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STUDYING THE DIAGNOSTIC VALUE OF RECOMBINANT *CAMPYLOBACTER JEJUNI* ANTIGENS

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Abstract

This article describes the results of a study on the diagnostic value of *Campylobacter jejuni* recombinant antigens, *Campylobacter* Omp18 protein and major outer membrane protein (MOMP). In the present study, these proteins were used as antigens in enzyme immunoassays to detect specific antibodies in the serum of cattle. Commercial native protein antigens were used to compare the effectiveness with similar studies. In total, 95 blood serum samples from cattle from various farms in the northern region of the Republic of Kazakhstan were used. The greatest number of positive results was observed when using a commercial native antigen (52.6%); 47.4% of sera reacted positively with respect to the Omp18 antigen, and 38.9% with respect to the MOMP antigen. Statistical analysis revealed a high correlation between the native and Omp18 antigens and, to a lesser extent, with MOMP32. Because native antigens contain many different cell-wall proteins, they are less specific. Therefore, for serological diagnosis of cattle using enzyme immunoassays, it is advisable to use the *C. jejuni* outer membrane recombinant antigen MOMP32, as it has valuable diagnostic properties.

Key words: Antigens; *Campylobacter jejuni*; campylobacteriosis; diagnostics; enzyme immunoassay; recombinant proteins; specific antibodies.

Basic position and Introduction

Campylobacteriosis is an infectious disease that affects animals of many species, caused by pathogenic microorganisms from the genus *Campylobacter*, and characterized by varying degrees of damage, severity of the

course and polymorphism of manifestations. Campylobacteriosis has recently been reported as a food-borne disease in countries worldwide [1,2].

The antigenic structure of *Campylobacter* is complex, as evidenced by a large number of cross-reactions between type strains and freshly prepared cultures. The differences in the antigenic structure of *Campylobacter* are used for serological typing. Antigenically, *Campylobacter* are heterogeneous; they are clearly differentiated by agglutination and indirect haemagglutination reactions. The structure of campylobacteriosis-pathogen antigens is represented by three thermostable O-antigens, seven thermostable K-antigens, and thermolabile H-antigens. *C. jejuni* and *C. coli* are the most common *Campylobacter* pathogens in humans and animals and are serologically heterogeneous. Fifty-five serogroups based on thermostable antigens have been previously described [3].

In diarrhoea caused by *Campylobacter*, there is a classic immune response. First, it is directed to membrane proteins and the flagellar antigen. Using the ratio of immunoglobulins of individual classes in the body, one can judge the presence of campylobacteriosis and the approximate timing of the disease course. High IgG titres persist for 3 months or more, and IgA titres persist for 1 month after illness [4].

At present, the existence of a "universal" *Campylobacter* antigen, that is, an antigen whereby antibodies would be detected in the serum of all patients in sufficiently high titres, remains unclear. Qian et al. suggested that for *C. jejuni,* this universal antigen is the p43 protein, whereas according to Blaser et al., it is the p44 protein [5,6].

To determine the role of various protein antigens in the immune response during campylobacteriosis, immunoblotting, which enables the simultaneous detection of antibodies against antigens with different molecular weights, is used. The intensity of the reaction and antibody titres increase significantly as the disease progresses and during the convalescence stage. Previously, immunoblotting revealed no cross-reactivity with 21 enterobacteria, staphylococci, and streptococci [7,8].

The most important surface antigens are the lipopolysaccharide and acid-soluble protein fractions. These antigens play a fundamental role in the serotyping of *C.fetus* and *C. jejuni,* and in the serological diagnosis of campylobacteriosis. Antigenic differences among bacteria of different serotypes are associated with the carbohydrate composition of the internal lipopolysaccharides of *Campylobacter*. The chemical composition of *C. jejuni* lipopolysaccharides are similar to that of the antigens of other enterobacteria. In addition to the similarity in chemical structure, immunoblotting and other methods have established a significant immunological relationship between *Campylobacter* lipopolysaccharides and other intestinal infection pathogens, including *Salmonella, Yersinia, Brucella, Shigella* [9,10,11].

Therefore, the purpose of our study was to determine the diagnostic value of recombinant proteins of the outer membrane of *C. jejuni* with molecular weights of 18kDA and 32 kDa, and their potential use in the serologi-

cal diagnosis of campylobacteriosis in animals.

Materials and Methods

Recombinant proteins of the outer membrane of *C. jejuni* (Omp18 and MOMP32) obtained from the laboratory of immunochemistry and immunobiotechnology of the National Center for Biotechnology, Astana, Kazakhstan [12], and a commercial native protein antigen *Campylobacter jejuni* antigen (Code: NAT41600-100, Native Antigen, UK) were used as antigens.

