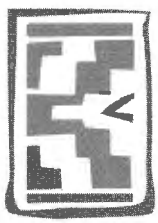

Strains of *Actinobacillus* spp. from diseases of animals and ostriches in Zimbabwe

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ABSTRACT

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Among the *Actinobacillus* spp. only *A. lignieresii* represents a homogenous and well studied taxon. However, haemolytic and non-haemolytic strains of *A. equuli* and *A. suis* are also isolated from a wide range of diseases in a variety of hosts. These isolates often pose problems in definitive identification. Consequently, several studies have been published, emphasizing the need for detailed studies to reclassify various members of this genus and also to assess their disease significance.

We isolated 48 strains of *Actinobacillus* from clinical cases in horses, cattle, sheep, cat, pigs and ostrich. In order to investigate the association of various taxa of *Actinobacillus* in different pathological conditions in these hosts, the Zimbabwean isolates were characterized in detail and assigned to the recently described taxa of *Actinobacillus*. Representative strains of different taxa were also confirmed at the Department of Veterinary Microbiology, Frederiksburg C, Denmark. Of the 48 isolates, 22 were identified as *A. lignieresii*, 13 as *A. equuli*; 8 as *A. suis*, four as belonging to Taxon 11 and one as belonging to Taxon 9. These two taxa were recently described. Details of pathological conditions in the respective hosts and the significance of isolation, are discussed. We have reported for the first time isolation of *A. lignieresii* from a post-operative wound in a cat and there is also a first report of isolation from an ostrich. We considered *A. equuli* to be the primary cause of equine abortion and septicaemia, and Taxon 9 as causing "sleepy foal" disease. We did not encounter any case of "wooden tongue", but isolated *A. lignieresii* from cases of superficial lymphadenitis in cattle and sheep.

This appears to be a first report of detailed descriptions of Zimbabwean strains of *Actinobacillus* spp.

Keywords: Abortion, *Actinobacillus*, *equuli*, *lignieresii*, septicaemia, *suis*

INTRODUCTION

A. liqnieresii is a homogenous species of *Actinobacillus* and its strains are identified with relative ease. But the members of the other two potentially pathogenic species, *A. equuli* and *A. suis*, represent clusters, the former of both haemolytic and non-haemolytic varieties which could not easily be identified . It appears that such strains represent a biotypic continuum with no dependable marker to help assign them to either of the two taxa with the use of routine diagnostic tests. Since all these three species are frequently encountered in diseases in different hosts, investigators have emphasized the need for in-depth studies in order to reclassify *Actinobacillus*-like organisms and also to ascertain their disease significance (Anonymous 1993; Bisgaard, Piechulla, Ying, Frederiksen & Mannheim 1984; Blackall, Bisgaard & McKenzie 1997; Jang, Biberstein & Hirsh 1987; Piechulla, Bisgaard, Gerlach & Mannheim 1985).

We often isolate *Actinobacillus* spp. from various disease syndromes in animals. We therefore decided

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TABLE 1 Disease syndromes in the hosts and *Actinobacillus* spp. isolated

Hosts	Abortion	Septicaemia	Pneumonia/ bronchitis	Polyarthritis/ serositis	Lympha-denitis	Surface wounds	Conjunctivitis
Horse	AE(6) T_1(1)	AE(2) T_9(1)[a]	AE(1) T_1(1)	–	–	–	AE(2)
Pigs	–	–	AE(1)	AS(6)	–	AE(1)	
Cattle	–	–	AL(8)	–	AL(9) AS(1)	–	
Sheep	–	–	–	–	AL(4): T1(1)	AS(1)	
Cat	–	–	–	–	–	AL(1)[b]	
Ostrich	–	–	T_2(1)	–	–	–	

AE: *A equuli :* 13 isolates
AL: *A. lignieresii :* 22
AS: *A. suis* : 8
T_1: Taxon 11 biovar 1:3 isolates
T_2: Taxon 11 biovar 2: one isolate
[a] T_9: Taxon 9: From "sleepy foal" disease; cultured from joints, heart blood,spleen and lungs
[b] Post-surgical sutured skin wound
Figures in parenthesis indicate number of isolate

TABLE 2 Differential properties of various taxa of Zimbabwean isolates of *Actinobacillus*

