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ADAPTION OF ELISA FOR THE DETECTION OF CAMPYLOBACTER ANTIBODIES AND ITS APPLICATION IN SEROEPIDEMIOLOGICAL STUDIES IN SHEEP AND CATTLE HERDS

By

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GRÖHN, K. and C. GENIGEORGIS: *Adaption of ELISA for the detection of Campylobacter antibodies and its application in seroepidemiological studies in sheep and cattle herds.* Acta vet. scand. 1985, *26*, 30—48. — ELISA was adapted for the study of antigenic relations among important campylobacters and for the presence of anti-campylobacter antibodies in 394 sheep and 265 cattle. Rabbit anti-C. jejuni, C. coli, C. fetus subsp. fetus and C. laridis heat-stable antigen sera were evaluated against 29 Campylobacter strains and 6 other bacteria. Anti-C. jejuni and C. coli reacted strongly with homologous antigens and weakly with C. fetus subsp. fetus, C. laridis and C. fecalis antigens. C. fetus subsp. fetus serum reacted mainly with its homologous antigen. C. laridis serum showed closer reactivity to C. jejuni than to C. fetus subsp. fetus, C. coli and C. fecalis. Insignificant cross-reactions were observed with Y. enterocolitica, S. dublin and E. aerogenes heat-stable antigens. Ewes vaccinated with C. fetus subsp. fetus bacterin showed higher ELISA titers against C. fetus subsp. fetus antigens than non-vaccinated ewes or rams. Twenty-five percent of the vaccinated animals showed titers as low as 95 % of the non-vaccinated animals. In cattle the lowest antibody titers against C. fetus subsp. fetus, C. jejuni, C. coli and C. laridis antigens were exhibited by the precolostrum sera followed by the postcolostrum and adult sera. These studies demonstrated the applicability of the ELISA test in seroepidemiological investigations concerning the distribution and significance of Campylobacter antibodies in food animal sera.

antigenic relations; abortions; classification; seroepidemiology; veterinary public health.

Campylobacter species are widely distributed in the animal kingdom both as pathogens and commensals (*Smibert* 1978), and a few have been recognized recently as important zoonotic agents. C. jejuni is a major cause of human enteritis (*Blaser* 1982, *Walder* 1982, *Public Health Laboratory Service* 1982) while

C. fetus subsp. fetus has been implicated occasionally in systemic infections, mainly in compromised individuals (*Bokkenheuser* 1970). The origin of human infections remains unclear. Foodborne transmission, handling of animal products and association with animals seem to contribute significantly to human disease (*Bokkenheuser & Mosenthal* 1981, *Blaser* 1982, *Skirrow* 1982, *Prescott & Munroe* 1982, *Norkrans & Svedhem* 1982, *Christenson et al.* 1983, *Blaser et al.* 1983).

C. fetus subsp. fetus has been isolated frequently from sheep feces and may be associated with enteric diseases in calves and cattle (*Garcia et al.* 1983). C. jejuni has been isolated from 2.5—100 % of asymptomatic cattle and is capable of inducing diarrhea in calves and sheep and mastitis in cattle (*Robinson* 1982, *Prescott & Munroe* 1982, *Firehammer & Myers* 1982, *Garcia et al.* 1983). Campylobacter species are also important abortion causes in cattle and sheep. About 90 % of all infertility and abortion problems in cattle are due to C. fetus subsp. venerealis and 10 % to C. fetus subsp. fetus (*Arthur et al.* 1982). The latter has been reported as causing sheep abortions in most European countries, in the USSR, the USA, New Zealand and Australia (*Gunnarsson et al.* 1976, *Jensen & Brinton* 1982). It seems that in the USA Campylobacter abortions in sheep are due equally to C. fetus subsp. fetus and C. jejuni (*Prescott & Munroe* 1982).

Serologic classification of Campylobacter is not well defined. *Penner & Hennessy* (1980) and *Lauwers et al.* (1981) developed a widely adopted system for C. jejuni and C. coli based on passive hemagglutination antigens. *Lior et al.* (1982) in their serotyping scheme for C. jejuni and C. coli used a slide agglutination test with heat labile protein antigens, which are more specific than the heat-stable antigens and allow strain indentification. *Hébert et al.* (1983) serotyped C. jejuni, C. coli, and C. fetus subsp. fetus by direct immunofluorescence.

In clinical diagnosis of Campylobacter infections, agglutination, bactericidal activity of sera, complement fixation, indirect fluorescent antibody and more recently ELISA (*Svedhem et al.* 1982, *Walder & Forsgren* 1982, *Kaldor et al.* 1983, *Garcia et al.* 1983, *Kosunen et al.* 1984, *Blaser & Duncan* 1984) have been utilized for antibody detection. ELISA has been used mainly as a diagnostic tool and to a lesser extent as a screening test for seroepidemiological studies.