To determine the molecular weight of the recombinant proteins, electrophoretic separation was performed according to the method described by Laemmli [13] on a polyacrylamide gel (11%) using a vertical electrophoresis apparatus (BioRad, USA). The activity of the recombinant proteins was determined by western blotting using positive control sera. Electrophoretic transfer of antigens from the gel to a nitrocellulose membrane (NCM) was performed using an immunoblotting device (Bio-Rad, Hercules, CA, USA) as previously described by Towbin et al. [14]. For immunochemical manifestation of specific antigens, NCM were incubated in a 1% solution of bovine serum albumin (BSA) overnight at 4°C. They were then washed three times with phosphate buffered saline (PBS) and PBS-Tween (Tw) and incubated for 1.5 h at 37°C in a solution of specific antibodies. The carrier was washed and incubated in the working dilution of peroxidase-labelled anti-species antibodies for 1 h at 37°C. The substrate was prepared before use:0.01 g 4-chloronaphthol (Sigma, USA) was dissolved in 2 ml of alcohol (90%), and mixed with 18 ml PBS (pH 7.2-7.4) and 0.01 ml of 3% hydrogen peroxide. After protein replicates appeared, the reaction was stopped by washing the membranes with distilled water.

Approximately 95 samples of blood sera were obtained from cattle from various farms in the Akmola, Karaganda and Kostanay regions.

Blood sera were analysed by enzyme-linked immunosorbent assay (ELISA). The wells of a polystyrene plate (Thermo Fisher Scientific, USA) were sensitised separately with the protein antigens, Omp18 and MOMP32, and a commercial antigen at a concentration of 0.001 mg/ml. After sensitisation, the active sites of the solid phase were neutralised with 1% BSA solution. Dilutions of blood serum in PBS-Tw were prepared in two wells 1:100-1:200, incubated for 1 h, and labelled anti-bovine IgG antibodies (Sigma-Aldrich, USA) were added after washing. A reaction was considered positive if the optical density (OD) of the serum was two or more times higher than the average OD of the control sample at a dilution of 1:100. Blood serum from healthy animals was used as the negative control. To exclude unreliable results, the experiments were performed in triplicate.

Statistical analysis. Statistical analyses were performed using GraphPad Prism version 9.3.1. (GraphPad software). The statistical tests used as well as the p-values are indicated in the figure captions. A p-value of 0.05was considered statistically significant.

Results

To determine the activity of the recombinant proteins in comparison with na-

tive commercial antigens, electrophoretic separation was performed, followed by immunoblotting using polyclonal antibodies (pAbs) commercial native *C. jejuni* antigen. Western blot analysis showed that the pAbs reacted specifically with the recombinant proteins (Figure 1).

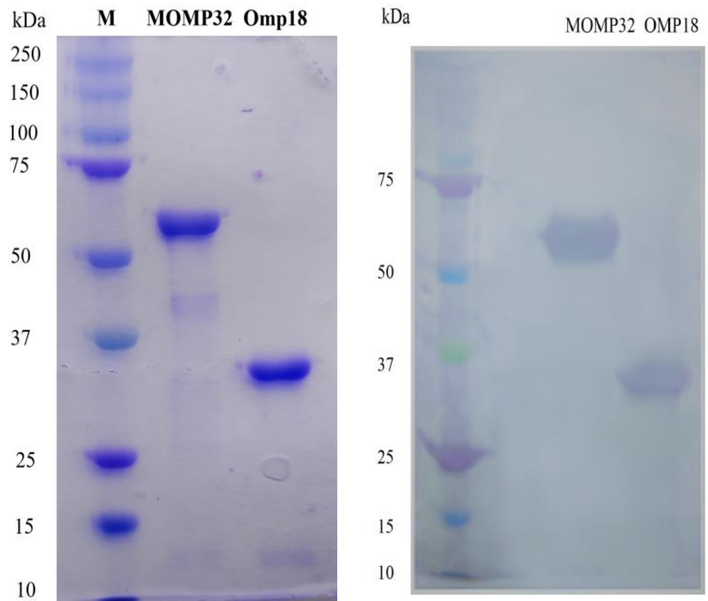

Figure 1 - Electrophoresis and western blot with recombinant antigens

As shown in Figure 1, the molecular weights of the recombinant *C. jejuni* OMPs were 36kDa and 64 kDa for OMP18 and MOMP32, respectively. The high molecular weights of the recombinant proteins were due to the presence of thioredoxin in the expression vector. These data were confirmed by Western blotting.

