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OUTBREAK OF "RED LEG" - AN AEROMONAS HYDROPHILIA INFECTION IN FROGS

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SUMMARY: Outbreaks of "Reg Leg", an *Aeromonas hydrophilia* infection occurred twice in a bullfrog farm in Johore. The first outbreak was an acute infection characterized by severe skin ulceration, petechial haemorrhages and corneal opacity with absense of any visceral lesions while in the second episode the symtoms were indicative of a more chronic infection. There were severe ascites, visceral enlargement and nervous disorders. Microscopic examination revealed numerous granulomas in the liver, spleen and kidney. The dominant organism isolated was *Aeromonas hydrophilia.*

Keys words: Bullfrog, Red-Leg, *Aeromonas hydrophilia*

INTRODUCTION

The first investigation on septicaemic disease of frogs was in 1890 (Kulp and Borden, 1942) in which an epizootic form of bacterial septicaemia was described. This disease, usually accompanied by cutaneous ulcerative condition was subsequently named "Red-Leg" (Emerson and Norris, 1905). The bacteria, *Aeromonas hydrophilia* has been involved in all the serious epidemics of this disease in the United States.

Frog farming in Malaysia is a backyard industry and little is known about disease problems. This paper describes the aetiological, clinical and pathological findings on an outbreak of "Reg-Legs" in frogs. This is believed to be the first report of this disease in Malaysia.

CLINICAL HISTORY AND NECROPSY FINDINGS

From February to October 1988, there were two severe disease outbreaks in a cultured bullfrog farm *(Rana catesbiana)* in Johore. In the first outbreak, the farmer reported that nearly 80% of his frogs from a total population of approximately 10,000 showed severe skin ulcerations and corneal opacity. It was observed that there was distincts sluggishness of the diseased frogs. Their skins were dull and the colour varied from dark to almost white which indicated cutaneous hyperpigmentation and depigmentation. On closer examination, most of the infected frogs revealed bright red mottling and petechial haemorrhages. There were red pustules and raw patches of erythematous skin ulcers on the lips, head, between the eyes, digits and on the back. In severe ulcerations, the feet and toes were eroded with bones exposed. There were also oedematous changes under the skin especially on the ventral portion of the thighs and abdomen. Severe bilateral or unilateral corneal opacity were observed in about 50% of the infected frogs. Gross pathological findings were minimal and limited to general

congestion of all organs. In the second outbreak in September1988, the frogs exhibited a wider range of symtoms. By this time, there was a marked drop in production and they appeared thin, stunted and some with postural abnormality. Besides the ulcerative skin condition which was mild, the more striking symptom was the severe ascites (Figure 1). The frogs appeared bloated, disinclined to move when prodded or was in a slumped posture with palms stretched out. Others showed neurological signs such as disequilibrium, incoordination and hyperaesthesia and circling movements on provocation. Post mortem examination revealed the presence of copious blood-tinged exudate and enlarge organs. The liver appeared to be the most severely affected, with pin point foci and marked nodular protrusions. Similar lesions were seen in the spleen, kidney and heart of some frogs. Some of the lungs were congested and a few intestines were thin-walled and greatly distended.

LABORATORY FINDINGS AND HISTOPATHOLOGY

Bacteriological examinations were done according to methods described by Carter (1978) and Glorioso *et al.* (1974a) and the organisms isolated were *Aeromonas hydrophilia, Aeromonas* spp., *Escherichia coli* and *α-haemolytic Streptococcus spp.* However, *Aeromonas hydrophilia* was recovered in large numbers and hence regarded as the dominant organism. Direct smears of infected lesions and ascitic fluid showed many Gram-negative baccilli. It was also negative for acid fast bacteria by Ziehl-Nielsen stain. No virus particles were detected on electron microscopy.

Stained sections of the liver, spleen and kidney showed degeneration and necrosis with lymphocytic infiltration and numerous granulomas with encapsulated fibrous capsule surrounding a necrotic centre (Figure 2). There were also lymphocytic accumulation in the heart and lungs. In the spinal cord, there were perivascular cuffing and oedema while in the brain, there was severe necrotizing meningitis. The conjunctiva was oedematous and with lymphocytic infiltration as well as necrosis. There was structural disruption of the muscle fibers, oedema, loss of contrast and a decreased affinity for eosin.

