NOTE

Serologic Typing of *Haemophilus Paragallinarum* Based on Serum Bactericidal Reactions

Akira SAWATA, Katsumi KUME¹⁾, and Toyotsugu NAKAI

Research Center for Veterinary Science of The Kitasato Institute, Kashiwa, Chiba 277, and ¹⁾The Kitasato Institute, 5-9-1 Shirokane, Minato, Tokyo 108, Japan

(Received 26 January 1984/Accepted 7 August 1984)

ABSTRACT. Attempts were made to detect serologic specificities involved in the serum bactericidal (BC) reaction with *Haemophilus paragallinarum* (Hpg). On the basis of serum BC reactions, ten Hpg isolates were classified into two distinct serotypes. Specificity of the BC activity toward the encapsulated organisms of Hpg defined by the BC assay is similar to serotype specificity defined by the hemagglutination-inhibition or by the agglutination tests.—**KEY WORDS:** *Haemophilus paragallinarum*, serotype.

Jpn. J. Vet. Sci. 46(6): 909–912, 1984

Page [13] originally classified his *Haemophilus paragallinarum* (Hpg) isolates into three serotypes A, B, and C, based on the agglutination reaction and this was followed by a similar classification by Hinz [3]. Sawata *et al.* [18] examined their isolates in Japan by an agglutination test, and claimed the existence of two agglutination serotypes 1 and 2. Furthermore, they [20] compared the serologic types of their isolates with those by Page [13] and indicated that Sawata's serotype 1 was identical with Page's serotype A, whereas their serotype 2 was identical with serotype C represented by strain "Modesto" [12]. Page's serotype B strains, 0222 and Spross, lacked serotype B-specific antigen, therefore, these were nontypable [20].

The organisms belonging to the serotypes 1 and 2 [18] possessed serotype-specific protein-like agglutinin designated as "L" on their surface [19]. The organisms also possessed [15, 16, 21] the hemagglutinin, designated as "HA-L", and the hemagglutinin appears to be closely related to "L" agglutinin [19]. Moreover, the specificity of the hemagglutination defined by the he-

magglutination-inhibition (HI) test [21] is similar to serotype specificity originally defined by Sawata *et al.* [18]. More recently, Kume *et al.* [6] classified Hpg strains into seven serotypes, designated as HA-1 to HA-7, by using the serotype-specific HA-L hemagglutinin system.

In a previous report [15, 17], we found that fresh immune chicken sera with homologous antibodies were bactericidal (BC) for encapsulated organisms of Hpg hemagglutination serotype HA-1. The BC activity was due to the antibodies against HA-L hemagglutinin [15, 16, 21] and/or L agglutinin [19] presented at the most outer part of outer membrane of the cell [16]. The present study deals with serologic typing of encapsulated Hpg based on the serum BC reactions, as compared with other serologic typing.

Ten strains of Hpg were used: strain 221 [4], 083 [14], 7682 [18], 7119 [18], and 7411 [18] were of the hemagglutination serotype HA-1 [6] (agglutination serotype 1 [18]); H-18, 1101, 7719, 7710, and FO-26 [7] were of the serotype HA-4 [6] (serotype 2 [18]). Only encapsulated hi variant [5, 15] obtained

Table 1. Effect of absorption of ICS with nontreated antigens originated from encapsulated variant of *H. paragallinarum* on antibody activities

Variant	ICS against	Absorbed with ^{a)}	HI antibody titers with		RPA titers with		BC titers ^{b)} in sera at reaction time of 3 hr at 37°C	
			221-HA-L hemagglutinin	H-18-HA-L hemagglutinin	221-L antigen	H-18-L antigen	221	H-18
221	221	None	1:640	<1:5	1:40	<1:5	1:125	<1:5
		221	<1:5	<1:5	<1:5	<1:5	<1:5	<1:5
		H-18	1:640	<1:5	1:40	<1:5	1:125	<1:5
H-18	H-18	None	<1:5	1:320	<1:5	1:40	<1:5	1:125
		221	<1:5	1:320	<1:5	1:40	<1:5	1:125
		H-18	<1:5	<1:5	<1:5	<1:5	<1:5	<1:5

a) Absorbed twice with 1×10^{12} cells/ml.

b) The highest serum-dilution showing positive BC effect was expressed as BC titer.

by the method described previously [15] were used throughout this work. Nontreated (NT) [15] or potassium thiocyanate (KSCN)-treated and sonicated (KSCN-S) [21] antigens were prepared from each strain according to the procedures described previously [15, 21]. Each NT antigen adjusted the concentration to a 5×10^9 cells/ml by spectrophotometry was used for injection in chicks to obtain immune chicken sera (ICS) for the BC assay after absorption onto 900 $\mu\text{g/ml}$ of $\text{Al}(\text{OH})_3$.

