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ISOLATION AND IDENTIFIACATION OF AEROMONAS HYDROPHILA FROM AN OUTBREAK OF HAEMORRHGIC SEPTICEMIA IN SNAKES (*)

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ABSTRACT

Aeromonas hydrophila was isolated during an outbreak of a fatal disease among snakes kept in the serpentarium of the Razi Institute. The organism was identified on the basis of morphological and biochemical properties, as well as pathogenicity for both warm blood and poikilothermic animals.

Résumé

Les auteurs ont isolé Aeromonas hydrophila à la faveur de l'éruption d'une maladie mortelle chez des serpents gardés dans le serpentarium de l'Institut Razi. Ils identifièrent ce microbe en se basant sur ses caractères morphologiques et biochimiques, ainsi que sur sa pathogénicité pour les animaux homéothermes et poïkilothermes.

The species of the bacteria involved in this experiment was first isolated from frog blood by Sanarelli (8) who found it pathogenic for frogs, toads, salamanders, fish, lizards, fresh water eels, rabbits, guinea pigs, dogs, cats, chickens and pigeons.

Further studies on the disease indicated that the causative microorganism was first named *Bacillus hydrophilus, Proteus hydrophilus,* and then *Aeromonas hydrophila* (1, 2, 5, 6, 9, 10, 11).

Sanarelli demonstrated water as the natural habitat because he isolated the organism from two of 26 samples of water that he examined. *Aeromonas hydrophila* has previously been reported as occurring naturally in frogs, brook trout and pike.

Kulp and Borden (3) made an extensive study of the morphological, cultural, biochemical, pathogenicity and immunological properties of this microorganism as the etiological agent of "Red Leg Disease" in frogs, while Reed and Toner (7) found *Aeromonas hydrophila* to be the causative agent of "Ulcer Disease" in hatchery reared brook trout and of "red sore" in pike.

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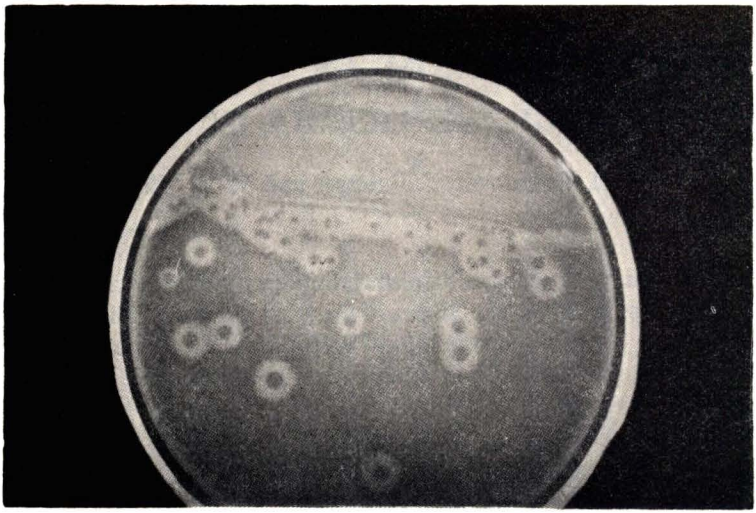
Marcus (4) mentioned that *Aeromonas hydrophila* was the causative agent for ulcerative stomatitis, pneumonia and septicemia in reptiles. The conspicuous haemorrhages characteristic of the septicemia in other reptiles are not visible in snakes owing to the thick coloured integument.