DISCUSSION

The immediate cause of death in the majority of cases in frogs is septicaemia caused by *Aeromonas hydrophilia* (Gibbs 1973). Other organisms have been implicated in natural and spontaneous frog disease from time to time (Gibbs et al., 1966; Gibbs, 1971; Reichenbach-Klinke and Elkan, 1965; Gloriosio et al (1974b) but the evidence was frequently inconclusive and the incidence low.

The clinical signs of this infection varied probably according to the virulence of the bacteria, the extent of lesions and the prophylactic measures attempted after the initial outbreak. In the earlier outbreak, mortality was higher and the disease appeared to be more acute whereas in the second outbreak, the desease was more chronic. The tadpoles during metamorphosis were the most susceptible. The disease in adult frogs seemed to be more insidious, lasting for more than 6 months before death or marketing. A good description of the symptoms was given by Kulp and Borden (1942) where the initial stage was haemorrhagic ulcer followed by extreme oedema in the final stages. Thus, the variability of symptoms actually indicate the different stage of infection.

The ubiquitous nature of *Aeromonas hydrophilia* in the environment suggests that the disease is derived from exogenous sources. Being an opportunist, a decline in the frogs' immunological defence led to the establishment of this organism and elaboration of toxins.

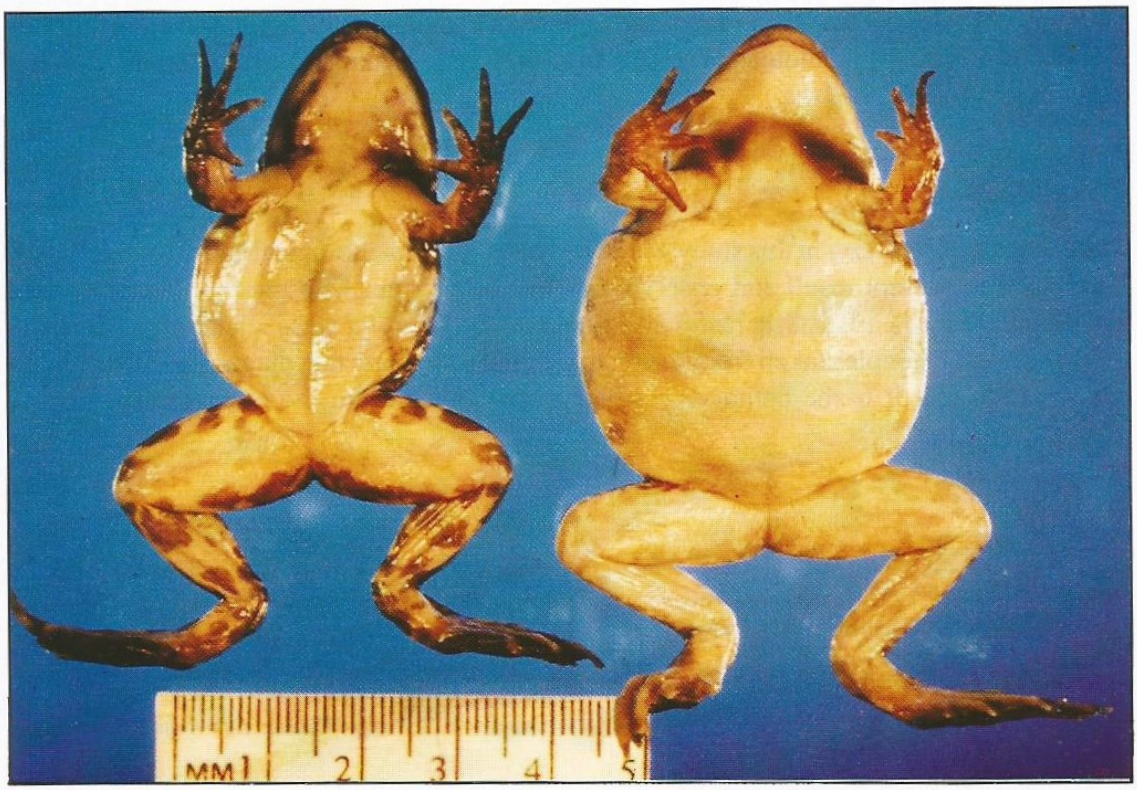

Fig 1. Severe ascites in the frog (on the right) due to *Aeromonas hydrophilia*.