The purpose of this study was to adapt the ELISA for the

detection of circulating antibodies against various heat-stable Campylobacter antigens and then use the test for seroepidemiological screening of sheep and cattle herds.

MATERIAL AND METHODS

Bacterial strains

Campylobacter species were obtained from a number of investigators. Species, strains and sources are presented in Table 1. Escherichia coli K12, Yersinia enterocolitica, Enterobacter aerogenes, Salmonella typhimurium, Salmonella dublin, Salmonella infantis, Staphylococcus aureus, and Staphylococcus epidermidis were obtained from the local reference collection.

Table 1. Campylobacter species and strains used in the present study.

Species (strains)	Source	Obtained from
C. jejuni (CB, GS, RH)	human	Dr. Midura, Berkeley
C. jejuni (105, 341, 372)	human	Dr. Kosunen, Finland
C. jejuni (60, 61, 63)	chicken	Dr. Kinde, Davis
C. fetus fetus (19619, 18440)	ovine fetus	Dr. Firehammer, Montana
C. fecalis (11362)	ovine fetus	Dr. Firehammer, Montana
C. fetus fetus (2, 3, 4)		Dr. Bokkenheuser, New York
C. jejuni (6, 7, 8)	human	Dr. Bokkenheuser, New York
C. jejuni (14, 15)	chicken	Dr. Bokkenheuser, New York
C. fetus fetus (82—123, 81—173)		Dr. Blaser, Denver
C. jejuni (LIO7, LIO15)	human	Dr. Lior, Ottawa
C. jejuni (LIO18)	chicken	Dr. Lior, Ottawa
C. coli (LIO8, LIO12)	human	Dr. Lior, Ottawa
"C. laridis" (LIO31)	human	Dr. Lior, Ottawa
"C. laridis" (LIO35)	sea gull	Dr. Lior, Ottawa
C. fetus fetus (VPI 15)		Dr. Lior, Ottawa

Preparation of heat-stable antigens

Campylobacters were grown in a nutrient broth (20 g polypeptone, 2 g yeast extract, and 5 g sodium chloride per l water) at 37°C (C. fetus subsp. fetus and C. fecalis), or at 42°C (C. jejuni, C. coli, C. laridis) in an atmosphere of 5 % O_2, 10 % CO_2 and 85 % N_2 for 24 h. Active log cells were harvested by centrifugation, washed in saline 3 times, and diluted in 0.05 mol/l carbonate-bicarbonate buffer, pH 9.6. Using a Spectronic 20 (Bausch & Lomb, Rochester, N.Y.) spectrophotometer, the cell suspension was standardized to OD_{600} nm of 1.0, boiled for 1 h, centrifuged

for 20 min at 17,000 × g and the supernatant was stored in the refrigerator.

Non-campylobacter bacterial antigens were prepared the same way from cells first grown on brain heart infusion agar in normal atmosphere at 37°C for 24 h.

Production of antisera

Single or pooled (in equal amounts) antigenic preparations were administered i.v. in New Zealand white rabbits. A typical injection schedule included 1 ml antigen the 1st and 4th day, and 2 ml the 7th, 10th, 13th, 15th, and 30th day. Bleeding was done on the 40th day. Preimmunization sera were taken from all rabbits.

Absorption of sera

Twofold dilutions of sera (1:80—1:640) were absorbed by the addition of equal volume of 1:100 dilution of heat-stable antigens for 30 min at 37°C. After centrifugation, the clear supernatant was tested immediately by ELISA for antibody titers (*Walder & Forsgren* 1982).

ELISA procedure

ELISA was performed with a microtiter modification of the indirect enzyme-linked immunosorbent assay (*Engwall & Perlman* 1972). Disposable flat bottom polystyrene microtiter plates (Immulon 1, Dynatech Laboratories, Inc., Alexandria, VA) were used as a solid phase for the adsorption of the heat-stable antigen. The antigen was diluted (usually 1:100) in 0.05 mol/l carbonate-bicarbonate buffer, pH 9.6, and 50 µl were added to each well. The plates were incubated at 4°C overnight, washed with ELISA washing solution (8.5 g NaCl, 1 l H_2O, 0.5 ml Tween 20) twice, and shaken dry. Fifty microliters serum diluted, usually 1:20, in 0.05 mol/l tris-buffer (6.05 g tris-buffer, 0.372 g EDTA, 1 g bovine albumin fraction V, 0.5 ml Tween 20, and 1 l H_2O), pH 7.4, was added into the wells and incubated at 37°C for 1 h. Then the plates were washed 3 times, shaken dry, and 50 µl of commercial specific anti-IgG horseradish peroxidase conjugate diluted in tris-buffer was added into each well. The plates were incubated at 37°C for 20 min, and then washed 3 times and shaken dry. The conjugated anti-rabbit IgG antibody (Cappel Laboratories, Coch-