The rapid plate agglutination (RPA) method was used for the agglutination test by using the KSCN-S antigen [21] as L agglutinin [19]. The HI test [6, 21] was performed by using glutaraldehyde (GA, Eastman Kodak)-fixed erythrocytes (RBC) [21] collected from 60-day-old line S chicks (Nisseiken Co., Yamanashi, Japan) and the KSCN-S antigen as HA-L hemagglutinin [21]. The NT antigen (whole cell) adjusted to a concentration of 1×10^{12} cells/ml were used as an absorbent. Absorption was done twice at 0°C for 1 hr as described previously, and the HI antibody, agglutinin, and BC antibody titers remaining in the resulting supernatant were titrated [17]. The BC assay was done by the procedures reported previously, and the result was read according to the criteria described in a previous paper [17].

The ICS prepared against strain 221 or

H-18 contained serotype-specific HI antibody and agglutinin. Each antiserum also showed BC activities to the homologous strain alone, indicating the serotype specificity of the BC reactions. These results were confirmed by the cross-absorption tests (Table 1). These results showed that the strain 221 and H-18 could be assigned to two distinct serotypes BC-1 and BC-2, respectively.

On the basis of the BC reactions using ICS against strains 221 and H-18, ten Hpg strains were differentiated into two distinct serotypes BC-1 and BC-2 (Table 2).

The BC system has been used as a method of serotyping of *Neisseria gonorrhoeae* [1, 2]. The results of the present investigation apparently showed that fresh ICS containing serotype-specific HI antibody and agglutinin can inactivate encapsulated organisms of Hpg of homologous type, but not of heterologous type. Specificity of the BC activity toward the encapsulated organisms of Hpg defined by the BC assay is similar to the serotype specificity defined by the HI [6, 21] or by the RPA [19] tests.

Sawata *et al.* [17] reported that fresh normal chicken serum can inactivate nonencapsulated organisms of Hpg, whereas fresh ICS containing the HI antibody against HA-L hemagglutinin and L agglutinin can inactivate both encapsulated and nonencapsulated or-

Table 2. Serologic typing of *H. paragallinarum* based on serum BC reactions

Variant	Hemagglutination serotype	Agglutination serotype	BC titers in sera at reaction time of 3 hr at 37°C	
			221-ICS	H-18-ICS
221	HA-1	1	1:125	<1:5
083	HA-1	1	1:125	<1:5
7682	HA-1	1	1:125	<1:5
7119	HA-1	1	1:125	<1:5
7411	HA-1	1	1:625	<1:5
H-18	HA-4	2	<1:5	1:125
1101	HA-4	2	<1:5	1:25
7719	HA-4	2	<1:5	1:25
7710	HA-4	2	<1:5	1:125
FO-26	HA-4	2	<1:5	1:125

ganisms. Therefore, careful selection of encapsulated variants for the BC assay is significantly required. A recommended method for obtaining the variants was previously described by Sawata and Kume [15].

Recently, Sawata *et al.* [17] pointed out that a BC antibody may be directed against the HA-L hemagglutinin [15, 16, 21] and/or L agglutinin [19] with serotype specificity by using Hpg strain 221 belonging to hemagglutination serotype HA-1 [6] (agglutination serotype 1 [19]). The strains belonging to agglutination serotypes 1 and 2 had a common L agglutinin, therefore, cross-reacted in varying extents in the agglutination test [19]. However, cross-reactivity was not demonstrated between the strains, when they were examined by the HI test [6, 21]. In the present investigation, cross-reactivity was also not observed between the two types of Hpg which was defined by the BC reactions. The BC assay system presented herein was found to be applicable for serologic typing of Hpg.

