Slov Vet Res 2004; 41 (1): 23-9
UDC 619:616.94:582.231:637

Original research paper

THE ROLE OF *AEROMONAS HYDROPHILA* BACTERIUM AS A CAUSATIVE AGENT OF SEPTICAEMIA IN DOGS

Irena Zdovc [1], Tanja Švara [2], Polona Juntes [2], Tina Kotnik [3], Matjaž Ocepek [1], Manica Černe [2], Milan Pogačnik [2]

Addresses of authors: [1] Insitute for Microbiology and Parasitology, [2] Institute for Patology, Forensic Administrative Veterinary Medicine, [3] Clinic for Surgery and Small Animals, Veterinary Faculty, Gerbičeva 60, 1000 Ljubljana, Slovenia

*Corresponding author. E-mail: irena.zdovc@vf.uni-lj.si

Summary: *Aeromonas hydrophila* is an organism commonly found in water, particularly during the warm season, though less commonly isolated as a pathogen in domestic animals and humans. The course of septicaemia caused by *Aeromonas hydrophila* in puppies is described to illustrate its bacterial pathogenic activity and the diagnostic procedures used. Seven of eight puppies, all clinically healthy at birth, died within ten days of birth, after receiving the same clinical course. Beside the gross pathology and histopathology, the internal organs were also bacteriologically examined. The puppies had diffuse acute fibrinous and necrotic bronchopneumonia. Infection with *A. hydrophila*, which was isolated from the liver, spleen, lungs and intestines, was determined as the cause of the sepsis and the consequent death. To establish the origin of the infection, cultures of milk, vaginal and rectal swabs taken from the dam were made and they were all negative for this bacterium. While the origin of the infection remains unknown, the underdevelopment of the puppies is thought to be a predisposing factor.

Key words: dog; septicaemia; bacterial pneumonia; *Aeromonas hydrophila*

Introduction

Aeromonas hydrophila is a non-fastidious, gram-negative rod-shaped, motile bacterium. The 1974 edition of Bergey's Manual includes it in the family *Vibrionaceae* (1). Its morphological and cultural characteristics are the same as is indicated for *Aeromonas caviae*, therefore the differentiation is difficult. As the species' name hydrophila ("water lover") indicates, the natural habitat of the microorganism is both fresh and sea water. It has been observed in numerous species of freshwater fish, the occasional salt-water fish, and in amphibians, reptiles, cattle and humans all over the world. *Aeromonas hydrophila* is recognized as an opportunistic pathogen or a secondary invader (2, 3, 4). There are some reports that describe the role of *Aeromonas hydrophila* in the pathology of mammals, however, most of them relate to humans (5, 6, 7) and there only a few describing its role in dogs (8, 9).

Received: April, 2004
Accepted for publication: June, 2004

The aim of this work is to show that infection with *A. hydrophila* can be severe enough to be considered a cause of death in mammals with weak immune responses.

Material and methods

Eight puppies were examined at birth and, at first, all appeared to be normal, healthy and strong. About 30 hours later, the first puppy showed signs of illness: it stopped sucking, became hypothermic and debilitated and died within 24 hours. All the puppies, except for one, developed the same symptoms and despite intensive care – warming, oxygen supplementation, supplemental feeding and glucose infusions – successively died within ten days.

The bitch, a five-year-old Doberman pinscher, in good general condition was admitted to the veterinary clinic due to difficulties in parturition. The act began on the 60th day of pregnancy and was run with caesarean section because of dystocia. During the operation the bitch was treated

