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IDENTIFICATION OF AEROMONADS IN FURRED ANIMALS

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Infectious diseases caused by species of the genus Aeromonas, especially in salmon, are frequently demonstrated. On the other hand little has been reported concerning the occurrence and importance of these organisms in warm-blooded animals. *Caselitz et al.* (1957/58) and *Caselitz & Buch* (1958) isolated aeromonads from osteomyelitis in man, and *Vasenius* (1963) described a case of septicaemia in Canadian beaver in Finland, caused by Aeromonas hydrophila.

The taxonomic position of these bacteria is still a subject of discussion (*Schubert* 1967). In *Bergey*'s Manual (1957) the genus Aeromonas is divided into 4 species: Ae. liquefaciens, Ae. punctata, Ae. hydrophila and Ae. salmonicida, whereas *Eddy* (1960) regarded the first 3 as synonyms. Since then Ae. proteolytica has also been proposed as a new species (*Merkel et al.* 1964).

In the present paper Ae. salmonicida and Ae. proteolytica are regarded as separate species, while the other 3 species are looked upon as the liquefaciens-hydrophila-punctata-group of Aeromonas (*Sandvik & Hagen* 1968).

This work deals with observations which indicate that organisms of the genus Aeromonas occur rather frequently in certain furred animals.

MATERIALS AND METHODS

Samples. The examined material includes organs from seal (Phoca groenlandia), mink and blue fox. Further details are given in Table 1.

Cultural examinations. The organs were examined according to general bacteriological procedures, and the organisms were identified on the basis of cultural and physiological properties given for the genus Aeromonas (*Bergey's* Manual 1957).

Flagella staining. A modified Casares-Gil method was used (*Society of American Bacteriologists* 1957).

Enzymoserologic examination. An immunoelectrophoretic method for serologic differentiation of extracellular bacterial proteinases (Casein Precipitating Enzymes) was used. Specific antiproteinases in immune rabbit sera were separated from normal proteinase inhibitors by paper electrophoresis, after which their antiproteolytic effect was demonstrated by inhibiting the corresponding enzyme from precipitating sodium caseinate in an agar medium (*Sandvik* 1962). Specific antiproteolytic sera produced against enzymes of Ae. salmonicida (ATCC 14174*) and Ae. liquefaciens (ATCC 14715) were used. Proteolytic enzymes were prepared by growing the organisms in litmus milk for 2 days at 30°C.

Animal inoculations. A 24-hour broth culture of the organism was injected, 0.2 ml in mice, 3.0 ml in blue fox and 0.5 ml in a silver fox.

RESULTS

Bacteriological investigations. Table 1 shows that out of 8 isolated strains of aeromonads, 6 were found in animals that showed no pathologic findings indicating an infectious cause of death. On the other hand, findings indicating a general infection were observed at least in 1 of the 2 blue foxes. The organism was isolated, however, in pure culture from both foxes.

All isolated strains were Gram-negative rod-shaped organisms, typical for the genus Aeromonas. The organisms grew well on ordinary blood agar at 30°C and at 37°C. The grayish-white, round and moist colonies were 2—3 mm i diameter, with a distinct β-haemolytic zone, at 24 hrs. Gelatin was rapidly hydrolyzed and the organisms produced proteolytic enzymes, which showed typical casein-precipitating abilities in a casein agar medium (*Sandvik* 1962). The catalase and oxidase tests were positive and the strains fermented carbohydrates, as listed for the

*) ATCC = American type culture collection.

Table 1. Isolated aeromonads from furred animals.

Case	Animal species	Organs	Diagnosis
1	Mink	Spleen	Anaemia and gastroenteritis
2	„	Spleen, liver	„
3	„	Spleen, liver	„
4	Seal (Phoca groenlandia	Spleen, liver, intestines	Emaciation and inadequate feeding
5	Mink	Intestines	Anaemia and gastroenteritis
6	„	Intestines	„
7	Blue fox	Liver, occipital joint	Polyarthritis, gastroenteritis, septicaemic bleedings, enlarged liver.
8	„	Liver	Gastroenteritis, enlarged liver

genus Aeromonas (*Bergey*'s Manual 1957). H_2S and indol were produced, but not urease. The citrate test was negative, NO_3^- was reduced to NO_2^-. The organisms were motile, and one polar flagellum was observed in flagell-stained smears.

