Characterisation of Australian isolates of *Actinobacillus capsulatus, Actinobacillus equuli, Pasteurella caballi* and Bisgaard Taxa 9 and 11

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Objective The objective of this work was to perform a comprehensive phenotypic characterisation of 16 isolates of bacteria previously identified as *Actinobacillus equuli.*

Design The 16 isolates that had been obtained from Australian animals – 15 from horses and one from a rabbit – were compared with reference strains of *A equuli, A capsulatus, Pasteurella caballi* and Bisgaard Taxa 9 and 11.

Results The characterisation study demonstrated that only nine of the isolates were *A equuli.* The other isolates were identified as *A capsulatus* (the isolate from rabbit), *P caballi* (one isolate), Bisgaard Taxon 11 (two isolates) and Bisgaard Taxon 9 (one isolate). The final two isolates could not be assigned to any recognised species or taxa.

Conclusion This study has highlighted the importance of a complete characterisation of *Actinobacillus*-like organisms isolated from horses and rabbits. The study represents the first time that *A capsulatus, P caballi* and Bisgaard Taxa 9 and 11 have been recognised as being present in Australia.

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Actinobacillus equuli, a member of the family *Pasteurellaceae,* is the causative agent of sleepy foal disease, an acute, highly fatal septicaemia of newborn foals.[1] The organism has been previously known as *'Shigella equirulis'* and *'S equuli'*.[2]

In the Australian context, *A equuli* was first isolated by Cottew and Ryley[3] from a foal that died of septicaemia. Bain[4,5] has reported the involvement of the organism in abortion, septicaemia and joint ill in young foals of less than 4 months of age and septicaemia and sudden death in foals of 6.5 to 9 months of age. *A equuli* has also been associated with abortion in mares[6] and peritonitis in adult horses.[2]

A range of other bacteria, all apparently members of the family *Pasteurellaceae,* have been isolated from horses, but not in Australia. Bacteria variously described as *Actinobacillus suis, A suis* –like, haemolytic *A equuli* and haemolytic *A ligniersii* have been reported in the literature.[7-10] It has been shown that at least some of these equine isolates known as *A suis* and haemolytic *A equuli* are, in fact, quite distinct from porcine isolates of *A suis,* with the designation Bisgaard Taxon 11 being proposed for such organisms.[11] In addition, a new member of genus *Pasteurella, P caballi,* has been recognised in horses.[12]

In the light of this knowledge of the presence of a range of members of the genera *Pasteurella* and *Actinobacillus* in horses, we have re-examined a collection of bacteria previously identified as *A equuli.* These isolates were all identified as *A equuli* during routine diagnostic disease investigations performed at the Animal Research Institute between 1963 and 1993. The isolates were obtained from 15 horses or foals (15 isolates) and one rabbit (one isolate).

Materials and methods

Bacteria

Sixteen isolates, all previously identified as *A equuli,* were studied. The available details on these isolates are provided in Table 1. The reference strains that were used in this study were *A equuli* NCTC 8529, *A capsulatus* NCTC 11408, *P caballi* ATCC 49197, Bisgaard Taxon 9 CCM 5500 and Bisgaard Taxon 11 biovar 1 CCUG 15573. All of these are type strains for their respective taxa.

Phenotypic characterisation

All the field isolates as well as the reference strains were subjected to a full phenotypic characterisation as previously described.[13]

Case histories

The available case hsitories were reviewed. In many instances, the details of the histopathogical findings were no longer obtainable. As well, for many of the cases, only a brief summary of the original findings could be located.