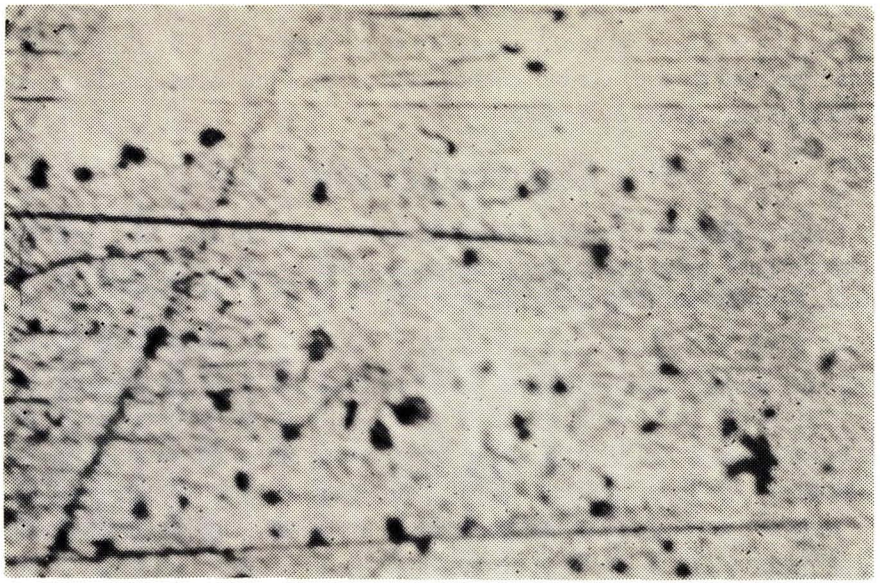


In August 1972, a disease characterized by high mortality appeared among snakes kept in the serpentarium of the Razi Institute. The affected snakes became very sluggish and normal reflexes, such as rearing back or darting out the tongue were decreased. In some cases snakes seemed to weaken and die, but in the majority of the cases there were convulsions with much thrashing about an hour before death.

The postmortem examination of snakes dead with the disease, revealed general haemorrhages in all organs of the body. In most specimens small areas of haemorrhages occurred on the lining of the mouth and on the serosal surface of the digestive tract. The kidneys and the adjacent fat bodies contained haemorrhages. The liver always appeared mottled and brownish in colour and had necrotic foci of varying size from pinpoint to pin-head. The lungs were red and usually filled with blood.

The ventricles of the heart were flaccid and pale due to the absence of blood, while the auricles were filled with dark and thick blood.

Dissections were made on dead snakes and cultures were made from heart blood and from other organs such as liver, kidneys and lungs. All the cultures were maintained at 37°C and readings were recorded at different intervals: 24, 48 and 72 hours. On nutrient agar the colonies were about 2 mm in diameter, translucent greyish to creamy white, raised, circular, butyrous in consistency



and the edges were entire. On nutrient agar slant the growth was spreading, abundant and creamy white with a smooth, glistening raised surface. On blood agar plates the colonies were about 1 mm to 2 mm in diameter and surrounded by a zone of beta haemolysis (Fig. 1). In nutrient broth the growth appeared dense, uniformly turbid with a surface pellicle in 24 hour old culture and a deposit was present which dissolved on shaking.

The microorganisms were pleomorphic, long and narrow, cocco-bacillary and long filamentous types. They were gram negative and some times showed bipolar staining. The bacteria had a single polar flagellum which was demonstrated by Leifson's staining method (Fig. 2) and was actively motile. The isolated cultures were stored in a refrigerator at 4°C at which temperature they remained viable with little loss of virulence.

Inoculations of these microorganisms were made into healthy frogs, lizards (*Lacerta chlonogaster*), rabbits, guinea pigs and white mice to confirm the identiifcation of the organism as *Aeromonas hydrophila* and to observe the pathogenicity for these animals. For inoculation I cc tuberculin hypodermic syringe with a 20 gauge needle was used. The injections in the above cited labo ratory animals were made intraperitoneally and intramuscularly. The inoculum used was a 24 hour old culture in nutrient broth. Each animal was given an injection of 0.2 ml per 100 gm of body weight. The lizards died within 18 hours post-inoculation, while one frog died within 18 hours. Mice, rabbits and guinea pigs died from 18 to 36 hours.

All dead experimental animals were dissected for pathological effects on the various organs, and cultures were made to find if the same bacteria could

recovered at necropsy. After isolation and recovery of the microorganism from heart blood and other organs of snakes dead with the disease, various differential media were used to study the biochemical properties of the bacteria and to identify them as *Aeromonas hydrophila.*

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