Enzymoserologic examination showed that all strains of the isolated organisms produced extracellular proteinases (Casein Precipitating Enzymes), typical for the liquefaciens-hydrophila-punctata group (*Sandvik & Hagen* 1968), in as much as they produced 1 enzyme fraction identical with the one specific for Ae. salmonicida (ATCC 14174) and 1 fraction typical for the liquefaciens-hydrophila-punctata group.

The organisms were strongly pathogenic for mice, which died within 15 hrs. after inoculation, but they were not observed to be pathogenic for blue fox and silver fox by the method used for testing the pathogenicity.

Post mortem examinations. Case 1—3 (from the same farm). The carcasses were anaemic. Catarrhal gastroenteritis with oedema in the intestinal wall was observed in all cases. One of the animals had ulcerations in the gastric mucosa, with considerable bleedings to the lumen.

Case 4. The animal was emaciated, with very little content in the digestinal tract. Both sides of the heart were dilated, and the lungs oedematous and emphysematous.

Case 5—6 (from the same farm). The animals were emaciated, with catarrhal gastroenteritis.

Case 7. The state of nutrition was below normal. It had a fibrinous polyarthritis. Numerous petechial haemorrhages were observed in the pleura and the peritoneum. The gastric and intestinal contents were very thin. The liver was enlarged and rich in blood, with some quite distinct pale spots in the parenchyma. Histologically the liver showed acute congestion, with the sinusoides dilated and with well marked haemorrhages. The pale spots were more anaemic areas.

Case 8 (from the same farm as case 7). The state of nutrition was normal. The digestive tract had the same appearance as seen in case 7. The liver was enlarged and congested.

DISCUSSION

The investigations seem to show that aeromonads are not unusual in furred animals. This may be due to the fact that furred animals are to a great extent fed on fish products which often contain Aeromonas species. As these organisms grow well at 20—30°C, it is reasonable to suppose that under certain conditions they can be enriched in the fodder.

The pathogenicity of the isolated organisms to furred animals is difficult to explain from this investigation, but in the case of the foxes the possibility cannot be excluded that aeromonads may be fatal in combination with predisposing factors. Thus distinct signs of a general infection were demonstrated in 1 of the 2 blue foxes examined. In the other fox, however, only gastroenteritis and congestion in the liver were found. As aeromonads were isolated in pure culture from the livers of both foxes, it seems reasonable to think that the organism may be of etiological significance in both cases. On the other hand, no symptoms could be observed after injection of the microbes into foxes, in spite of their high pathogenicity for mice.

The enzymoserologic investigations turned out to be of great importance in the identification of the otherwise problematically identified Aeromonas species. Thus the diagnosis could be made without any considerable biochemical and morphological study, including flagella staining. The procedure, however, requires specific antisera. It is likely that these organisms may often be wrongly classified, because of their resemblance to other haemolytic bacteria in the material in question.

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SUMMARY

Altogether 8 strains of aeromonads were isolated from various furred animals; 2 of the strains may have had fatal significance. The investigations showed that the identification of these bacteria can be based on enzymoserologic study of their proteolytical enzymes by specific antiproteolytic sera, which inhibits the biocatalytical effect of the enzymes in a casein agar medium.

ZUSAMMENFASSUNG

Nachweis von Aeromonaden bei Pelztieren.

Insgesamt wurden 8 Stämme von Aeromonas von verschiedenen Pelztieren isoliert. In 2 Fällen schienen sie von Bedeutung bei den Todesfällen gewesen zu sein.

Die Untersuchungen zeigten, dass die Identifikation dieser Bakterien in erheblichem Grade auf die enzymserologischen Studien ihrer proteolytischen Enzyme basiert werden können. Diese Studien erfolgen mit Hilfe von spezifischen antiproteolytischen Seren, die den biokatalytischen Effekt der Enzyme in einem kaseinenthaltenden Agarmedium hemmen.

SAMMENDRAG

Påvisning av aeromonader hos pelsdyr.

En har isolert i alt 8 stammer av aeromonader fra forskjellige pelsdyr, og i 2 tilfelle synes de å ha vært av betydning for dødsfallene.

Undersøkelsene viste, at identifikasjonen av disse bakterier i vesentlig grad kan baseres på enzymoserologiske studier av deres proteolytiske enzymer ved hjelp av spesifikke antiproteolytiske sera, som hemmer enzymenes biokatalytiske effekt i et kaseinatholdig agarmedium.

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