Results

All 16 field isolates were Gram-negative, non-motile rods that fermented glucose and did not produce indole. The isolates had no requirements for X- or V-factors in vitro, were unable to utilise citrate, mucate or malonate, did not produce H_2S, did not produce any pigment, could not grow in KCN and were negative in the methyl red and Vogues-Proskaur tests. All the isolates could reduce nitrate but lacked the ability to hydrolyse arginine, decarboxylate lysine or ornithine and could not hydrolyse gelatin, Tween 20 or Tween 80. None of the isolates could grow on McConkey agar or deaminate phenylalanine but all showed phosphatase activity. All the isolates were negative for α-fucosidase, β-glucuronidase and α-mannosidase activity but all were positive for alanine aminopeptidase and β-galactosidase activity. None of the isolates produced acid from adonitol, D(+) arabitol, dulcitol, meso-erythritol, D(+) fucose, D(+) glycogen, inulin, D(+) melezitose, L(+) rhamnose, L(-) sorbose, D(+) turanose, xylitol or L(-) xylose. All the isolates produced acid from D(-) fructose, D(+) glucose, D(+) mannose, lactose, raffinose, D(-) ribose and sucrose. The isolates differed in a number of properties and the details of these distinguishing properties are given in Table 2, which also shows the properties of the type strains used in this study.

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On the basis of the differential properties shown in Table 2, the field isolates were identified as *A equuli* (five isolates), L(+) arabinose+ *A equuli* (four isolates), *A capsulatus* (one isolate), Bisgaard Taxon 11 biovar 1 (two isolates), Bisgaard Taxon 9 (one isolate) and *P caballi* (one isolate). The remaining two isolates could not be assigned to any currently recognised taxon in the family *Pasteurellaceae*.

Table 1 provides a summary of the relevant information obtained from the review of the case histories. The *A equuli* isolates (both arabinose positive and negative) were commonly associated with the typical infectious conditions linked to this organism - shigellosis, septicaemia and abortion. The single *P caballi* isolate was obtained from an abortion in which there was no evidence of an infectious process being involved. The single Bisgaard Taxon 9 isolate was obtained from a horse in which there was histological evidence of a bacterial septicaemia. For the two Bisgaard Taxon 11 isolates, there is no evidence for or against their involvement in any pathogenic process. Of the two unclassifiable isolates, one (H4272/4) was obtained in pure culture from a case in which there was histological evidence of an infection. This histological evidence plus an absence of other potential pathogens suggests that this isolate may have been primary pathogens. The *A capsulatus* isolate was obtained as a pure culture from a dead rabbit but there is no record of any histological examination that may have been performed.

Discussion

This study highlights the problems confronting diagnostic laboratories attempting to identify members of the family *Pasteurellaceae*. The isolates examined in this study were all originally identified as *A equuli*, using a battery of tests considered acceptable for diagnostic laboratories. Many of the original identifications were in fact performed by one of us (PJB). A close re-examination with a more extensive battery of tests and a better knowledge of the alternative possible identifications has resulted in only nine of the 16 isolates being confirmed as *A equuli*. The remaining seven isolates have been confirmed as either belonging to other formally recognised species (*A capsulatus* and *P caballi*), other recognised but un-named taxa (Bisgaard Taxa 9 and 11) or appear to have not previously been described in the literature. The finding that the original identifications were incorrect should not be seen as a condemnation of the original workers. It is a reflection of the fact that identification of members of the family *Pasteurellaceae* is a difficult task that requires considerable effort.

P caballi has been recognised as an inhabitant of various mucous membranes of horses, particularly of the upper respiratory tract.[12] It has been suggested that the organism can play a significant role in upper respiratory tract infection, pneumonia and peritonitis.[12,14,15] This is the first isolate of *P caballi* from a foetus, although the organism has been reported before from the female reproductive tract.[12] There was no histological evidence of an infectious process contributing to this abortion, indicating that the *P caballi* isolate may have been part of the normal flora. This is the first time that *P caballi* has been reported as being present in horses in Australia. Veterinary microbiologists should carefully examine all suspect *A equuli* isolates to determine if they are *P caballi*. Clinical microbiologists should also be aware of the existence of *P caballi* as the organism has recently been isolated, along with *Escherichia coli*, from an infected wound on a veterinary surgeon.[16]

Table 1. Source, field information and final identification of the isolates used in this study.

