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ISOLATION OF A HEMOLYTIC *Actinobacillus* FROM WATERFOWL

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Abstract: A previously undescribed species of hemolytic *Actinobacillus* was isolated from six waterfowl, three with periocular serous exudation and two with airsacculitis and bronchopneumonia. Cultural and biochemical characteristics were compared with those of *Actinobacillus* and *Pasteurella* spp, using a numerical technique.

INTRODUCTION

In 1974, an unusual organism was isolated on five occasions from captive waterfowl. Four ducks originated from the Kortright Waterfowl Park near Guelph, and a swan from a private pond some 48 Km distant. The organism was again isolated in 1976 from a duck from the Metro Toronto Zoo, 100 Km from Guelph. The biochemical reactions of this organism placed it in either *Pasteurella* or *Actinobacillus,* but it could not be assigned to a recognized species. The possibility that the waterfowl organism was an undescribed species of *Actinobacillus,* and its presence in internal organs, justified further biochemical studies despite the rarity of the organism.

Only two references were found of the isolation of *Actinobacillus* spp. in birds. Bisgaard[1] described an atypical *A. lignieresii* from ducks with salpingitis and peritonitis, and cited the isolation by Pacheco of an *Actinobacillus* from a parakeet in 1934.

MATERIALS AND METHODS

Pathology

The carcasses of four 2 to 12 week old captive ducks, one Mandarin (*Aix galericulata*), one Wood Duck (*Aix sponsa*), and two Old-Squaw (*Clangula hyemala*); an adult Australian Shellduck (*Tadorna tadornoides*); and an 18 month old captive mute swan (*Cygnus olor*) were submitted to the Wildlife Disease Laboratory for postmortem examination. Selected tissues of four were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin for microscopic examination.

Bacteriology

Bovine blood agar and MacConkey agar cultures were prepared from various specimens and were incubated aerobically at 37 C for 72 hrs. Biochemical tests were performed on isolates according to standard procedures.[3] Carbohydrate utilization was determined in phenol red broth base, and sensitivity to penicillin using 2u discs.

Numerical Taxonomy

Numerical taxonomy methods were those described by Lockhart and Liston[8] and McAllister and Carter.[10] Using several sources,[2,4,5,6,7,9,10,12,13,14] thirty characteristics were compared for those *Pasteurella* and *Actinobacillus* species described in the 8th edition of Bergey's Manual. Also included were *A. suis* listed under *species incertae sedis* in Bergey's Manual, and *P. aerogenes* recently described by McAllister and Carter. Difficulty was encountered in finding properties not common to all members of this

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closely-related group. The characteristics studied were hemolysis of bovine blood agar; growth on MacConkey's agar; adherent growth on blood agar; sensitivity to penicillin; production of catalase, indole, urease, gelatinase, and ornithine decarboxylase; methylene blue reduction; utilization of Simmon's citrate; Voges-Proskauer reaction; acid production in litmus milk; esculin hydrolysis; H_2S detection using lead acetate paper; production of gas from glucose; and acid production from arabinose, dextrin, dulcitol, glycerol, inositol, lactose, maltose, mannitol, mannose, raffinose, rhamnose, salicin, sorbitol, trehalose, and xylose. Similarity coefficients were determined for each pair of organisms using the following formula: S = [Sum of positive, negative, and variable matches/(Total no. of tests—No. of valid tests)] x 100. Invalid tests are those in which the result is unknown for either or both members of a pair. With variable results, a match was recorded only if both members of the pair were listed as variable.

Pathogenicity

Pathogenicity of the waterfowl organism was briefly studied by intraperitoneal injection of three domestic ducks and four laboratory mice with 1 ml of a 24 hr culture in brain heart infusion broth; a fourth duck was inoculated per os and intraocularly.

RESULTS

Pathology

There was no clear indication of the pathologic significance of the organism from the postmortem findings. Three of the ducklings had clinically evident eye problems characterized by matting of periocular feathers. The only eye examined microscopically was autolyzed but apparently normal. Other lesions present in these ducklings (slipped tendons, staphylococcal arthritis, bacteremia) had etiologies apparently unrelated to the waterfowl organism. Both the remaining 12 week old old-squaw duck and the 18-month mute swan had fibrinous airsacculitis and bronchopneumonia. The Australian shellduck had an acute necrotizing proventriculitis, but no lung lesions attributable to the organism.

Bacteriology

A mixed bacterial flora including the unknown organism was isolated from the ocular exudate of each of the three ducklings. No significant bacteria were isolated from liver, intertarsal joint, or intestine of the old-squaw duck; the unknown organism and other bacteria were isolated from the airsac, lung and brain. No bacteria were isolated from the liver and spleen of the swan; a mixed flora again including the unknown organism was isolated from the airsac. *Escherichia coli* was isolated from the liver, spleen, kidney, and intestine of the shellduck, and *E. coli* and the waterfowl organism from the lung.

The organism was gram negative, predominantly rod-shaped, but with occasional spherical forms. It measured 0.8 μm in width, and up to 3.7 μm in length. Bipolar staining was not evident with methylene blue. After 24 hr at 37 C on blood agar, colonies were white with an irregular outline and a raised center, 1 mm in diameter, and surrounded by a narrow zone of complete hemolysis 1.5 mm in diameter. After 72 hr, colonies attained a maximum diameter of 3 mm. They were firmly adherent to the agar and could be removed only with the surrounding media; on repeated subculture this property was lost, but was often regained following inoculation into brain heart infusion broth. Generally there was no growth on MacConkey's agar; however, a heavy inoculum occasionally resulted in a sparse growth of small pink colonies. Growth in brain heart infusion broth after 24 hr at 37 C was abundant and viscid with variably sized lumps suspended in the medium. A strand of sticky material could be drawn out between the surface of the broth and an inoculating loop. Growth was weak in tryptone broth. Cultures grown on blood agar, and in brain heart infusion and tryptone broths were viable for up to 14 days at 37 C, whereas those in glucose broth were found nonviable at 72 hr. Biochemical properties are given in Table 1.

