Biochemical and Serological Examination of β -hemolytic Streptococci Isolated from Slaughtered Pigs

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(Received 15 April 1997/Accepted 5 August 1997)

ABSTRACT. A total of 170 β -hemolytic streptococci isolated from lesions in slaughtered pigs during 1988 to 1995 were identified by biochemical and serological examinations. Of these, 132 strains (77.6%) were *Streptococcus* (*S.*) *dysgalactiae* and 38 strains (22.4%) were *S. porcinus*. The largest serological group of streptococci was group C (78 strains, 45.9%), followed by group L (43 strains, 25.3%), group U (14 strains, 8.2%), group G (11 strains, 6.5%), group E (5 strains, 2.9%), and group P (5 strains, 2.9%). Most of isolates from endocarditis (61 strains) and arthritis (25 strains) were group C *S. dysgalactiae*, but about 33.3% of the isolates from lymphadenitis were group L *S. dysgalactiae* (28 strains), followed by group C (14 strains, 16.7%), group U *S. porcinus* (14 strains, 14.3%), and group G (10 strains, 11.9%). — **KEY WORDS:** epidemiology, β -hemolytic streptococci, swine.

J. Vet. Med. Sci. 60(1): 129–131, 1998

β -hemolytic streptococci are known to be causative agents of septicemia, endocarditis, arthritis, abortion and lymphadenitis in pigs [3, 7, 12]. Most of the β -hemolytic streptococci isolated from lesions in pigs belong to Lancefield's serogroups C, E, G, L, P, U and V [16].

Recent genetical and biochemical studies have led to important modifications in the classification and nomenclature of broad β -hemolytic streptococci isolated from pigs and other animals. Farrow and Collins [6] showed that *S. dysgalactiae*, "*S. equisimilis*" and streptococci of Lancefield's serological groups C, G and L are a single species, and they suggested that an emended description of the species *S. dysgalactiae* be given. Collins *et al.* [4] reported that streptococci of serological groups E, P, U and V formed a single DNA-DNA homology group and represented a new species for which the name *S. porcinus* was proposed. In a later study, β -hemolytic group G streptococci from cows with mastitis and from dogs with different pathological conditions were characterized and named *S. canis* by Devriese *et al.* [5].

There are no detailed studies of biochemical and serological examinations of β -hemolytic streptococci from diseased pigs in Japan. In accordance with these new classifications, we investigated the biochemical and serological properties of β -hemolytic streptococci from endocarditis, arthritis and lymphadenitis in slaughtered pigs.

A total of 170 isolates of β -hemolytic streptococci were collected between 1988 and 1995 from endocarditis (61 strains), arthritis (25 strains) and lymphadenitis (84 strains) of pigs slaughtered at the Sendai Meat Plant. Culture of the tissues was carried out on 5% horse blood agar plates, incubated at 37°C in 5% CO₂ for 24 hr. Colonies of catalase-negative, gram-positive cocci exhibiting β -hemolysis were subinoculated onto 5% sheep blood agar plates and incubated at 37°C in 5% CO₂ for 24 hr. The strains were identified by using API 20 STREP (BioMerieux, France) according to instructions of the

manufacturer. Growth characteristic in a brain heart infusion (BHI, Oxoid, U.K.), CAMP tests and hyaluronidase tests were examined by the method of Hommez *et al.* [9].

Serological grouping was performed by the precipitation in capillary tubes described by Lancefield [14]. Rabbit hyperimmune grouping sera A to V were prepared with heat-killed whole streptococcus cells using the reference group strains of the WHO International Center for Streptococcus Typing, Prague, Czech Republic. The antigen for serogrouping was extracted by autoclaving as described by Rantz and Randall [15]. Serological identification was further carried out using Streptex (Wellcome, U.K.) and Phadebact Strep test (Pharmacia, U.S.A.), which are coagglutination reagents for identification of serogroup G.

The results of serogrouping by the capillary precipitation test were as follows. Of the 61 strains from endocarditis, 49 strains (80.4%) were identified as group C and 7 strains (11.5%) as group L. Of the 25 strains from arthritis, 15 strains (60.0%) were identified as group C and 8 strains (32.0%) as group L. The majority of the isolates from lymphadenitis were group L streptococci (28 strains; 33.3%). The remaining strains belonged to groups C (16.7%), E (6.0%), P (4.7%) and U (14.3%) streptococci. Twenty-five strains (14.7%) were ungroupable.

The biochemical properties of all 170 strains are shown in Table 1. All of group C (78 strains) and group L streptococci (43 strains) were biochemically identified as *S. dysgalactiae* and group E (5 strains), group P (5 strains), group U (14 strains) were identified as *S. porcinus*. Eleven of the 25 ungroupable isolates were biochemically identified as *S. dysgalactiae* and 14 were identified as *S. porcinus*. The 11 strains biochemically identified as *S. dysgalactiae* were further examined for serological identification using commercial kits. Those of ungroupable strains were serologically identified as group G streptococci.