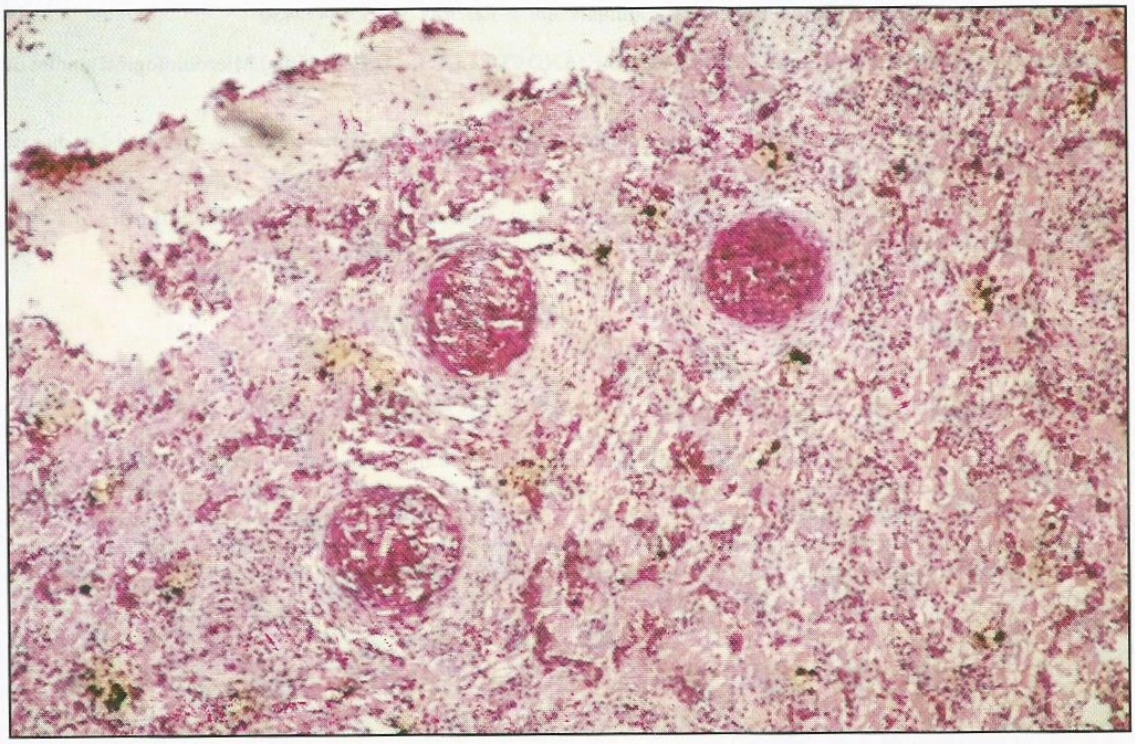

Fig 2. Hepatocytic necrosis, lymphocytic infiltration and granuloma of frogs due to *Aeromonas hydrophilia*.

The resultant release of toxins and enzymes irritates and damages the host tissues and the body reacted by forming granulomas. The severe necrotizing meningitis explained the nervous signs.

CONCLUSION

Diagnosis of Red-Leg in this study was based on clinical, pathological and microbiological findings. The present result and observations were consistent with the findings of various authors mentioned earlier. The fact that *Aeromonas hydrophilia* was isolated from the frogs as well as water samples taken from the residing ponds supports this diagnosis, although one has to prove that it is the pathogenic strain that is involved in the outbreak and not the saprophytes.

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RINGKASAN

WABAK "RED-LEG" - SEBUAH JANGKITAN AEROMONAS HYDROPHILIA PADA KATAK

Wabak penyakit "Reg Leg" yang disebabkan oleh Aeromonas hydrophilia berlaku sebanyak dua kali di ladang ternakan katak di Johor. Wabak pertama merupakan jangkitan akut dimana terdapat ulser kulit yang teruk, pendarahan petekia dan kelegapan kornea tanpa lesi visera yang ketara, manakala episod yang kedua menunjukkan jangkitan kronik. Terdapat petanda seperti asites, pembesaran visera dan gangguan saraf. Pengamatan mikroskopi menunjukkan granuloma di hati, limpa dan ginjal. Organisma utama diasingkan adalah Aeromonas hdrophilia.