TABLE 1. Biochemical Properties of the Waterfowl Organism.

Test	Result	Test	Result
Cytochrome oxidase	+	Acid production:	
Catalase	w+	arabinose	—
Fermentative	+	dextrin	+
Motility	—	dulcitol	—
H_2S (lead acetate strip)	—	glycerol	—
Indole	—	inositol	—
Simmon's citrate	—	lactose	—
Urease	w+	levulose	+
Esculin hydrolysis	+	maltose	+
Gelatinase	—	mannitol	v[b]
Methyl red	—	mannose	+
Voges-Proskauer	—	raffinose	—
Lysine decarboxylase	—	rhamnose	—
Arginine decarboxylase	—	salicin	+
Ornithine decarboxylase	—	sorbitol	+
Nitrate reduction	+	sucrose	+
Litmus milk	RC[a]	trehalose	+
Methylene blue reduction	+	xylose	—
Gas from glucose	—		
Sensitivity to penicillin	+		

[a] Reduction and clot

[b] 2 of 4 isolates tested positive

Numerical Taxonomy

Using a single linkage procedure, a shaded similarity matrix (Fig. 1) was prepared. In the similarity matrix, the waterfowl organism had the strongest similarities to *P. ureae* and *A. suis; P. ureae* in turn had a strong similarity to *A. lignieresii.*

Pathogenicity

No clinical illness nor gross nor microscopic lesions were seen in the laboratory animals killed 14 days after experimental inoculation of the organism, and the organism was not isolated from the carcasses.

DISCUSSION

Whether or not there was any casual relation between the organism and the eye problems in the ducklings and the bronchopneumonia in the older waterfowl was not determined. In our experience, bronchopneumonia is an unusual lesion in wild birds. The presence of both this lesion and the organism may be more than coincidence. A similar organism was isolated in 1973 from ocular exudate from captive Canada geese (*Branta canadensis*) at Brighton, Ontario, approximately 320 km from Guelph.[2] This isolate is no longer available for study.

[2] Personal communication, Dr. A. W. Gough, Ontario Ministry of Agriculture and Food, Guelph, Ontario.

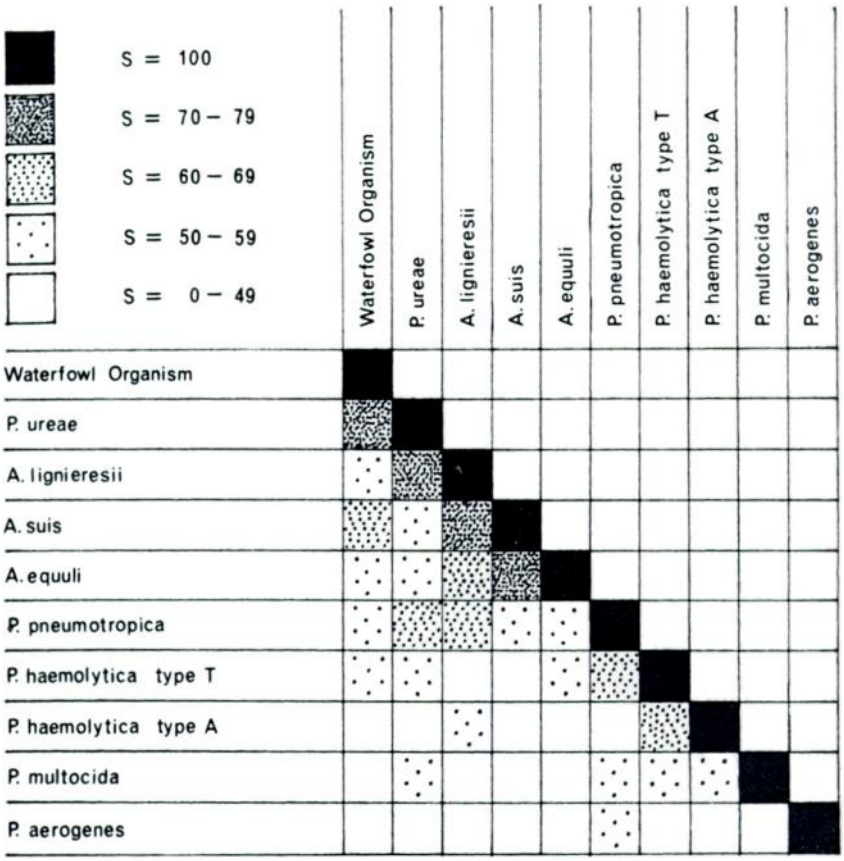

FIGURE 1. Shaded similarity matrix for **Pasteurella** and **Actinobacillus** spp.

The highest similarity coefficient between any pair of species studied was between the waterfowl organism and *P. ureae*. The waterfowl organism was originally thought to be *P. ureae*, a species primarily reported from the human respiratory tract,[11] but it varied from this species in certain key characteristics. *P. ureae* does not produce true hemolysis, does not adhere to the agar or have white colonies, and does have a characteristically strong urease reaction.[15] The waterfowl organism had a weak urease reaction that was still incomplete at 24 hr. Strong adherence to agar is a property described for *Actinobacillus* spp. The genera *Pasteurella* and *Actinobacillus* are closely related and Bergey's Manual gives no indication how the two should be separated. Although these two genera may well be considered one in the future, the authors consider that the results of this preliminary taxonomic evaluation justify placing the waterfowl organism in the genus *Actinobacillus*.

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