Onderstepoort J. vet. Res., 54, 613–615 (1987)

THE DEMONSTRATION OF THE K-ANTIGEN OF *CAMPYLOBACTER FETUS VENEREALIS* USING A MICROTITRE AGGLUTINATION TEST

MARTHA L. VAN DER WALT, Veterinary Research Institute, Onderstepoort 0110

ABSTRACT

VAN DER WALT, MARTHA L., 1987. The demonstration of the K-antigen of *Campylobacter fetus venerealis* using a microtitre agglutination test. *Onderstepoort Journal of Veterinary Research*, 54, 613–615 (1987).

Using specific antisera prepared against heat-treated and untreated cells in a microtitre system, 7 strains of *Campylobacter fetus venerealis* were examined for the presence of K-antigen. The technique was effective for distinguishing between strains and it was found that 4 out of 7 isolates contained microcapsules, therefore being potentially suitable for vaccine production.

INTRODUCTION

The K-antigen (microcapsule) of *C. fetus* was recognized by Berg, Jutila & Firehammer (1971) as an important factor in the immunity to bovine genital campylobacteriosis. This antigen is an extracellular microcapsule around the organisms (Winter, McCoy, Fullmer, Burda & Bier, 1978). It has antiphagocytic properties (McCoy, Doyle, Burda, Corbeil & Winter, 1975) and renders the organisms resistant to phagocytosis in the genital tract. Only after opsonizing antibodies specific for this antigen have bonded with the antigen will the organisms be phagocytized. A vaccine against bovine genital campylobacteriosis should elicit an opsonin response to be effective against microcapsule-carrying campylobacters. Vaccine strains should thus have microcapsules. A number of *C. fetus venerealis* strains, whose microcapsule status was unknown, were examined serologically to select a possible vaccine strain.

The microcapsule covers the somatic (O-) antigens of *C. fetus* organisms (Berg *et al.*, 1971) and organisms with microcapsules are therefore not agglutinated by O-specific antibodies (Border, Myers & Firehammer; 1974; Ristic, White, Doty, Herzberg & Sanders, 1957). The microcapsule is a glycoprotein (Winter *et al.*, 1978) and is destroyed by heat at 121 °C for 120 min (Berg *et al.*, 1971). Antisera produced from such heat-treated cells will have only O-antibodies and will not cause agglutination of cells with microcapsules, as these cover the O-antigen.

Several workers have done serum agglutination tests with *C. fetus* (Marsh & Firehammer, 1953; Berg & Firehammer, 1978; Ristic *et al.*, 1957; Schurig, Hall, Burda, Corbeil, Duncan & Winter, 1973; Walsh & White, 1968; Winter & Dunne, 1962), all of whom performed the tests in tubes. The disadvantages of their systems were: (i) long incubation periods (20 h), (ii) manual performance of the test which could result in inaccuracy, and (iii) large volumes of sera and antigen were needed. To overcome these disadvantages a serum agglutination test that could be performed in microtitre plates (Herr, Te Brugge & Guiney, 1982) was therefore investigated.

MATERIALS AND METHODS

Bacterial strains

The origin of the 7 *C. fetus venerealis* strains used in the study are listed in Table 1. Catalase positive isolates with typical *Campylobacter* morphology were identified as *C. fetus venerealis* as a result of absence of H_2S-production and inability to grow in the presence of 1 % glycine and 3,5 % NaCl (Garcia, Eaglesome & Rigby, 1983).

Antigen production

The organisms were grown on blood tryptose agar plates for 48 h at 37 °C in anaerobic jars[1], containing a microaerophilic atmosphere (5 % O_2, 10 % CO_2 and 85 % N_2).

TABLE 1 Origin of *C. fetus venerealis* strains

Strain No.	Origin
796/1	Sheath washing, VRI, OP
6829	Institute Pasteur, Paris
1336	Florent, Paris
157/5	Sheath washing, VRI, OP
725/2	Sheath washing, VRI, OP
311/2	Sheath washing, VRI, OP
413/17	Sheath washing, VRI, OP

Untreated antigens: Growth from the agar plates was suspended in 1 % formol saline and washed 3 times by centrifugation for 10 min at 1 000 *g*. The cells were finally resuspended in formol saline and stored at 4 °C until used for antiserum production or in the agglutination test.