ranville, PA) was used in dilution 1:3000 for testing rabbit sera. Affinity purified antibodies against ovine IgG and bovine IgG produced in rabbits (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) were used at 1:1000 and 1:2000 dilution to test sheep and cattle sera respectively. After the addition of the conjugate, ABTS (2-2′ azino-di-(3-ethyl benzothiazolin suffone-6) diammonium salt) was used as the substrate. Stock solution (274.4 mg ABTS in 12.5 ml, distilled H_2O) was diluted 1:100 in 0.1 mol/l citrate buffer, pH 4.0. Fresh 2.5 % H_2O_2 was also added to get a 1:250 dilution. One hundred µl of this substrate solution was added to each well and the plate was incubated on a shaker at room temperature for 10 min. The reaction was stopped by adding 100 µl 0.1 mol/l hydrofluoric acid, pH 3.3 into each well. The OD_{405} of the color reaction was read by a Dynatech HR580 Microelisa Auto Reader (Dynatech Instruments, Inc., Santa Monica, CA) connected to a Radio Shack TRS-80 model II microcomputer (Radio Shack, Fort Worth, TX).

In each test plate, a conjugate and a positive and negative serum control were included. All the samples and controls were tested as duplicates and if the standard deviation of the OD of the duplicates exceeded the value of 0.05, the test was repeated. The OD values of the unknown serum samples were automatically measured and compared to the OD of the positive control sample on the plate whose ELISA OD was designated as 100 %. Thus, a relative ELISA titer for all unknown sera could be obtained.

Animal sera

Sheep and cattle sera were obtained from a variety of sources as specified later and kept in the freezer along with produced rabbit antisera until examined.

Statistical methodology

Distribution free statistical methods were applied (*Reed et al.* 1971) if an appropriate test was available. The BMDP (*Dixon* 1981) program was used to perform the Kruskal-Wallis test for comparing three or more populations. Joint ranking was used as a post test in comparison of the groups. Two-way analysis of variance (BMDP2V Program, *Dixon* 1981) was performed to compare the mean relative ELISA titers against 3 different antigen preparations in 4 dairy herds.

RESULTS

Evaluation of rabbits sera

Four rabbits were immunized with heat-stable Campylobacter antigens. Rabbit 1 received C. jejuni strains 105, and 341; rabbit 2 received C. coli strains LIO8, and LIO12; rabbit 3 received C. fetus subsp. fetus strains 19619, 18440, 82-123, and 81-173; and rabbit 4 received C. laridis strain LIO31.

Hyper-immune and pre-immune titers were measured by ELISA. A checkerbord titration was used for the selection of optimum antigenconcentration for plate coating. Final selection was based on the highest ELISA OD ratio of positive to negative sera. A 1:100 dilution of antigen was found to be best. Stocks of antigens were good even after a 6-month storage at 4°C. Typical curves relating preimmune and immune antiserum dilutions to ELISA OD for each antigenic preparation are shown in Fig. 1. One of the pre-immune sera at 1:10—1:20 dilution exhibited

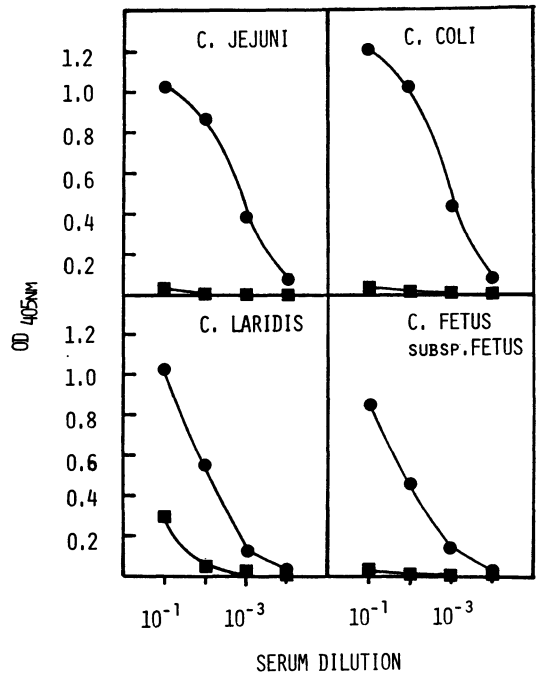

Figure 1. ELISA end point titration curves of preimmune and specific antisera against their corresponding heat-stable antigens used for plate coating at 1:100 dilutions (●—● immune sera, ■—■ pre-immune sera).

ELISA OD values against the C. laridis antigen high enough to suggest that this rabbit had previous exposure to C. laridis.