Tests for	AE(13)	AL(22)	AS(8)	T_9(1)	T_1(3)	T_2(1)
Beta-haemolysis	–	–	+	–	+	+
Catalase	+	+	+	+	+	+
Oxidase	+°	+	+	+	+	+
Motility at 22 °C	–	–	–	–	–	–
O/F glucose	F	F	F	F	F	F
Phosphatase	+	+	+	+	+	+
Urease	+	+	+	+	+	+
Indole	–	–	–	–	–	–
H2S in TSI	–	–	v	+√	–	–
Aesculin hydrolysis	–	–	+	+√	–	–
ONPG	+	+	+	+	+	+
Arginine dihydrolase	–	–	–	–	–	–
Lysine decarboxylase	–	–	–	–	–	–
Ornithine decarboxylase	–	–	–	–	–	–
Nitrate → Nitrite	+	+	+	+	+	+
Acid in PW sugars:						
L-Arabinose	v	v	+	+[t]	–	–
Cellobiose	–	–	+	–	+	+
Dulcitol	–	–	–	–	–	–
Glucose	+	+	+	+	+	+
Inositol	–	–	–	–	–	–
Mannitol	+	+	–	+	–	+
Salicin	–	–	+	–	+	+
Sucrose	+	+	+	+	+	+
Trehalose	+	–	+	–	+	+
Xylose	+	+	+	+	+	+

AE = *A. equuli.* Total number of isolates indicated in parenthesis
AL = *A. lignieresii*
AS = *A. suis*
T_9 = Taxon 9
T_1 = Taxon 11 biovar 1
T_2 = Taxon 11 biovar 2
° = The isolate from the outbreak of conjunctivitis was oxidase-negative
√ = Expected to be negative
t = Tested negative in our laboratory but positive in Denmark

to study the phenotypes of the Zimbabwean strains in detail, with a view to obtaining information on whether the Zimbabwean strains are similar to those reported from other countries and also to determine their disease potential. Results of this retrospective study are presented in this paper.

MATERIALS AND METHODS

Actinobacillus spp. were cultured from horses, cattle, pigs, sheep, cat and ostrich showing various disease syndromes. Clinical specimens for routine bacteriological culture comprised swabs, in Stuart's transport medium, from cases of conjunctivitis, superficial lymphadenitis and skin-surface wounds; foetal organs/uterine exudate from abortion; synovial aspirate from polyarthritis/serositis; and tracheal wash from bronchitis. Isolation from cases of septicaemia was post mortem from heart blood, spleen, joint aspirate and lungs, while from the ostrich it was from the choanal swabs. Cultures from several cases of pneumonia were obtained post mortem from pneumonic lungs. Specimens were cultured within 1 h after they had been received. Methods used for isolation have previously been described (Mohan, Sadza, Madsen, Hill & Pawandiwa 1994). These included the use of of 10% sheep blood (BA) and chocolate agar (CA) prepared with Blood Agar Base no. 2, and MacConkey agar (MA), both from Oxoid Company, incubated in candle jar for elevated CO_2 at 37°C. Specimens from pneumonia, abortion, conjunctivitis and polyarthritis were also cultured for mycoplasma, and the techniques described earlier were employed (Mohan, Foggin, Muvavarirwa, Honywill & Pawandiwa 1995). Suspected colonies of *Actinobacillus* spp. and other potentially pathogenic bacteria were picked up for purification onto BA/CA and incubated likewise. For detailed phenotypic characterization of different isolates, essentially methods described by Barrow & Feltham in *Cowan & Steel's Manual* (1993) were followed. Initial identification was based on haemolysis on BA, colonial characteristics and results of O/F test; and tests for catalase, oxidase, urease, indole, aesculin, H_2S in TSI, motility, nitrate, arginine,lysine, ornithine and acid in Andrade's peptone water with arabinose, cellobiose, dulcitol, inositol, mannitol, salicin, sucrose, trehalose and xylose; ONPG and growth on MA. Representative strains were further characterized and confirmed in the Department of Veterinary Microbiology, the Royal Veterinary and Agriculture University, Denmark, with the use of a battery of tests described elsewhere (Bisgaard *et al.* 1984).

RESULTS

The isolates, respective number within parenthesis, were cultured from horses (14), cattle (18), pigs (8), sheep (6), cat (1) and ostrich (1). Details of disease syndromes in those hosts and the isolates cultured are shown in Table 1. Results of important tests for identification were as expected and conformed to the characteristics of the *Actinobacillus* spp. (Phillips 1984) except for the following aberrant results. Two isolates of *A. suis* and the isolate identified as Taxon 9 produced H_2S in TSI and the latter also produced acid in arabinose and hydrolysed aesculin. Also the strain from conjunctivitis tested oxidase-negative. Differential characters of various taxa are shown in Table 2. Stickiness of the colonies was consistent with isolates of *A. lignieresii,* but all 48 isolates discerned "Morse code" appearance in Gram-stained smears, although typical bi-polar staining reaction in a large proportion of cells of Taxon 9 and the *A. equuli* strain from cases of conjunctivitis was observed. The Taxon 9 strain was also a rapid grower and discerned mucoid surface growth on BA similar to *Klebsiella* spp. Several isolates of *A. equuli* split urea, on initial isolation, within hours, but on repeated subcultures and after storage, the urease reaction got delayed from few hours to one day. *Actinobacillus* spp. were isolated in pure culture from cases of lymphadenitis and septicaemia, including "sleepy foal" disease; but from cases of pneumonia in different hosts, a mixed infection was often recorded, such as *Rhodococcus equi* in horses, while mostly *Streptococcus* and or coryneform bacteria was recorded in other animals. *Mycoplasma* could be cultured from only two cases of polyarthritis in pigs. Foetal organs from equine abortion yielded pure culture but cultures from uterine exudate yielded *Actinobacillus* mixed with both Gram-positive and -negative pyogenic bacteria. From a mild outbreak of conjunctivitis in foals, oxidase-negative *A. equuli* and beta-haemolytic group-C *Streptococcus* were simultaneously isolated. The choanal culture from the ostrich consisted of Taxon 11 biovar 2 and *Pasteurella multocida* (*sensu stricto*).