In *N. gonorrhoeae* [11, 22], the BC system has been advocated as a method of immunotyping of the organisms because of its significant correlation with protective immunity in *in vivo* studies. Previously, Kume *et al.* [7-10, 21] found that two immunotypes 1 and

2 corresponded to the agglutination serotypes 1 and 2 [18] among Hpg strains, but that cross-protection was not observed between them. The types of ten strains classified based on the BC reactions significantly correlated with the protective immunity in *in vivo* studies which were conducted previously by Kume *et al.* [7-10, 21]. Recently Kume *et al.* [6] classified Hpg strains into seven serotypes by using the serotype-specific HA-L hemagglutinin system. This system was found more useful to discriminate among serotypes [6]. However, the correlation between the HA-L hemagglutinin specificity and immunologic specificity has not been studied well except for the serotypes HA-1 and HA-4 [21]. The BC assay method developed by us would be employed as a useful tool to analyse the immunotypes of Hpg *in vitro*.

ACKNOWLEDGEMENTS. The authors thank Dr. M. Matsumoto, School of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97331, for reviewing the manuscript and Mrs. N. Kaneko, Kitasato Institute, for technical assistance.

REFERENCES

1. Glynn, A. A., and Word, M. E. 1970. *Infect. Immun.* 2: 162-168.
2. Gold, R., and Wyle, F. A. 1970. *Infect. Immun.*

- 1: 479-484.
3. Hinz, K. H. 1973. *Avian Pathol.* 2: 269-278.
4. Kato, K., and Tsubahara, H. 1962. *Bull. Natl. Inst. Anim. Health* 45: 21-26 (in Japanese).
5. Kume, K., and Sawata, A. 1984. *Jpn. J. Vet. Sci.* 46: 47-54.
6. Kume, K., Sawata, A., Nakai, T., and Matsumoto, M. 1983. *J. Clin. Microbiol.* 17: 958-964.
7. Kume, K., Sawata, A., and Nakase, Y. 1978. *Jpn. J. Vet. Sci.* 40: 65-73.
8. Kume, K., Sawata, A., and Nakase, Y. 1980. *Am. J. Vet. Res.* 41: 97-100.
9. Kume, K., Sawata, A., and Nakase, Y. 1980. *Am. J. Vet. Res.* 41: 757-760.
10. Kume, K., Sawata, A., and Nakase, Y. 1980. *Jpn. J. Vet. Sci.* 42: 673-680.
11. Mark, J. A., and Wang, S. P. 1978. *Infect. Immun.* 22: 403-410.
12. Matsumoto, M., and Yamamoto, R. 1978. *Am. J. Vet. Res.* 36: 579-582.
13. Page, L. A. 1962. *Am. J. Vet. Res.* 23: 85-95.
14. Rimler, R. B., Davis, R. B., and Page, R. K. 1977. *Am. J. Vet. Res.* 38: 1587-1589.
15. Sawata, A., and Kume, K. 1983. *J. Clin. Microbiol.* 18: 49-55.
16. Sawata, A., Kume, K., and Nakai, T. 1984. *Jpn. J. Vet. Sci.* 46: 19-27.
17. Sawata, A., Kume, K., and Nakai, T. 1984. *Jpn. J. Vet. Sci.* 46: 805-813.
18. Sawata, A., Kume, K., and Nakase, Y. 1978. *Jpn. J. Vet. Sci.* 40: 645-652.
19. Sawata, A., Kume, K., and Nakase, Y. 1979. *Am. J. Vet. Res.* 40: 1450-1453.
20. Sawata, A., Kume, K., and Nakase, Y. 1980. *Am. J. Vet. Res.* 41: 1901-1904.
21. Sawata, A., Kume, K., and Nakase, Y. 1982. *Am. J. Vet. Res.* 43: 1311-1314.
22. Wong, K. H., Arko, R. J., Logan, L. C., and Bullard, J. C. 1976. *Infect. Immun.* 14: 1297-1301.

要 約

免疫血清の殺菌作用にもとづく *Haemophilus paragallinarum* の血清型別 (短報): 澤田 章・久米勝巳¹⁾・中井豊次 (北里研究所附属家畜衛生研究所, ¹⁾北里研究所) —— *H. paragallinarum* に対する免疫血清の殺菌作用の特異性を検討したところ, 供試10菌株は2血清型に型別され, 赤血球凝集抑制反応や凝集反応にもとづく型別と一致した。