The specificity of the immune sera produced was then tested against antigens (1:100 dilution) representing the 5 Campylobacter and the other non-compylobacter bacteria. The results are presented in Fig. 2. To simplify the comparison among strains and species, ELISA OD given by an antigen preparation and its corresponding immune serum is recorded as 100 % positive ELISA. Fig. 2 shows the close similarity of C. jejuni and C. coli antigens, some degree of cross reaction of these antigens with anti-C. laridis serum and the low reactivity of anti-C. fetus subsp.

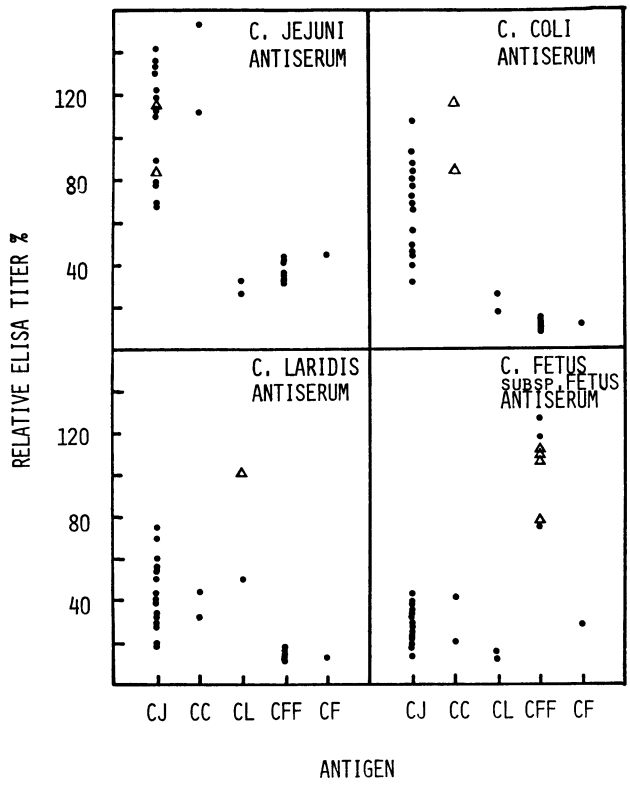

Figure 2. Relative ELISA responses of individual heat-stable antigens from 5 Campylobacter species against anti-Campylobacter rabbit sera. ELISA OD-value of each antigen preparation used for rabbit immunizations is recorded as 100 % positive ELISA. (CJ = C. jejuni, CC = C. coli, CL = "C. laridis", CFF = C. fetus subsp. fetus, CF = C. fecalis, △ = antigens used for immunization, ● = other antigens).

fetus serum with heterologous antigens. The other bacterial antigens showed insignificant cross reactions with anti-Campylobacter sera.

Effect of absorption of the specific sera on their ELISA titer

Twofold dilutions of hyper-immune sera produced against C. jejuni, C. coli and C. fetus subsp. fetus were each absorbed with a variety of Campylobacter, Y. enterocolitica, S. dublin, E. aerogenes and E. coli antigens and the ELISA OD titer of the absorbed sera was determined. The results are presented in Table 2.

The effect of each absorption is expressed as a percent decrease of the ELISA titer of the unabsorbed serum set as 100 %. The absorption of C. jejuni antiserum (1:640 dilution) with C. jejuni, C. coli, C. laridis, C. fetus subsp. fetus, S. dublin and Y. enterocolitica antigens decreased the ELISA OD by 55, 36, 13, 9 and 8 %. The behaviour of C. coli antiserum at 1:640 dilution

Table 2. Effect of serum absorption with different Campylobacter and some other gram negative bacteria heat-stable antigens on the ELISA response of anti-C. jejuni, anti-C. fetus subsp. fetus immune sera.

Specific antiserum (antigen used)	Specific antiserum dilution	Non-adsorbed titer		Antiserum titer decrease (%) when absorbed with the following heat-stable antigens							
		%	(OD_{405nm})	CJ (RH)	CC (LIO8)	CL (LIO31)	CFF (81-173)	SD	YE	EA	EC
C. jejuni	1:80	100	1.153	21	9	1	4	3	0	0	0
(105, 341)	1:160	100	0.991	33	13	2	8	3	0	0	0
	1:320	100	0.966	44	24	9	11	8	6	3	2
	1:640	100	0.776	55	36	13	13	9	8	6	0
C. coli											
(LIO8, LIO12)	1:80	100	0.657	39	45	24	9	10	1	11	9
	1:160	100	0.433	43	51	14	2	8	6	1	0
	1:320	100	0.374	47	52	12	1	0	5	0	17
	1:640	100	0.257	51	49	20	14	10	8	11	2
C. fetus	1:80	100	0.547	0	1	2	27	0	0	0	0
subsp. fetus	1:160	100	0.418	0	0	0	28	0	0	0	0
(19619, 18440,	1:320	100	0.360	13	0	9	45	9	14	3	0
82-123, 81-173)	1:640	100	0.242	26	10	14	57	14	16	16	9