Heat-treated antigens: Growth from the agar plates was suspended in 0,1 M phosphate buffered saline (PBS), pH 7,2 and autoclaved for 2 h at 121 °C. Cells were then washed in PBS, as described for untreated antigens, resuspended in formol saline and stored at 4 °C, until used for antiserum production.

Antigen concentration for the agglutination test

Untreated antigens were used in the agglutination test. Antigen solutions were adjusted to an optical density of 0,6 absorbance at 420 nm with a Bausch & Lomb Spectronic 21 spectrophotometer[2].

Antiserum production

Rabbits were hyperimmunized, following the method of J. H. Bryner (personal communication, 1978) as set out in Table 2. Rabbits were exsanguinated on Day 16, serum was separated and stored in 2 mℓ aliquots at −18

TABLE 2 Injection schedule[(1)] for the production of antisera raised to heat-treated and untreated cells

Day	0	2	4	7	9	11	14
Antigen concentration in % light transmission at 420 nm[(2)]	40	40	40	40	20	20	20
Amount injected intravenously	1 mℓ	2 mℓ	2 mℓ	2 mℓ	2 mℓ	2 mℓ	2 mℓ

(1) J. H. Bryner, personal communication, 1978

(2) Bausch & Lomb Spectronic 21 spectrophotometer light transmission (L.T.) readings at wave length 420 nm

[1] B.B.L. anaerobic jars, Laboratory and Scientific Equipment Co. (Pty) Ltd., P.O. Box 4512, Mayfair, Johannesburg 2108

[2] Protea Laboratories, P.O. Box 39127, Bramley, Johannesburg 2018

Received 26 June 1987—Editor

TABLE 3 Serological reactions of strains to homologous and heterologous antisera

Untreated antigens	Antiserum															
	796/1		6829		1336		157/5		725/2		311/2		413/17			
	Untreated	Heat-treated	Untreated	Heat-treated	Untreated	Heat-treated	Untreated	Heat-treated	Untreated	Heat-treated	Untreated	Heat-treated	Untreated	Heat-treated		
796/1	5 120	1 280	10 240	—	10 240	2 560	1 280	—	640	—	2 560	10 240	640	—		
6829	5 120	5 120	10 240	160	—	—	2 560	—	5 120	—			—	—		
1336	20	60	320	80	2 560	1 280	—	—	—	80	—	4	—	—		
157/5	5 120	10 240	10 240	—	5 120	—	2 560	—	—	—	2 560	—	—	—		
725/2	2 560	—	640	—	—	—	640	—	5 120	—	640	—	10 240	2 560		
311/2	10 240	320	160	—	—	—	320	—	1 280	—	10 240	160	—	—		
413/17	5 120	10 240	2 560	10 240	10 240	10 240	640	40	2 560	—	10 240	10 240	320	160		

°C until used. Untreated and heat-treated cells from each strain were used to produce antisera. The 2 antisera in each case were tested against homologous and heterologous untreated strains used as antigen.

The microtitre serum agglutination test

Aliquots of 50 $\mu\ell$ sera were placed in the 1st row of wells of microtitre plates[3]. Sera (5 $\mu\ell$) were carried over to the 2nd row of wells with a Micro Compu-Pet[4] Multi Diluter adjusted to a 1/10 dilution. The dilution was then changed to 1/2 and a serial twofold dilution of the serum was carried out.

Antigen (50 $\mu\ell$) was added to each well, the contents were mixed and the plates were incubated for 3 h on a shaker at 37 °C. The plates were then centrifuged for 1,5 min at 650 *g*.

The tests were read against a dark background with illumination from the sides. A positive reaction was taken as the granular agglutination of the cells with a clear supernatant. The titre of the reaction was taken as the highest serum dilution where complete agglutination of the antigen occurred. Both antisera raised to heat-treated and untreated cells of each strain were tested against untreated antigen preparations of all the strains.

RESULTS

The results of the homologous and heterologous agglutination reactions of antisera raised to heat-treated and untreated cells with untreated antigen preparations of the various strains are given in Table 3.

It was fairly easy to differentiate between a positive and a negative reaction. In a positive reaction agglutination was seen as a clearly visible clumping of cells, with either a granular mat or a smooth shield of agglutinated cells. In the case of positive reactions, the supernate was clear. The negative reaction showed no agglutination, formed a button of cells in the wells and had a cloudy supernate.

