Serological characterisation of *Haemophilus parasuis* isolates from Australian pigs

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SUMMARY: A total of 31 isolates of *Haemophilus parasuis* obtained from Australian pigs were serotyped by the Kielstein-Rapp-Gabrielson scheme. The isolates were assigned to serovar 1 (1 isolate), serovar 2 (1 isolate), serovar 4 (4 isolates), serovar 5 (7 isolates), serovar 9 (2 isolates), serovar 10/7 (4 isolates), serovar 12 (1 isolate) and serovar 13 (6 isolates). The remaining 5 isolates could not be assigned to a serovar. Two different serovars (5 and 13) were detected in one herd. The only 2 isolates obtained from clinically normal pigs (from the same herd) were serovar 9. The common serovars were isolated from pigs with pneumonia as well as from pigs with conditions of the Glässer's disease type. The serological heterogeneity amongst Australian isolates of *H parasuis* has important implications for the use of vaccines to control Glässer's disease.

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Introduction

Haemophilus parasuis, an organism dependent upon nicotinamide adenine dinucleotide (NAD) or V-factor for *in-vitro* growth, is the causative agent of porcine polyserositis and arthritis (Glässer's disease) (Nicolet 1992). The principal lesions associated with this disease are fibrinous or serofibrinous meningitis, serositis, pleuritis, pericarditis, peritonitis and arthritis that can occur in various combinations or occasionally singly (Nicolet 1992). While early literature reports describe the causative agent of Glässer's disease as *"H influenzae suis"* or *"H suis"* (organisms requiring both haemin and NAD), the extensive taxonomic study of Kilian (Kilian 1976) has shown that all such strains are, in fact, *H parasuis*. *H parasuis* is commonly found in the nasal cavities of pigs (Harris *et al* 1969), only rarely in normal pig lungs, but commonly in lung lesions suggestive of enzootic pneumonia (Little 1970).

Considerable antigenic heterogeneity has been recognised among *H parasuis* isolates, resulting in confusion over the serological classification of this species. The initial serological classification was performed by Bakos (1955) who recognised serovars A to D on the basis of a precipitation test. Three additional serovars were proposed by Schimmel *et al* (1985). An alternative serological classification system, based on heat-stable antigens detected in an immunodiffusion test, that recognised 7 serovars (1 to 7) was developed by Nicolet and colleagues (Morozumi and Nicolet 1986; Nicolet *et al* 1986) and has now replaced the Bakos scheme. Independent studies in Germany (Kielstein *et al* 1991) and the United States of America (Rapp-Gabrielson and Gabrielson 1992), both based on immunodiffusion tests, suggested the existence of further new serovars, a total of 6 and 5, respectively. Subsequently, it has been shown that some of these new German and American serovars were identical, and that some of the German serovars were based on organisms that were not *H parasuis* (Kielstein and Rapp-Gabrielson 1992). The serological classification of *H parasuis* has now been rationalised with the formal proposal of the Kielstein and Rapp-Gabrielson (KRG) scheme. The KRG scheme builds on the original proposal of Nicolet and colleagues, is based on heat-stable antigens detected in an immunodiffusion test, and currently recognises 15 serovars (Kielstein and Rapp-Gabrielson 1992).

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In the Australian context, Glässer's disease was first definitely diagnosed in 1947 in Queensland (Sutherland and Simmons 1947). There have been subsequent reports of the classic form of the disease in Victoria (Pullar 1958) and Tasmania (King 1968). Septicaemic cases with no serosal inflammation have been reported from Victoria (Riley *et al* 1977) and Western Australia (Peet *et al* 1983). Recent biochemical characterisation studies of haemophilic organisms from Australian pigs have confirmed that *H parasuis* is present in pigs in all Australian states (Eaves *et al* 1989; Blackall and Pahoff 1995). A small collection of Australian *H parasuis* isolates has been serotyped, with serovars 2 (2 isolates), 4 (1 isolate), 5 (6 isolates) and 13 (1 isolate), being identified among the 10 isolates examined (Rapp-Gabrielson and Gabrielson 1992).

In this paper, we describe the serological characterisation, using the KRG scheme, of a further 31 Australian isolates of *H parasuis* and the clinical conditions associated with them. As the ability to serotype *H parasuis* is not yet available in Australia, this study was performed in a collaborative manner, involving 2 laboratories in Australia and one in the United States of America.

Materials and Methods

Isolates

The 31 *H parasuis* isolates used in this study were collected during the period 1989 to 1992 and all have been previously identified (Eaves *et al* 1989; Blackall and Pahoff 1995). The geographical origin of these isolates and of the 10 isolates that were serotyped in a previous study (Rapp-Gabrielson and Gabrielson 1992) are given in Table 1. The geographic origin of these latter 10 isolates has not been previously published.

Medium

TM/SN, a medium capable of supporting the growth of V factor-dependent haemophili, was prepared as described previously (Reid and Blackall 1987) and used to grow the organisms from which the antigens were extracted for serological testing. Incubation was at 37°C in air.