The immunoblotting results demonstrated that pAbs reacted with both recombinant proteins.

Recombinant proteins were used as antigens when establishing an indirect variant of an enzyme immunoassay with blood sera from cattle on farms in the Karaganda region. A native commercial antigen was used for comparison. The test results are presented in Table 1.

Table 1- Results of testing bovine blood sera using various antigens

OD multiplicity values OD of the test sample/OD of the control sample	Commercial native antigen	MOMP32	OMP 18
	Number of samples	Number of samples	Number of samples
Total	95	95	95
0-0,999	37	45	32
1,000-1,999	8	13	18
2,000 and higher	50	37	45

Note: OD ratio is the ratio of the optical density of the reaction medium relative to the optical density of the negative control. Values of optical multiplicity from 2 and above show that the samples are positive

As shown in Table 1, when using a commercial native antigen, 50 positive serum samples were detected, which represented 52.6% of the total number of samples tested. Specific antibodies to the recombinant MOMP32 antigen were reliably confirmed in 37 sera samples (38.9%), and an interaction with serum antibodies was recorded in 45 samples (47.4%) in the case of the OMP18 antigen.

To determine the diagnostic value of the recombinant antigens, the OD distribution of positively reacting blood serum samples with recombinant and native antigens was analysed. The results of this analysis are shown in Figures 2 and 3.

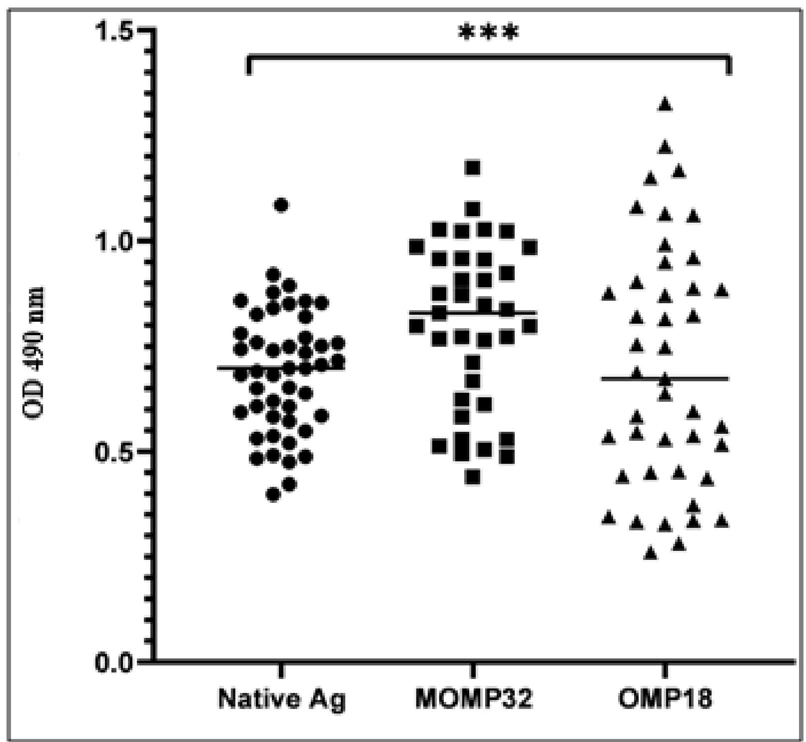

Figure 2 - Analysis of the distribution of optical parameters of positive sera with recombinant and commercial native antigens. Note: each character represents an optical parameter. Statistical analysis was performed with log-transformed data using the Student's t-test: ***p, 0.005.