Isolate	Source	Syndrome	Identification	Pathogen	Other pathogens
R3011/3	Horse heart	?	*A equuli*	?	Yes[a]
R4922/6	Horse liver	?	*A equuli*	?	No
R4922/7	Horse kidney	?	*A equuli*	?	No
I4963	Horse joint	Septicaemia	*A equuli*	Yes	No
93/107376	Foal kidney	Septicaemia	*A equuli*	Yes	No
S5632/4	Horse kidney	?	L(+) arabinose+ *A equuli*	?	No
I5445/4	Foal liver	Septicaemia	L(+) arabinose+ *A equuli*	Yes	No
J786	Foal liver	Septicaemia	L(+) arabinose+ *A equuli*	Yes	Yes[b]
J1959/4	Horse foetal lung	Abortion	L(+) arabinose+ *A equuli*	Yes	No
V2766	Horse foetal stomach	Abortion (placentitis)	*P caballi*	No	No
G6526/4	Horse lung	Septicaemia	Bisgaard Taxon 9	Yes	No
H5132	Horse faeces	Diarrhoea	Bisgaard Taxon 11 biovar 1	No	No
88/138011	Horse sinus	Fistulous withers	Bisgaard Taxon 11 biovar 1	?	Yes[c]
E1668/4	Rabbit Uterus	Death	*A capsulatus*	?	No
H4272/4	Foal spleen	Abortion	Unclassified	Yes	No
I5054/5	Foal Brain	Septicaemia	Unclassified	Yes	No

? = No available information

Yes = histopathological evidence of involvement of bacteria morphologically consistent with *Actinobacillus* spp in the foal, foetus, placenta or other tissue; No = no evidence that isolate involved in any infectious disease process; ? = no available information

[a]The other potential pathogen isolated was *Streptococcus equi* subspecies *zooepidemicus*

[b]The other potential pathogen isolated was *Escherichia coli*

[c]The other potential pathogen isolated was *Staphylococcus aureus*

A capsulatus is recognised as a primary pathogen of rabbits, having been originally isolated from septicaemic caged rabbits in Sri Lanka.[17,18] The organism has subsequently been isolated from snowhares[19] and European brown hares,[20] and has also been associated with purulent infections in jaws of European hamsters.[21] While well recognised as a pathogen of rabbits and hares outside Australia, *A capsulatus* does not appear to have been previously reported in Australia. Unfortunately, there are no existing diagnostic records that indicate if the isolate described in this study was associated with any infectious disease process.

A range of papers has reported the presence of haemolytic actinobacilli variously identified as haemolytic *A equuli, A suis* or *A suis*–like.[7-16] The taxonomy of all these various organisms has not been fully resolved. We have shown that the only two haemolytic isolates present in our study exactly match the properties of the reference strain for Bisgaard Taxon 11. Hence we are confident that our isolates are Taxon 11. DNA homology and 16S rRNA sequencing studies have confirmed that Taxon 11 is a close relative of *A. equuli, A suis* and *A capsulatus*.[22,23] While the final taxonomic resolution of the status of Taxon 11 is not likely until more extensive phenotypic and genetic studies are completed, it is important that diagnostic laboratories perform as complete an identification as possible on all haemolytic *Actinobacillus*–like organisms isolated from horses. Such studies will allow a more complete picture to be assembled of the distribution of these organisms in horse populations.

Taxon 11 is regarded as a part of the normal flora of the horse.[11] The available information provided no evidence for or against the pathogenic role of the isolates reported in this study (Table 1). There is a continuing need for laboratories to confidently separate *A equuli*, a significant pathogen, from Bisgaard Taxon 11, an organism that probably is part of the normal flora and is apparently, at best, an opportunistic pathogen.

A equuli is the only species of *Actinobacillus* to have been reported as being present in Australian horses, making our report the first formal recognition of the presence of Taxon 11 in Australian horses. However, a careful reading of the published literature indicates that haemolytic actinobacilli have been

Table 2. Differential properties and identity of Australian isolates of *A equuli* and related organisms.