High titres, ranging from 2 560 to 10 240, were encountered in the homologous reactions of untreated antigens with untreated antisera (Table 3). This was the case for all strains except 413/17, whose titre was lower (Table 3).

In the heterologous reactions, titres varied from low to high. The heat-treated antisera gave more negative reactions than the untreated antisera. The degree of cross-agglutination was high. The heat-treated antisera generally reacted with lower titres to both homologous and heterologous antigens than did untreated antisera (Table 3).

The untreated antigen preparation of strain 413/17 showed the lowest titre of all the untreated antigens in the homologous agglutination reactions. This antigen showed high titres by heterologous antisera, although antisera of this strain agglutinated the antigens of the other strains poorly, except for antigen 725/2 (Table 3).

The untreated antigen preparations of strains 796/1, 1336 and 413/17 in their homologous reactions were agglutinated to almost the same titres by both their untreated and heat-treated antisera.

The untreated antigenic forms of strains 6829, 157/5, 311/2 and 725/2 were either not agglutinated or showed lower titres by their homologous heat-treated antisera, and they reacted equally poorly with heat-treated antisera of these 3 strains in the heterologous reactions (Table 3).

[3] Linbro U-bottom microtitre plates, Seravac, P.O. Box 5933, Johannesburg 2000

[4] General Diagnostics, Warner Pharmaceuticals (Pty) Ltd, P.O. Box 1718, Cape Town

Discussion

The serum agglutination test performed in microtitre plates has many advantages over a manually performed test:

(i) the results are available earlier because of a shorter incubation period (3 h as opposed to 20 h).

(ii) the accuracy and reproducibility are superior because of the very accurate microdiluter apparatus.

(iii) only small quantities of antiserum and antigen are required. This is of great benefit when the slow-growing *C. fetus* is used for antigen.

The test was developed with the aim of determining the presence of the K-antigen (microcapsule) among various *C. fetus venerealis* strains. The microcapsule is heat-labile (Berg *et al.*, 1971) and covers the somatic antigens of an organism, rendering it inagglutinable in somatic-antigen-specific antisera.

Antisera produced from untreated organisms which possess microcapsules would contain antibodies against the microcapsule, flagellar antigens and, if some somatic antigens are exposed, antibodies against these as well.

Antiserum produced from untreated strains which do not have this microcapsule in their natural state would contain antibodies against the exposed somatic antigens, as well as against flagellar antigens. Thus, antisera produced from such an untreated strain, as well as antisera from any heat-treated strain, would both contain antibodies against somatic antigens and would give similar agglutination reactions. However, it should be kept in mind that antisera from untreated strains contain antibodies against flagellar antigens, while flagellar antigens are destroyed by the heating process (Berg *et al.*, 1971). Also, some incompletely covered somatic antigens may elicit an antibody response, even in untreated strains with a microcapsule.

On the basis of the inagglutinability of strains 6829, 157/5, 725/2 and 311/2 by their heat-treated antisera (Table 3), it could be assumed that these strains do possess microcapsules which cover somatic antigens. Strains 796/1, 1336 and 413/17 are agglutinated to more or less the same degree by homologous heat-treated and untreated antisera (Table 3). It seems as if these latter 3 strains do not possess microcapsules.

The high degree of cross-agglutination can be expected, as an antigenic relationship exists between all strains of *C. fetus* (Winter & Dunne, 1962). All *C. fetus venerealis* strains possess the same somatic antigen (Berg *et al.*, 1971), thus heat-treated antisera of all *C. fetus venerealis* strains would have the same specificity. The titres as well as the degree of cross-agglutination were comparable with the results of other workers (Berg *et al.*, 1971; Walsh & White, 1968; Winter & Dunne, 1962; Ristic *et al.*, 1957).

All strains of *C. fetus venerealis*, except strain 413/17, seem to be highly immunogenic. Apparently all *C. fetus venerealis* strains have microcapsules while in their natural host. A strain which seemed not to have a microcapsule was mentioned only once in the available literature (Winter & Dunne, 1962; Winter, 1963). *C. fetus venerealis* strain 796/1 could have been laboratory passaged and adapted, but have lost, through mutation, its ability to form a microcapsule. Strain 1336, a strain obtained in 1972, could have lost its microcapsule through the same mechanisms as strain 796/1. All the other strains used in the study were more recent field isolates or imports (6829) which had not been passaged to the same extent as strain 796/1.