These data are summarized in Table 2. From the biochemical and serological examinations, 132 (77.6%) of

Table 1. Biochemical properties of β -hemolytic streptococci from slaughtered pigs

Biochemical properties	Lancefield serogroup						
	C (78) ^c	L (43)	G ^a (11)	E (5)	P (5)	U (14)	UG ^b (14)
Sediment in BHI	+	+	+	–	–	1/14	–
VP reaction	–	–	–	+	+	+	+
Hippurate	–	–	–	–	–	–	–
Esculin	–	–	–	+	4/5	13/14	8/14
Pyrrolidonyl arylamidase	–	–	–	–	–	–	–
α -Galactosidase	–	–	–	–	–	–	–
β -Glucuronidase	+	+	+	+	+	+	+
β -Galactosidase	–	–	–	–	–	–	–
Alkaline phosphatase	+	+	+	+	+	+	+
Leucine arylamidase	+	+	+	+	+	+	+
Arginine dihydrolase	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+
Arabinose	–	–	–	–	–	–	–
Mannitol	–	–	–	+	+	+	+
Sorbitol	–	–	–	+	+	–	12/14
Lactose	70/78 ^d	29/43	2/11	–	3/5	+	–
Trehalose	+	+	+	+	+	+	+
Inulin	–	–	–	–	–	–	–
Raffinose	–	–	–	–	–	–	–
Starch	+	+	+	+	4/5	+	+
Glycogen	42/78	+	+	–	–	–	–
CAMP reaction	–	–	–	+	+	+	+
Hyarulonidase	+	+	+	–	–	1/14	2/14

a) Serological identification using commercial kits. b) UG: Ungroupable strains. c) Number of examined strains. d) Number of positives/number of strains.

Table 2. The biochemical and serological identification of 170 β -hemolytic streptococci isolated from slaughtered pigs

Bacterial species	Lancefield serogroup	Source of streptococcal strains			
		Endocarditis	Arthritis	Lymphadenitis	Total
<i>Streptococcus dysgalactiae</i>	C	49 (80.4%)	15 (60.0%)	14 (16.7%)	78 (45.8%)
	G	1 (1.6%)		10 (11.9%)	11 (6.5%)
	L	7 (11.5%)	8 (32.0%)	28 (33.3%)	43 (25.3%)
<i>Streptococcus porcinus</i>	E			5 (6.0%)	5 (3.0%)
	P	1 (1.6%)		4 (4.7%)	5 (3.0%)
	U	1 (1.6%)	1 (4.0%)	12 (14.3%)	14 (8.2%)
	Ungroupable	2 (3.3%)	1 (4.0%)	11 (13.1%)	14 (8.2%)
Total		61	25	84	170

the 170 β -hemolytic streptococci were identified as *S. dysgalactiae* and 38 (22.4%) strains were identified as *S. porcinus*. The largest serogroup of β -hemolytic streptococci was group C (45.8%), followed by group L streptococci (25.3%). The main bacterial species in slaughtered pigs with endocarditis, arthritis or lymphadenitis was *S. dysgalactiae*.

This study showed that most of the β -hemolytic streptococci isolated from slaughtered pigs with endocarditis, arthritis or lymphadenitis belong to *S. dysgalactiae* and that the main serogroup is group C. Similar results were obtained by Jones [7], Krantz and Dunne [12] and Kunter [13]. Although *S. suis* infection in pigs has

been attracting considerable attention lately [16], the isolation rate of β -hemolytic streptococci from swine diseases is still high.

Streptococci have been mainly classified according to serological grouping, biochemical properties and hemolysis type. However, DNA-DNA hybridization in recent taxonomic studies has led to nomenclature or reclassification of streptococcal species, regardless of Lancefield's serogroups [4–6]. However, our results indicate that serological grouping is important for epidemiological studies because of the relationship between the prevalence of disease and the serological properties of the bacterial isolates.

Regarding β -hemolytic streptococcal infections in Japan, Hara *et al.* [8] reported that group C *S. dysgalactiae* were only found in 10 of 145 samples from diseased pigs in the field. On the other hand, Ichikawa *et al.* [10] reported that group C streptococci were the most prevalent (34.9%) bacteria isolated from slaughtered pigs with endocarditis over a 3-year period. In our previous study on bacterial endocarditis in slaughtered pigs, *S. dysgalactiae* was isolated from 75 (15.2%) out of 495 pigs [11], and it was confirmed that those bacteria mostly belonged to group C streptococci.

In the capillary precipitation test, some isolates could not be serologically identified. However, those strains were identified as group G streptococci using commercial serological kits. Human group G streptococci were found to belong to the *S. dysgalactiae* homology group by Farrow and Collins [6]. On the other hand, animal group G streptococci have been associated with different pathological conditions in dogs [1] and with mastitis in cow [2]. Those strains were differentiated from *S. dysgalactiae* by simple physiological and biochemical tests and named *S. canis* [5]. All of the group G streptococci isolated from swine endocarditis and lymphadenitis in the present study were identified as *S. dysgalactiae* by physiological tests, and it was clarified that group G *S. dysgalactiae* can cause various diseases in the swine.

Streptococcal lymphadenitis of the swine caused by group E streptococci, also known as jowl abscess, cervical lymphadenitis, feeder boils, or swine strangles, is a serious problem for the swine industry in the United States because of the losses from trimming and condemnation of infected carcasses [16]. The present study was the first study to isolate group E streptococci *S. porcinus* from lymphadenitis in pigs in Japan. This is a major disease in the United States but is rarely seen in other countries [17]. In the cases of lymphadenitis in slaughtered pigs caused by group E streptococci, macroscopic findings were only mild lymphadenitis without jowl abscesses. In Japan, there has been no report of jowl abscess or cervical lymphadenitis

caused by group E streptococci. As this study has shown the presence of group E streptococci in lymphadenitis in pigs, more care should be taken concerning streptococcal lymphadenitis in Japanese swine herds.

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