	A equuli			*A capsulatus*		*P caballi*		Taxon 9		Taxon 11 Biovar 1		H4272/4	5045/5
	Ref	Field (5)	Field A (4)	Ref	Field (3)	Ref	Field (1)	Ref	Field (1)	Ref	Field (2)	Field (1)	Field (1)
Catalase	+	d	d	+	d	-	-	+	+	w	+	+	+
Oxidase	+	+	d	+	+	-	-	+	+	+	+	+	-
Haemolysis	-	-	-	-	-	-	-	-	-	+	w	-	-
Urease	+	+	+	+	+	-	-	+	+	+	+	+	+
ODC	-	-	-	-	-	+	+	-	-	-	-	-	-
β-glucosidase	-	-	-	+	+	-	-	-	-	+	+	-	-
α-galactosidase	+	+	+	+	+	-	-	+	+	+	+	+	+
α-glucosidase	+	+	+	-	+	-	-	-	-	+	+	-	-
β-xylosidase	+	+	+	-	(+)	-	-	-	-	+	+	-	(+)
Acid from:													
Aesculin	-	-	-	+	+	-	-	-	-	+	+	-	-
Amygdalin	-	-	-	+	+	-	-	-	-	(+)	(+)	-	-
D(-) arabinose	-	-	-	-	-	-	-	-	-	-	-	+	+
L(+) arabinose	-	-	+	+	+	-	-	-	-	-	-	-	+
Arbutin	-	-	-	+	+	-	-	-	-	+	+	-	-
Cellobiose	-	-	-	+	+	-	-	-	-	+	+	-	-
Dextrin	+	+	+	+	+	+	-	+	+	+	+	-	-
L(-) fucose	-	-	-	-	-	-	-	-	-	-	-	+	+
D (+) galactose	+	+	+	+	+	+	+	(+)	+	+	+	-	+
Gentibiose	-	-	-	+	(+)	-	-	-	-	+	(+)	-	-
Glycerol	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	-	(+)	(+)	-	(+)
Meso-inositol	-	-	-		-	-	-	+	-	-	-	-	-
Maltose	+	+	+	+	+	+	-	+	+	+	+	-	-
D(-) mannitol	+	+	+	+	+	+	+	+	+	-	-	+	+
D(+) melibiose	+	+/(+)	+	(+)	+	-	-	(+)	(+)	+	(+)	-	+
Salicin	-	-	-	+	+	-	-	-	-	+	+	-	-
D(-) sorbitol	-	-	-	+	+	-	-	(+)	+	-	-	-	+
Trehalose	+	+	+	+	+	-	-	-	-	+	+	-	-
Xylose	+	+	+	+	+	+	-	+	+	+	+	+	+
Gas from glucose	-	-	-	-	-	+	+	-	-	-	-	-	-

Ref = Reference strain
Field = Australian field isolate or isolates (number in brackets)
Field A = Australian field isolates of *A equuli* that are L-arabinose positive (number in brackets)
+ = positive within 1-2 days
(+) = late positive > 3 days
w = weak positive
d = variable, some isolates positive and some negative
- = negative after 14 days

encountered before. Cottew and Francis[24] reported that some of their *A equuli* isolates were haemolytic. Unfortunately, the Cottew and Francis[24] cultures are no longer available, making it impossible to re-evaluate the identification of these isolates.

Taxon 9 isolates have rarely been reported in the literature.[20] On the basis of DNA relatedness, Taxon 9 has been excluded from *A equuli* sensu stricto.[25] There is insufficient information yet available to indicate if Taxon 9 is a pathogen or a commensal. The retrospective nature of our study has meant that there is little detailed evidence on the pathogenic role of the Taxon 9 isolate reported here. The available diagnostic records note that the isolate was obtained in pure culture from a condition that appeared typical of septicaemia due to *A equuli*. To assist in establishing the true role of Taxon 9, it is important that all suspect *A equuli* isolates from horses be carefully examined. As there are only a few characters that separate these two taxa (see Table 2), these differential tests should be included when identifying suspect *A equuli* isolates.

Two of the isolates examined in this study (H4272/4 and I5404/5) could not be assigned to any currently recognised species or taxa and apparently represent new taxa. This is further evidence of the need for more extensive studies, based on both phenotypic and genotypic properties, of the actinobacilli that can be isolated from horses.

In summary, this study has confirmed for the first time in Australia, the presence of four new species or taxa - *P caballi*, *A capsulatus*, Bisgaard Taxon 11 and Bisgaard Taxon 9. As all four species/taxa have only been recognised retrospectively by a re-examination of isolates previously identified as *A equuli*, our study emphasises the need for a careful examination of all *Actinobacillus*-like organisms from horses and rabbits. The detailed identification table provided in this study (Table 2) should assist laboratories in this task of extended characterisation.

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