C. fetus has 7 antigenically different heat-liable (microcapsular) antigens (Berg *et al.*, 1971; Corbeil, Schurig, Bier & Winter, 1975). Since an organism can have between 2 and 5 of the different antigenic forms (Berg *et al.*, 1971), untreated antisera from strains 6829, 311/2, 157/5 and 725/2 would not have exactly the same microcapsule specificity. This explains why these 4 strains did not show agglutination reactions identical to those of different heterologous untreated antisera.

The microtitre serum agglutination test was developed to examine *C. fetus venerealis* strains antigenically to determine which strains possess microcapsules. Such strains would be suitable to be used as vaccine strains. This study shows that strains 6829, 311/2, 157/5 and 725/2 all possess microcapsules. However, to use a strain like 157/5, whose antisera did not give non-specific cross-reactions, would be an advantage. Further studies should be carried out on strains 157/5, 725/2, 6829 and 311/2, before the final selection of a vaccine strain could be made.

References

Berg, R. L. & Firehammer, B. D., 1978. Effect of interval between booster vaccination and time of breeding on protection against campylobacteriosis (vibriosis) in cattle. *Journal of the American Veterinary and Medical Association*, 173 (5), 467–471.

Berg, R. L., Jutila, J. W. & Firehammer, B. D., 1971. A revised classification of *Vibrio fetus*. *American Journal of Veterinary Research*, 32 (1), 11–22.

Border, Maryon, Myers, L. L. & Firehammer, B. D., 1974. Bactericidal activity of blood of rabbits vaccinated with homologous antigens of *Campylobacter fetus (Vibrio fetus)*. *Infection and Immunity*, 10 (3), 416–421.

Corbeil, L. B., Schurig, G. D., Bier, P. J. & Winter, A. J., 1975. Bovine venereal vibriosis: antigenic variation of the bacterium during infection. *Infection and Immunity*, 11, 240.

Garcia, M. M., Eaglesome, M. D. & Rigby, C., 1983. Campylobacters important in veterinary medicine. *Veterinary Bulletin*, 53, 793–818.

Herr, S., Te Brugge, Lesley A. & Guiney, M. C. M., 1982. The value of the microtitre serum agglutination test as a second screening test in bovine brucellosis. *Onderstepoort Journal of Veterinary Research*, 49, 23–28.

Marsh, H. & Firehammer, B. D., 1953. Serological relationships of twenty-three ovine and three bovine strains of *Vibrio fetus*. *American Journal of Veterinary Research*, 14, 396.

McCoy, E. C., Doyle, D., Burda, K., Corbeil, L. B. & Winter, A. J., 1975. Superficial antigens of *Campylobacter (Vibrio) fetus:* characterization of an antiphagocytic component. *Infection and Immunity*, 11 (3), 517–525.

Ristic, M., White, F. H., Doty, R. B., Herzberg, M. & Sanders, D. A., 1957. The characteristics of agglutinating antigens of *Vibrio fetus* variants. I. Effects of heat and formalin on serological activity. *American Journal of Veterinary Research*, 18, 764–770.

Schurig, G. D., Hall, C. E., Burda, M. S., Corbeil, L. B., Duncan, J. R. & Winter, A. J., 1973. Persistent genital infection with *Vibrio fetus intestinalis* associated with serotypic alteration of the infecting strain. *American Journal of Veterinary Research*, 34, 1399–1403.

Walsh, A. F. & White, F. H., 1968. Biochemical and serological characteristics of *Vibrio* isolants from cattle. *American Journal of Veterinary Research*, 29 (7), 1377–1384.

Winter, P. J., 1963. An antigenic analysis of *Vibrio fetus*. II. Further characterization of precipitating antigens. *American Journal of Veterinary Research*, 24, 193–198.

Winter, A. J. & Dunne, H. W., 1962. An antigenic analysis of *Vibrio fetus*. I. Properties of soluble extracts of the organism. *American Journal of Veterinary Research*, 23, 150–158.

Winter, A. J., McCoy, E. C., Fullmer, C. S., Burda, K. & Bier, P. J., 1978. Microcapsule of *Campylobacter fetus:* chemical and physical characterization. *Infection and Immunity*, 22 (3), 963–971.