CJ — C. jejuni
CC — C. coli
CL — "C. laridis"
CFF — C. fetus subsp. fetus
SD — S. dublin
YE — Y. enterocolitica
EA — E. aerogenes
EC — E. coli

was similar with ELISA OD decreases of 51, 50, 20, 14, 10 and 8 % for the corresponding antigens. The C. fetus subsp. fetus antiserum was affected mainly by its corresponding antigen. The absorption studies demonstrated again the close antigenic relationship of C. jejuni and C. coli and the limited relationship of these species to C. fetus subsp. fetus, C. laridis, and the other gram negative bacteria examined.

Examination of sheep sera for Campylobacter fetus subsp. fetus antibodies

Two hundred and fifty sera originated from an Idaho sheep ranch which experienced an epidemic of abortions due to C. fetus subsp. fetus 2 years ago were collected. These sera included 74 samples from non-vaccinated rams and 176 samples from ewes vaccinated with ovine Campylobacter fetus subsp. fetus bacterin every spring and with a combination vaccine of Enzootic Abortion and C. fetus subsp. fetus (Colorado Serum Company Laboratories, Denver, Colorado) each fall. Also 40 and 104 sera were obtained from non-vaccinated ewes from 2 different California sheep ranches.

To adapt the ELISA for the detection of C. fetus subsp. fetus antibodies in sheep sera, we prepared first single and pooled antigens (1:100 dilution) from 5 C. fetus subsp. fetus strains for plate coating. These antigens were tested against a positive serum from a vaccinated herd, a negative serum from a non-vaccinated

Table 3. Impact of 5 single or pooled C. fetus subsp. fetus antigen preparations on the relative ELISA titers of eight sheep sera.

C. fetus subsp. fetus strain or strains used for preparation of antigen	Relative ELISA titer %								
	Vaccinated sheep		Non-vaccinated sheep	Aborted sheep					
	Titer	OD_{405nm}		1	2	3	4	5	6
3	100	0.232	3	6	3	32	6	3	10
81-173	100	0.291	2	7	5	27	7	3	10
82-123 + 81-173	100	0.291	2	6	5	30	8	3	10
2, 3, 4	100	0.325	1	4	4	23	5	3	8
2, 3, 4 + 82-123 + 81-173	100	0.294	3	6	6	27	7	3	9

herd, and 6 sera from ewes suffering abortions of unknown etiology. Table 3 shows that the responses of sera to the 5 C. fetus subsp. fetus antigenic preparations were similar. Because of this, all other sheep sera were examined against the antigen of strain 81-173.

The distribution of C. fetus subsp. fetus antibodies in 4 sheep groups is shown in Fig. 3. The titer distribution of the vaccinated sheep was skewed to the left with 25 % of the animals giving a

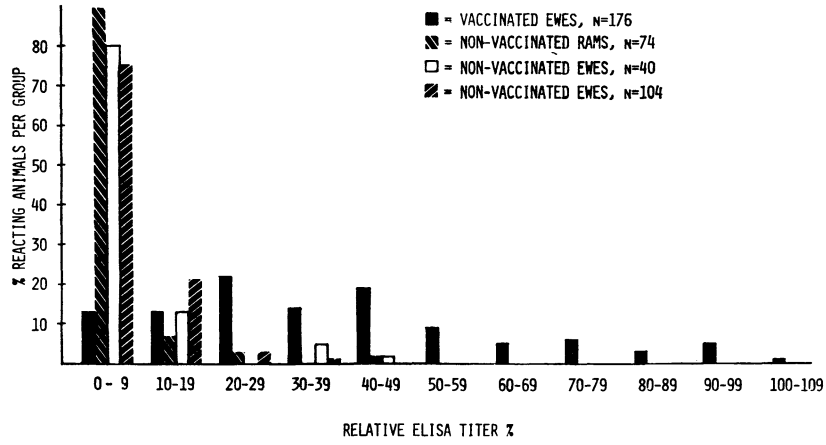

Figure 3. Relative ELISA titers (based on the positive control ELISA OD as 100 %) of 4 groups of sheep sera tested against C. fetus subsp. fetus (strain 81-173) heat-stable antigen.

relative ELISA titer of less than 20 % (100 % ELISA being the positive control serum). Ninety-five percent of the animals in the 3 non-vaccinated groups exhibited titers of less than 20 % which are significantly different ($P < 0.01$) from the titers of vaccinated sheep. The non-vaccinated groups did not differ from each other ($P > 0.05$). These studies demonstrated the utility of the ELISA test in measuring antibody titers in sheep vaccinated with C. fetus subsp. fetus.