Serotyping

Antigens were prepared as described previously (Rapp-Gabrielson and Gabrielson 1992), with some modification. Briefly, each isolate was heavily inoculated onto 3 TM/SN plates from a fresh overnight culture. The plates were incubated overnight and the resultant growth harvested into 2 mL of sterile saline. The suspension was then autoclaved at 121°C for 2 hours, centrifuged (14 000 *g*, 5 min) and

TABLE 1
Geographical origin and serovar of Australian isolates of *Haemophilus parasuis*[*]

Origin	Serovar									
	1	2	4	5	9	10/7	12	13	NT[†]	Total
New South Wales	–	–	–	2	–	–	–	–	–	2
Queensland	–	1	–	2 (3)	2	–	–	1 (1)	1	7 (4)
South Australia	–	–	1	2 (3)	–	–	–	1	1	5 (3)
Tasmania	1	–	–	–	–	–	–	–	–	1
Victoria	–	– (2)	1 (1)	1	–	4	–	4	2	12 (3)
Western Australia	–	–	2	–	–	–	1	–	1	4
Total	1	1 (2)	4 (1)	7 (6)	2	4	1	6 (1)	5	31 (10)

* Results of the current study, combined with those of Rapp-Gabrielson and Gabrielson (1992) in parentheses.
† NT = non-typable.

TABLE 2
Relationship between serovar of *H parasuis* and isolation site [*]

Serovar	Respiratory	Systemic[†]
1	0	1 (Joint)
4	2	1 (Brain)
5	2	4 (Brain, Joint -2, Liver)
9	2	0
10/7	0	2 (Brain - 2)
12	1	0
13	3	3 (Heart, Joint - 2)
NT[‡]	3	1 (Joint)
Total	13	12

* Respiratory = isolates from respiratory sites of pigs without signs of septicaemia
† Systemic = isolates from normally sterile sites of pigs with signs of septicaemia. Site indicated in parentheses
‡ NT = non-typable

the supernatant held at 4°C. Immunodiffusion testing was performed as described by Rapp-Gabrielson and Gabrielson (1992).

Results

The serovars of the 31 isolates of *H parasuis* are shown in Table 1. Most of the isolates could be assigned to a serovar, but 5 failed to react with any of the 15 type antisera. The commonest serovars were 5 (7 isolates) and 13 (6 isolates) followed by serovar 4 (4 isolates). Preparations from 4 isolates reacted with antisera to both serovars 7 and 10. Whether these cross-reactions were reactions of identity or partial identity could not be determined with certainty in the immunodiffusion test. The remaining serovars found, 1, 2, 9 and 12, were each represented by only a few isolates. Two of the isolates from South Australia were obtained from different pigs in the same herd, but were different serovars, 5 and 13. While only small numbers of isolates were available from some States, serovar 5 was shown to be widely distributed, being present in New South Wales, Queensland, South Australia and Victoria.

For 25 of the 31 isolates, the information submitted with the isolates was sufficient to classify them according to whether they were obtained from the respiratory tract with no recorded signs of a septicaemic disease, or from a normally sterile site such as joint, heart or brain with indications of a Glässer's disease-like condition. The source of these isolates from either pigs with only respiratory disease or pigs with septicaemic disease is shown in Table 2. Only 2 isolates were obtained from clinically normal pigs. These were the only 2 serovar 9 isolates encountered in the study and were recovered from nasal swabs of different pigs in the same high-health status herd. This herd has a long history of being free of both respiratory disease and Glässer's disease.

Discussion

This study has extended our knowledge of the serological characteristics of Australian isolates of *H parasuis*. Previously, serovars 2, 4, 5 and 13 have been recognised as being present in Australian pigs (Rapp-Gabrielson and Gabrielson 1992). The current study has established that serovars 1, 9, 10/7 and 12 are also present in Australian pigs. As well, 5 isolates that could not be assigned to one of the 15 currently recognised serovars were encountered. It is possible that non-typable isolates represent a new serovar or serovars. Alternatively, these isolates may produce only small amounts of the serovar specific antigens, and these were not detected in the gel diffusion test.

Antigenic heterogeneity within serovar 7 has been reported previously (Rapp-Gabrielson and Gabrielson 1992). However, this is the first report of possible cross-reactions between serovars 7 and 10. Additional testing with cross-absorbed sera will be necessary to define the antigenic relationships among isolates identified as serovars 7 or 10.

The KRG scheme has been used previously on *H parasuis* isolates from Canada and the USA (Rapp-Gabrielson and Gabrielson 1992), as well as from the former German Democratic Republic (Kielstein *et al* 1991; Kielstein and Rapp-Gabrielson 1992). The results of our study have similarities to these previous studies. All studies have confirmed the presence of a number of different serovars in a broad geographical area - 12 in the USA, 10 in Canada and 14 in the German Democratic Republic (Kielstein and Rapp-Gabrielson 1992; Rapp-Gabrielson and Gabrielson 1992). The smaller number of serovars identified in Australia (8 in total) is probably explained by the smaller number of strains examined (41 in total), compared with the studies on isolates from Canada (108), the USA (120) and the former German Democratic Republic (290). It is probable that other serovars of this species may be present in Australian pigs. In all studies, serovars 4 and 5 were among the most common serovars encountered, whilst a significant percentage of isolates could not be serotyped.