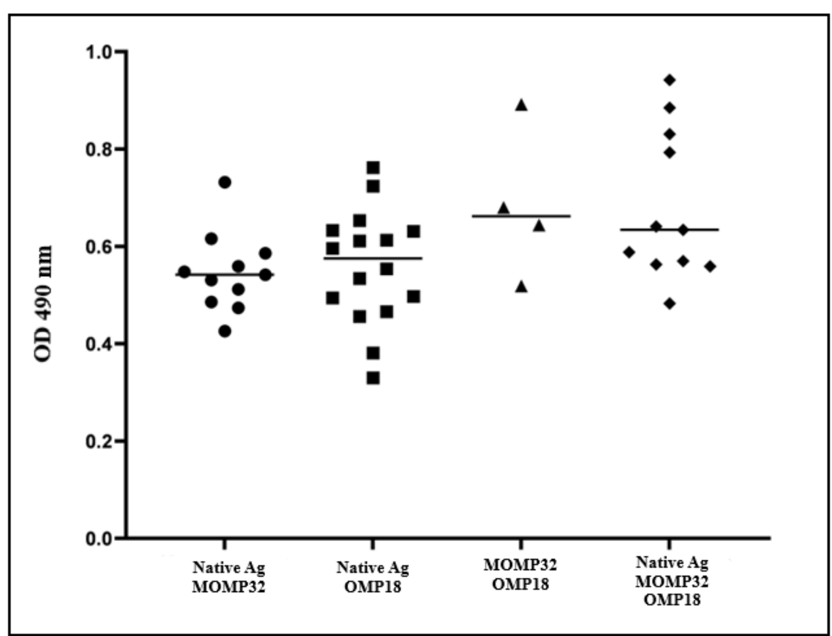

Figure 3 - Analysis of the distribution of coincidences with regards to optical parameters of positive sera in the reaction with recombinant and commercial native antigens

The analysis suggested that the most compact distribution of the optical parameters of bovine sera was observed for the commercial native antigen, with an average of 0.700 OD.The optical reaction parameters of sera with recombinant MOMP32 were also distributed compactly, but with higher OD values equal to an average of 0.800. The same sera containing the recombinant OMP18 antigen showed a wide range of optical reaction parameters ranging from 0.4 to 1.4 OD. This scattering resulted in a decrease in the mean OD values compared to the OD values of the commercial native and MOMP32 antigens. Despite the differences between the OD of OMP18, commercial native, and MOMP32 antigens, the results demonstrated the suitability and interchangeability of antigens for use as components of ELISA in the detection of campylobacteriosis.

This assumption was confirmed by analysing the distribution of optical reaction parameters that coincided with different antigens. Figure 3 shows that the maximum agreement between the results was recorded when using the commercial native antigen and the recombinant OMP18 antigen, which was noted when testing 16 blood serum samples (16.84%). When comparing positive results using the commercial native antigen and the recombinant MOMP32 antigen, a match was found in 10 cases (10.52%). When comparing the results of the interaction of antibodies in the sera with recombinant and native antigens, the coincidence increased slightly for up to 11 positive samples. Notably, the most insignificant coincidence accounted for the variant of the interaction of sera with the two recombinant antigens, which was established in only four cases.

Discussion

Campylobacteriosis is one of the main causes of food poisoning in humans and manifests as diseases of the gastrointestinal tract. It has been estab-

lished that the main source of infection is the pathogen *C. jejuni*, which circulates in animals and inseminates livestock products during slaughter and butchering of carcasses.

At present, the standard diagnostic method for detecting *C. jejuni* is the bacteriological method; the limitation of this method lies in the complexity of isolating the culture, the duration of cultivation, and the need for special media. Of the modern diagnostic methods, PCR diagnostics can also be noted, but this method requires special conditions, the availability of qualified specialists, and expensive equipment.

Improvements in serological diagnostic methods, simple execution, and effective performance will allow for the detection of specific antibodies that indicate the circulation of campylobacteriosis pathogens in the body of a given animal. One of these methods is the enzyme immunoassay; however, the specificity and sensitivity of the method depends on the quality of the antigen, which is the main component of the diagnosticum. The use of recombinant antigens is a promising approach for serodiagnosis [15,16].

As our studies have shown, recombinant antigens, as ELISA components in the diagnosis of campylobacteriosis, and native commercial antigens, have high specificity and sensitivity.

Conclusion

As a result of the study, the diagnostic value of previously obtained recombinant antigens of the outer membrane of *Campylobacter jejuni* was studied, and the possibility of their use in ELISA for the serological diagnosis of campylobacteriosis was investigated.

The effectiveness of the recombinant antigens was tested by western blotting and ELISA and compared with native commercial antigens. Western blot analysis showed that the recombinant MOMP32 (64 kDa) and Omp18 (36 kDa) antigens reacted with pAbs. ELISA of 95 bovine sera samples revealed 50 positive samples for the commercial native antigen, 37 samples for the recombinant MOMP32 antigen, and 45 samples for the Omp18 antigen.

When analysing the results, it was found that the highest correlation was observed between native and Omp18 antigens, since matches were recorded with 16 samples. Antigen coincidence between native and recombinant MOMP32 was detected in 10 blood serum samples. It can be assumed that the difference in the number of positive results was dependent on the fact that many different cell wall proteins are present in native antigens; therefore, they are less specific.

Therefore, we recommend the use of the recombinant antigen MOMP32 from the outer membrane of *Campylobacter jejuni* in enzyme immunoassays for the serological diagnosis of campylobacteriosis in cattle.

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