Examination of cattle sera for Campylobacter antibodies

One hundred twenty serum samples were collected from 3 California dairy herds having no particular abortion problem. From a herd with an abortion problem over several years, 145 samples were also obtained. These included samples from 66 normal cows, 16 aborted cows taken in 2 weeks after abortion,

and repeated samples from 4 of these cows taken 4 weeks later. In addition, 32 samples were taken from newborn calves before suckling colostrum to explore possible neonatal Campylobacter infection, and 27 samples were taken 3 days after administration of colostrum.

Using ELISA, all sera were tested for the presence of Campylobacter antibodies against a pooled antigenic preparation (1:100 dilution) made of 3 C. jejuni strains (RH, 372 and 9) and 1 C. coli strain (LIO 8), an antigenic preparation of C. fetus subsp. fetus (81-173) and 1 of C. laridis (LIO 31). Since we did not have known positive control cows sera against the 3 antigenic preparations, 3 high ELISA titer sera (OD 0.688, 0.876 and 0.780) against S. fetus subsp. fetus, C. jejuni/C. coli and C. laridis antigens, respectively were selected and their OD values were designated as 100 % ELISA response. The rest of the sera were compared to these controls. The results are presented in Fig. 4.

The median relative ELISA titer for cows in the normal herds (A) for the normal cows in the problem herd (B) and the calves in the problem herd (C) was 40 %, 51 % and 11 %, respectively against C. fetus subsp. fetus antigen, 13 %, 19 % and 4 % against

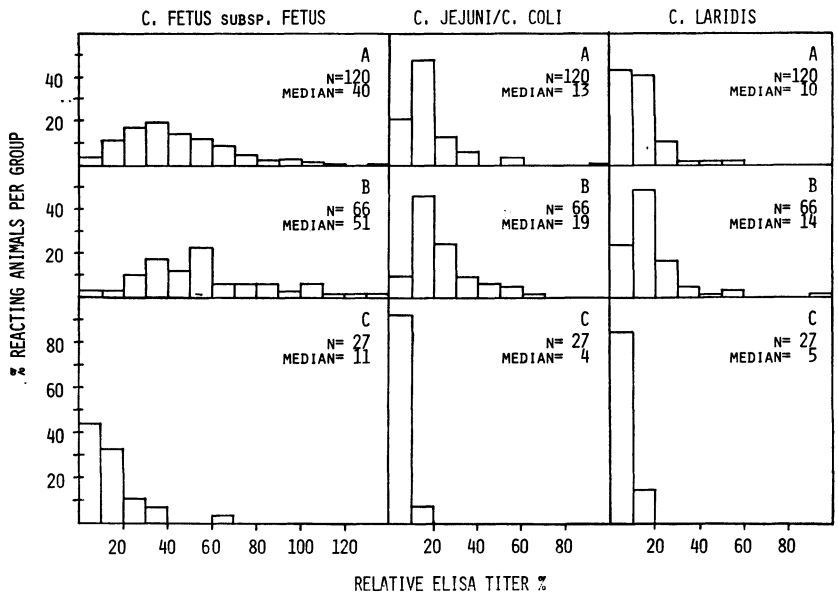

Figure 4. Relative ELISA titers (based on the positive control ELISA OD as 100 %) of 3 groups of cattle sera tested against C. fetus subsp. fetus, C. jejuni/C. coli, and "C. laridis" heat-stable antigens.

C. jejuni/C. coli antigens and 10 %, 14 % and 5 % against C. laridis antigen, respectively. Only 1 calf exhibited a titer of 63 % while the rest were less than 40 % against C. fetus subsp, fetus antigen. The precolostrum sera gave titers equal or less than 5 % against the 3 antigen preparations in all but 1 sample (titer 8 %). The difference between precolostrum and postcolostrum titers, and between postcolostrum calf and adult dairy cow titers was significant ($P < 0.05$).

Two of the normal herds had titers significantly lower ($P < 0.05$) than the problem herd, while the third normal herd exhibited titers similar to those of the problem herd against C. fetus subsp. fetus.

The relative ELISA titers from the 16 aborted cows are given in Fig. 5. The median titers against C. fetus subsp. fetus, C. jejuni/C. coli, and C. laridis antigens are 34 %, 14 % and 11 %, respectively. Repeated serum samples were also collected from 4 cows within 2—4 weeks from the first sampling. Although a noticeable increase in titer, especially against C. fetus subsp. fetus antigen