In one instance, we found 2 different serovars (5 and 13) simultaneously present in one pig herd. This herd had a history of deaths in weaners, with meningitis and septicaemia being the predominant post-mortem finding. Rapp-Gabrielson and Gabrielson (1992) have reported similar findings, noting that more than one serovar can be

isolated from the one pig. As noted by Rapp-Gabrielson and Gabrielson (1992) and Kielstein and Rapp-Gabrielson (1992), the presence of more than one serovar is not unexpected as *H parasuis* is commonly found in the nasal cavities of swine (Harris *et al* 1969). The potential for heterogeneity in the *H parasuis* population within a pig herd also has been confirmed by DNA restriction endonuclease analysis (REA), where isolates having between 2 to 4 different REA patterns were recovered from 9 of 10 herds (Smart *et al* 1988).

Of the 31 isolates examined in this study, 25 were accompanied by detailed clinical information. With 2 exceptions, all 25 isolates were obtained from pigs with either pneumonia alone or polyserositis with or without pneumonic involvement. The most common serovars (4, 5 and 13) were associated with both conditions. Others have also reported on the association of *H parasuis* with pneumonia (Little 1970; Rapp-Gabrielson and Gabrielson 1992) but whether *H parasuis* is a primary or secondary pathogen in pneumonia remains unclear. We found a range of serovars (1, 4, 5, 10/7 and 13) were associated with septicaemic conditions, a finding also reported previously (Rapp-Gabrielson and Gabrielson 1992). Further, the most common serovars (4, 5 and 13) were associated both with pneumonia and septicaemic conditions. Hence, our results do not suggest any particular association between serovar and pneumonia or septicaemia, a conclusion also reached by Rapp-Gabrielson and Gabrielson (1992).

Only 2 *H parasuis* isolates were obtained from clinically normal pigs from the same high-health status herd and both were serovar 9. A high prevalence of *H parasuis* carrier pigs has been recorded previously in "excellent" specific pathogen-free herds in Ontario, Canada, with 16 of 19 herds containing carrier pigs (Smart *et al* 1989). As the literature clearly indicates that isolates of *H parasuis* can show marked differences in virulence (Kielstein and Rapp-Gabrielson 1992; Nicolet 1992; Rapp-Gabrielson *et al* 1992, 1994b), the presence of *H parasuis*, in the absence of any disease, needs careful interpretation. It is interesting to note that the type strain for serovar 9 has been shown to be non-pathogenic (Kielstein and Rapp-Gabrielson 1992).

Vaccines have been shown to be an effective means of controlling Glässer's disease, although there is conflicting evidence on whether the protection from inactivated vaccines is serovar specific or indeed even strain specific (Riising 1981; Smart and Miniats 1989; Kielstein and Rassbach 1991; Miniats *et al* 1991). Some of the uncertainty on the degree of cross-protection has arisen as some of the reported vaccination-protection trials have been performed on strains that have not been serologically characterised. Recently, it was reported that an inactivated vaccine prepared from serovars 4 and 5 protected pigs from challenge with heterologous strains of the same serovars (Rapp-Gabrielson *et al* 1994a). In addition, a serovar 4 and 5 inactivated vaccine has been shown to protect against challenge from serovars 13 and 14 but not serovars 2 and 12 (Rapp-Gabrielson *et al* 1994a). In contrast, it has been demonstrated that monovalent, inactivated vaccines prepared from different strains of either serovar 2 or 12 varied in their ability to provide serovar-specific protection (Kocur *et al* 1994). In the light of this uncertainty in the literature, our finding that a wide variety of serovars are present in Australian pigs has important implications for the development and use of vaccines against diseases due to *H parasuis* in Australian pigs. At the very least, inactivated vaccines should contain the serovars prevalent in the target pig population, or be produced from isolates of *H parasuis* obtained from the target herd. As we and others have found that more than one serovar of *H parasuis* can be present in a herd at the same time, the choice of strains and serovars to include in a vaccine is of prime importance.

Currently, the ability to serotype *H parasuis* is not available in Australia. The serological heterogeneity that we encountered among Australian isolates indicates that there is a need for an Australian based service if any directed attempts are to be made to develop effective prevention and control programs for the diseases associated with this organism. There is currently considerable interest in the Australian pig industry in the management technique of segregated early weaning, particularly to control porcine pleuropneumonia (Thornton 1995). However, extensive experience with this technique in the United States of America shows that segregated early weaning does not control Glässer's disease (Henry 1995). Hence, effective vaccines against Glässer's disease, supported by an ongoing knowledge of the serovars of *H parasuis*, will remain as needs of the Australian pig industry into the foreseeable future.

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