DISCUSSION

It has been reported that the strains considered as the haemolytic variety of *A. equuli* and the strains identified as *A. suis* but cultured from hosts other than porcine, possess distinct characteristics and should be assigned to the proposed Taxon 11 (Bisgaard *et al.* 1984; Jang *et al.* 1987). Taxon 9 has been proposed to accommodate certain non-haemolytic strains of *A. equuli* (Blackall *et al.* 1997). Nonetheless, African isolates of *Actinobacillus* spp. do not appear to have been studied in the context of these proposals. Our study, therefore, assumes significance.

We consider our two isolates of *A. suis* producing H_2S as aberrant strains, but similar strains have been reported earlier (Wetmore, Theil, Herman & Harr 1963). Oxidase-negative *A.equuli* strains have also been recorded (Blackall *et al.* 1997). We do not know,

however, whether the aberrant results recorded (Table 2) are of any taxonomic significance.

Actinobacillus spp. have been incriminated in diseases of a variety of hosts; most of the reports related to diseases in horses, cattle and pigs (Blackall *et al.* 1997; Carman & Hodges 1982; Carter, Marshall & Jolly 1971; Gollard, Hodgson, Hodgson, Brownlow, Hutchins, Rawlinson, Collins, McClintock & Raisis 1994; Hebeler, Linton & Osborne 1961; Jang *et al.*,1987; Odin & Helie 1993; Varshney & Uppal 1993). We did not come across any reported isolation from cat. Our isolation of *A. lignieresii* from a sutured wound following fracture in a cat, was probably a post-surgical contamination. It appears to be a first report of isolation from a cat, but corroborates a report of isolation of *A. lignieresii* by De Kruif, Mitjen, Haesebrouck, Hoorens & Devriese (1992) from post-surgical, contaminated wounds after caesarian sections in bovines in which the spread was attributed to the operating surgeon. Strains of *A. lignieresii* could be considered potential post-operative contaminants. We could not, however, record any case of "wooden-tongue" in cattle for which *A. lignieresii* is considered a putative agent. We cultured this species mostly from cases of superficial lymphadenitis in cattle and sheep. In an outbreak of actinobacillosis in a dairy herd, Hebeler *et al.* (1961) also reported isolation of *A. lignieresii* only from lymph nodes and lesions on the gums but not on the tongue. We have subsequently been informed that cases of "wooden tongue" have been confirmed clinically in Zimbabwe (Madzima, W.N., personal communication 1996).

A. equuli-related abortion and septicaemia have been reported by several investigators (Carter *et al.* 1971; Larson 1974; Nelson, Darien, Konkle & Hartman 1996). Having isolated *A. equuli* in pure cultures from a majority of cases of equine abortion and septicaemia in foals, we confirm that *A. equuli* should be considered the primary cause of these diseases. Taxon 9 was isolated in pure culture from a septicaemic foal with "sleepy foal" disease. This corroborates an Australian report of isolation of Taxon 9 from a horse in which there was histological evidence of bacterial septicaemia (Blackall *et al.* 1997).

Taxon 11 strains are reported to constitute the part of the microflora of the oral cavity of horses but their pathogenic role is considered to be questionable (Bisgaard *et al.* 1984; Blackall *et al.* 1997). We cultured Taxon 11 biovar 1 from the aborted equine foetus in pure culture, and therefore consider it to be of primary disease significance. The other two strains of Taxon 11 biovar 1 and the solitary isolate of biovar 2 from the ostrich might be considered secondary invaders. This appears to be the first reported isolation of *Actinobacillus* from an ostrich, but actinobacillosis-related septicaemia and airsacculitis have been reported in black swans and waterfowls (Hacking & Sileo 1977; Onderka & Kierstead 1979).

Pigs suffer a variety of diseases mostly due to *A. suis* infection (Odin & Helie 1993; Pederson 1977). Significantly, we isolated only *A. suis* from the joint aspirate in cases of polyarthritis/serositis and consider them to be the isolations of disease potentials. The other isolates from pigs could be the secondary invaders in respective diseases.

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