INFECTION AND IMMUNITY, Feb. 1974, p. 470-471

Vol. 9, No. 2
Printed in U.S.A.

Antibody Against *Clostridium perfringens* Type A Enterotoxin in Human Sera

TAKASHI UEMURA, CONSTANTIN GENIGEORGIS, HANS P. RIEMANN, AND CHARLES E. FRANTI

Department of Veterinary Science, College of Agriculture, University of Osaka Prefecture, Osaka 591, Japan, and Department of Epidemiology and Preventive Medicine, School of Veterinary Medicine, University of California, Davis, California 95616

Received for publication 11 September 1973

Antibody against *Clostridium perfringens* type A enterotoxin was found in 82% of Brazilian and 65% of American serum samples.

Food poisoning *Clostridium perfringens* type A strains produce an enterotoxin during sporulation (3). The enterotoxin has been purified (5, 8, 9; G. Sakaguchi, T. Uemura, and H. P. Riemann, submitted for publication), which has made it possible to develop serological methods for quantification of enterotoxin and anti-enterotoxin.

Passive hemagglutination (PHA) is a sensitive, specific, and quantitative test which has been used in surveys of serum antibodies against several microbial toxins (4, 7). We used this method, as well as the microslide immunodiffusion test (MID) to survey human sera from 115 Brazilian, 81 American, and 5 Japanese volunteers for presence of antibody against *C. perfringens* enterotoxin.

The PHA test was carried out with Formalin-treated sheep red blood cells (SRBC) that had been sensitized with enterotoxin purified from a sporulating culture of *C. perfringens* type A NCTC 8798 (G. Sakaguchi, T. Uemura, and H. P. Riemann, submitted for publication). Sensitization occurred with the aid of bis-diazotized benzidine (1). Nonspecific agglutination of SRBC was eliminated by absorbing the sera with three volumes of 2.5% nonsensitized SRBC suspended in phosphate-buffered saline containing 0.25% bovine serum albumin, pH 7.2. The absorbed sera were diluted 1:80 (vol/vol) and subjected to the PHA test. Twofold dilutions were tested to determine antibody titer.

The MID test was carried out according to Unterman's modification (10) of Casman's method (2). Sera diluted 1:4 (vol/vol) by the absorption procedure were placed in the outer wells. One outer well was filled with 1:100 (vol/vol) diluted rabbit immune serum, which served as reference, and 2 μg of enterotoxin per ml in saline was placed in the center well.

Serum samples were obtained from students and employees at the Schools of Veterinary Medicine, University of Minas Gerais, Belo Horizonte, Brazil, and University of California, Davis. Five persons in a laboratory of the Department of Veterinary Science, University

TABLE 1. *Distribution of serum titers against C. perfringens type A enterotoxin*

Nationality and sex[a]	Reciprocal of PHA titer							
	<160[b]	160	320	640	1,280	2,560	5,120	Total
Brazilian								
Male	19	5	19	30	13	11	6	103
Female	2	1	1	3	2	2	1	12
Total	21	6	20	33	15	13	7	115
Percentage	18	5.2	17	29	13	11	6.1	
American								
Male	21	10	10	10	8	5	1	65
Female	8	2	4	1	5			20
Total	29	12	14	11	13	5	1	85
Percentage	34.7	14	16	13	15	5.9	1.2	

[a] The chi-square test showed no significant difference between sexes ($P > 0.1$).
[b] Sera negative for PHA at a dilution of 1:160.

TABLE 2. *Persistence of antibody against enterotoxin in sera of normal human subjects*

Subject	Reciprocal of PHA titer (time in weeks)			
	0	4	14	17
SK	2,560	1,280	1,280	1,280
SS	320	320	320	640
YH	640	640	640	320
GS	320	320	160	320
KH	160	320	160	320

TABLE 3. *Sensitivity of MID compared with PHA in detection of serum antibody of 31 persons against enterotoxin*

No. of sera tested	No. of positive MID tests	Reciprocal of PHA titer
17	0	640
4	0	1,280
5	4	2,560
5	4	5,120

of Osaka Prefecture, Japan, submitted several serum samples during a period of 17 weeks.

Eighty-two percent of Brazilian and 65% of American serum samples showed a PHA titer higher than 1:160 (vol/vol), the maximum titer for both groups was 1:5,120 (vol/vol) (Table 1). The difference between the two nationality groups was statistically significant ($P < 0.01$), but no significant differences were found with respect to sex, residence (urban versus rural), or a few recorded food habits (e.g., drinking raw versus pasteurized milk and eating raw or rare meat versus never eating undercooked meat). Antibody level seemed to remain fairly stable over a period of 17 weeks (Table 2).

It is not possible at the present time to explain the high prevalence of enterotoxin antibody in human sera nor is the reason for the observed national difference known. Antibody production might be induced during acute *C. perfringens* food poisoning but could possibly also be due to prolonged absorption of enterotoxin in symptomless carriers who harbor high numbers of *C. perfringens* type A.

The superior sensitivity of PHA compared with MID in detecting serum antibody is illustrated in Table 3.

This work was supported by Public Health Service grant no. 5-R01-FD-004-Tox and a grant-in-aid from the American Meat Institute Foundation.

The authors acknowledge the assistance of P. Brant, R. Reis (School of Veterinary Science, Federal University of Minas Gerais, Belo Horizonte, Brazil), and C. Stormont (University of California, Davis) in obtaining serum samples.

LITERATURE CITED

1. Butler, W. T. 1963. Hemagglutination studies with Formalinized erythrocytes. J. Immunol. **90**:663–671.
2. Casman, E. P., R. W. Bennet, A. E. Dorsey, and J. E. Stone. 1969. The microslide gel double diffusion test for the detection and assay of staphylococcal enterotoxins. Health Lab. Sci. **6**:185–198.
3. Duncan, C. L., and D. H. Strong. 1968. Improved medium for sporulation of *Clostridium perfringens*. Appl. Microbiol. **16**:82–89.
4. Finkelstein, R. A., and J. W. Peterson. 1970. In vitro detection of antibody to cholera enterotoxin in cholera patients and laboratory animals. Infect. Immunity **1**:21–29.
5. Hauschild, A. H. W., and R. Hilsheimer. 1971. Purification and characterization of the enterotoxin of *Clostridium perfringens* type A. Can. J. Microbiol. **17**:1425–1433.
6. Hochstein, H. D., J. C. Feeley, and W. E. DeWitt. 1970. Titration of cholera antitoxin in human sera by microhemagglutination with Formalinized erythrocytes. Appl. Microbiol. **19**:742–745.
7. Sakaguchi, G., S. Sakaguchi, T. Uemura, H. Kondo, M. Kubonoya, H. Iida, and E. Kodama. 1971. Distribution of type E botulinum antitoxin among Japanese people. Jap. J. Vet. Sci. **33**:82.
8. Stark, R. L., and C. L. Duncan. 1971. Biological characteristics of *Clostridium perfringens* type A enterotoxin. Infect. Immunity. **4**:89–96.
9. Stark, R. L., and C. L. Duncan. 1972. Purification and biochemical properties of *Clostridium perfringens* type A enterotoxin. Infect. Immunity **6**:662–673.
10. Unterman, F. 1972. Diagnostik der staphylokokken Lebensmittelvergiftung. Fleischwirtschaft **52**:500–503.