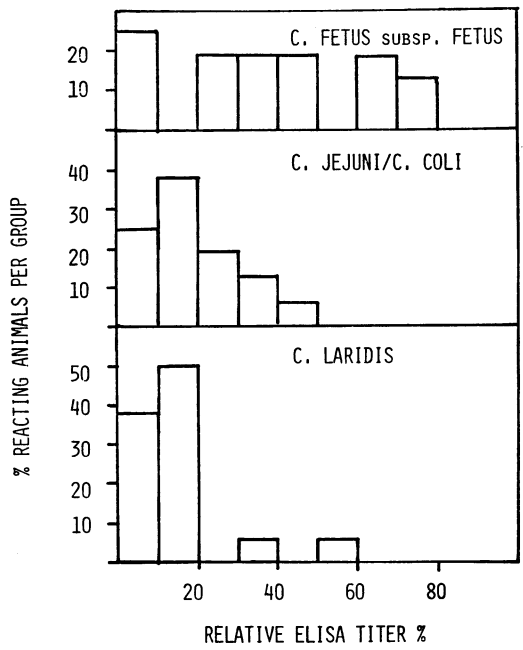

Figure 5. Relative ELISA titers (based on the positive control ELISA OD as 100 %) of a group of sera collected from 16 aborted dairy cows and tested against C. fetus subsp. fetus, C. jejuni/C. coli, and "C. laridis" heat-stable antigens.

was demonstrated, the median titers of 52 %, 21 %, and 14 % for the second sampling did not differ from the median of 51 %, 19 %, and 14 % of the normal cows in the problem herd for the respective C. fetus subsp. fetus, C. jejuni/C. coli and C. laridis antigens.

DISCUSSION

Practical methods, specific for the detection of antibodies against Campylobacter sp. are needed for epidemiological surveys on the distribution of Campylobacter antibodies in food animals and indirectly of the degree of apparent and inapparent infections.

Use of agglutination, bactericidal activity and complement fixation assays had rather limited success (*Jones et al.* 1981, *Watson & Kerr* 1982, *Garcia et al.* 1983). ELISA is one of the most often applied tests for serological diagnosis of various infectious diseases today. This very sensitive test can measure the total amount of antibody bound to an antigen in question and not just functional subgroup of antibody, such as that which gives rise to the agglutination, complement fixation, or precipitation reactions (*Hill & Matsen* 1983). Such functional antibody diversity is the reason that results from different serological tests are not always comparable.

Svedhem et al. (1982), used DIG-ELISA with a glycoprotein antigen to study a large waterborne C. jejuni outbreak in Sweden. *Walder & Forsgren* (1982) applied ELISA in clinical diagnosis of C. jejuni and C. coli. They used both purified lipopolysacharide antigens and formalinized whole cell antigens to detect antibodies in human and rabbit sera. The authors were able to develop a combination antigen from 2 strains which was able to react with 24 rabbit sera produced against 24 different C. jejuni strains.

Kaldor et al. (1983) used a heat-stable antigen pool of 6 C. jejuni strains to study the presence of circulating antibodies in the sera of patients with enteritis and positive C. jejuni isolations from their stools. The authors found that 80—90 % of the patients exhibited detectable levels of specific immunoglobulins to C. jejuni. *Kosunen et al.* (1984) used ELISA in analysis of C. jejuni antigens with monoclonal antibodies. *Blaser & Duncan* (1984) adapted ELISA to measure IgA, IgG and IgM antibodies to C. jejuni in human sera. In veterinary medicine, *Gill et al.*

(1983) applied ELISA to test antibodies to C. fetus subsp. venerealis in bovine vaginal mucus.

The present study was based on the use of heat-stable antigens for ELISA since we were interested more in the distribution of antibodies to species rather than to strains. The findings, which agree with the literature, show that some degree of antigenic diversity exists within the C. jejuni strains. The close antigenic relationship between C. jejuni and C. coli was also shown. Since only 2 C. coli strains were used as antigenic preparations the relative ELISA titer spreading is not seen in Fig. 2 as it was seen for C. jejuni strains.

All 8 C. fetus subsp. fetus antigens tested reacted uniformily. On the basis of heat-stable somatic antigens and tube agglutination, *Berg* (1971) identified C. fetus subsp. venerealis as belonging to serotype A, C. fetus subsp. fetus to A or B and C. jejuni to serotype C.

Hébert et al. (1983), using direct immunofluorescence, identified 2 serotypes A and B, within the C. fetus subsp. fetus and C. fetus subsp. venerealis species. The 2 subspecies were not found to differ serologically since both could be either type A, or B.

C. fetus subsp. venerealis was not included in this study because immunoglobulin response from natural infection is local in the vaginal mucus rather than systemic (*Garcia et al.* 1983). In addition to this, C. fetus subsp. venerealis is a very rare cause of human infections associated with compromised hosts (*Blaser & Reller* 1981, *Garcia et al.* 1983).

