT). Antonie Van Leeuwenhoek **51,** 321–331.

Ramadass, P., S. Meerarani, and V. D. Padmanaban, 1993: Dipstick enzyme immunoassay for rinderpest antibody in cattle. Vet. Microbiol. **36,** 385–388.

Rautelin, H., 1987: Acid and alkaline *Campylobacter* extracts in the demonstration of antibody response in hyperimmunized rabbits. Acta Path. Microbiol. Immunol. Scand. Sect. B **95,** 103–108.

Rautelin, H., and T. U. Kosunen, 1983: An acid extract as a common antigen in *Campylobacter coli* and *Campylobacter jejuni* strains. J. Clin. Microbiol. **17,** 700–701;.

Rautelin, H., and T. U. Kosunen, 1987: Single and multiple acid extract antigens in *Campylobacter* serology. Acta Path. Microbiol. Immunol. Scand. Sect. B **95,** 277–281.

Svedhem, A., H. Gunnarsson, and B. Kaijser, 1983: Diffussion in gel enzyme-linked immunosorbent assay for routin detection of IgG and IgM antibodies to *Campylobacter jejuni*. J. Infect. Dis. **148,** 82–92.

Watson, K. C., E. J. C. Kerr, and S. M. MacFadzean, 1979: Serology of human campylobacter infections. J. Infect. **I,** 151–158.

Yardımcı, H., B. Boynukara, M. Akan, and K. S. Diker, 1998: Van çevresindeki koyunlarda *Campylobacter* antikorlarýnýn ELISA ile saptanması Y.Y.Ü. Vet. Fak. Derg. **1–2,** 5–8.

Yardımcı, H., K. S. Diker, and M. Akan, 1994: Use of ELISA for detection of *Campylobacter* antibodies in sheep. Tr. J. Vet Anim Sci. **18,** 129–133.