Cross-reactions of campylobacters with other bacteria have not been investigated systematically. Using a hemagglutination test, *Bokkenheuser* (1972) did not get any cross-reactions between C. fetus subsp. fetus antigens and E. coli, Shigella, Salmonella, Streptococcus, Mycoplasma and Brucella immune sera. In the present study some cross-reactivity of C. fetus subsp. fetus antisera and heat-stable antigens of Y. enterocolitica, S. dublin and E. aerogenes was observed. E. coli, S. aureus, S. epidermidis, S. infantis and S. typhimurium antigens did not show any cross-reactions. Sera produced against C. jejuni, C. coli and C. laridis showed very low ELISA reactivity with the antigens produced by non-Campylobacter bacteria.

C. fetus subsp. fetus is a common source of abortion in sheep. The abortions usually occur if the ewes are infected after the

120th day of the pregnancy. Infected animals build an immune response. Use of bacterins is common in areas where C. fetus subsp. fetus abortions are frequent (*Garcia et al.* 1983). The broad range of the relative ELISA titers of vaccinated sheep in this study indicates that the bacterin used for immunization may not always be able to give a high antibody response to somatic antigens.

Since symptoms due to C. fetus subsp. fetus infection appear only during the later part of the pregnancy, a screening test such as ELISA, can be useful in identifying foci of infection. In applying ELISA for clinical diagnosis more research is needed to determine the antibody response with respect to time, in sheep infected with C. fetus subsp. fetus, and the relationship between antibody titer after vaccination and protection.

Like C. fetus subsp. fetus, C. jejuni has long been known to cause abortion in sheep, usually during the last 6 weeks of pregnancy. Heavy losses can occur in susceptible flocks during the lambing season following the introduction of infection, after which a solid immunity is developed with fewer losses in subsequent years. In the present study, the distribution of C. jejuni antibodies in the sera of the flocks under investigation was not tested.

C. fetus subsp. venerealis is the main of Campylobacter abortions in cattle. C. fetus subsp. fetus has been found in about 10 % of those abortions, and occasionally C. jejuni has been isolated in bovine fetuses or bovine placenta (*Arthur et al.* 1982). In this study, C. jejuni was isolated once from an aborted fetus in the herd with the abortion problem. Unfortunately, the isolate was lost and thus it was not included as an antigen in the present study.

A variety of aerotolerant Campylobacter-like organinsms have been isolated also from bovine abortions (*Neill et al.* 1979), but their actual contribution to abortions is not well defined.

In the present study sera from the herd with an abortion problem and from the aborted cows did not have higher C. jejuni/C. coli antibody titers than the other normal herds. C. fetus subsp. fetus antibodies were widely distributed, more than C. jejuni/C. coli and C. laridis antibodies in all 4 cattle herds studied. This may indicate a higher significance of C. fetus subsp. fetus to bovine infections than the other campylobacters.

Calf sera collected before the administration of colostrum,

gave very low Campylobacter titers, indicating the absence of apparent neonatal infection. The titers were significantly increased within 3 days after the adminiration of the colostrum indicating the mother's contribution to passive immunity. As with sheep sera, the use of ELISA in the detection of Campylobacter antibodies in the dairy cattle herds demonstrated the utility of the adapted ELISA for seroepidemiological investigations concerning Campylobacter infections in food animals.

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SAMMANFATTNING

Tillämpning av ELISA för påvisning av antikroppar mot Campylobacter samt användning i seroepidemiologiska studier i får- och nötbesättnigar.

ELISA tillämpades för att studera antigeniteten inom viktiga stammar av campylobacter samt förekomsten av anti-campylobacter antikroppar hos 394 får och 265 nötkratur. Kanin anti-C. jejuni, C. coli, C. fetus subsp. fetus och C. laridis termo-stabila antigen sera analyserades mot 29 Campylobacter stammar och 6 andra bakterier. Anti-C. jejuni och C. coli reagerade starkt med homologa antigener och svagt med antigener av C. fetus subsp. fetus, C. laridis och C. fecalis. C. fetus subsp. fetus serum reagerade främst med sitt homologa antigen. C. laridis serum uppvisade en närmare reaktivitet med C. jejuni än med C. fetus subsp. fetus, C. coli och C. fecalis. Osignifikanta korsreaktioner kunde observeras med termostabila antigener av Y. entero-

colitica, S. dublin och E. aerogenes. Tackor vaccinerade med C. fetus subsp. fetus bacterin visade högre ELISA titrar mot C. fetus subsp. fetus antigener än icke vaccinerande tackor eller baggar. 25 % av de vaccinerade djuren hade titrar så låga som 95 % av de icke vaccinerade djuren. Hos nöt konstaterades de lägsta antikroppstitrarna mot C. fetus subsp. fetus, C. jejuni, C. coli och C. laridis antigener i prekolostrala sera, följd av postkolostrala sera och sera från fullvuxna djur. Detta arbete har visat tillämpbarheten av ELISA-testen i seroepidemiologiska undersökningar angående fördelningen och betydelsen av Campylobacter antikroppar i sera av husdjur för animalieproduktion.

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