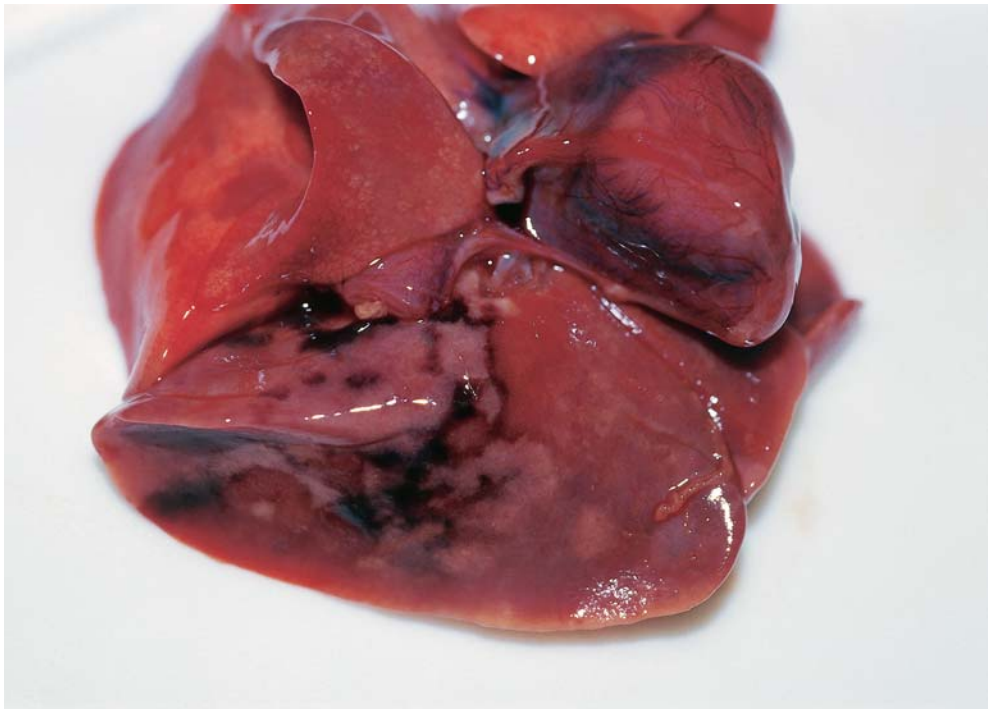

Figure 1: Lung. Acute diffuse fibrinous bronchopneumonia with multifocal areas of necrosis and haemorrhages

with antibiotics amoxycillin+clavulanic acid (Amoksiklav, Lek) and gentamicin (Gentamicin, Lek).

Two puppies were submitted for post-mortem examinations immediately after death: a female (body weight 400 g) and a male (body weight 500 g). A necropsy was performed and several tissue specimens were taken for further laboratory examinations. For the histological examinations, tissue samples from the brain, kidneys, heart, liver, lungs, spleen and intestines were fixed in a 10 % buffered formalin, routinely processed in paraffin and then stained with haematoxylin and eosin (HE). The lung and spleen samples were also treated with Grocott's methenamine silver staining and periodic acid Schiff reaction (to exclude mycotic infections), as well as Goodpasture's stain method (gram staining for tissues). Cryostatic tissue sections of the lungs, liver and kidneys were stained with Sudan III for fat. Imprints of the pleural surface were prepared for cytology, air dried, fixed in methanol and stained with Giemsa.

Samples for bacteriological examinations were taken from the liver, spleen, lungs and intestines. The material was inoculated on nutrient agar (Oxoid) supplemented with 5 % of ovine blood (BA) and Drigalski agar (DA) and incubated at 37 °C for 24 h, and on Sabouraud Dextrose Agar (SDA, bioMerieux, France) at 37 °C for five days. Subcultures for *A. hydrophila* identification were made on the BA. Simultaneously, the milk of the dam was examined and a few days later rectal and vaginal swabs were also taken from the dam. Bacteria that grew on the culture media were Gram stained (Difco-BBL) and tested for catalase and oxidase activity (Difco-BBL). The biochemical characteristics of the isolated bacteria were tested using classical biochemical tests and the Api 20NE, Api 20E and API Staph commercial systems (bioMerieux, France) in accordance with the manufacturer's instructions.

Results

The post-mortem findings of both animals were similar. The thoracic cavity was filled with a small amount of a serofibrinous, opaque inflammatory exudate. There were pulmonary lesions characteristic of acute fibrinous and necrotic bronchopneumonia with acute fibrinous pleuritis (Fig. 1). The pleura was covered with a thick layer of fibrin, the lung texture was firm, and the majority of the pulmonary tissue was heavily congested and oedematous with multifocal grey areas of necrosis on the pleural and cut surfaces. Both the liver and the kidneys were enlarged and congested, the spleen was enlarged and the intestines displayed acute catarrhal enterocolitis.

Microscopic lesions of the lung were consistent with the gross pathology findings – acute fib-

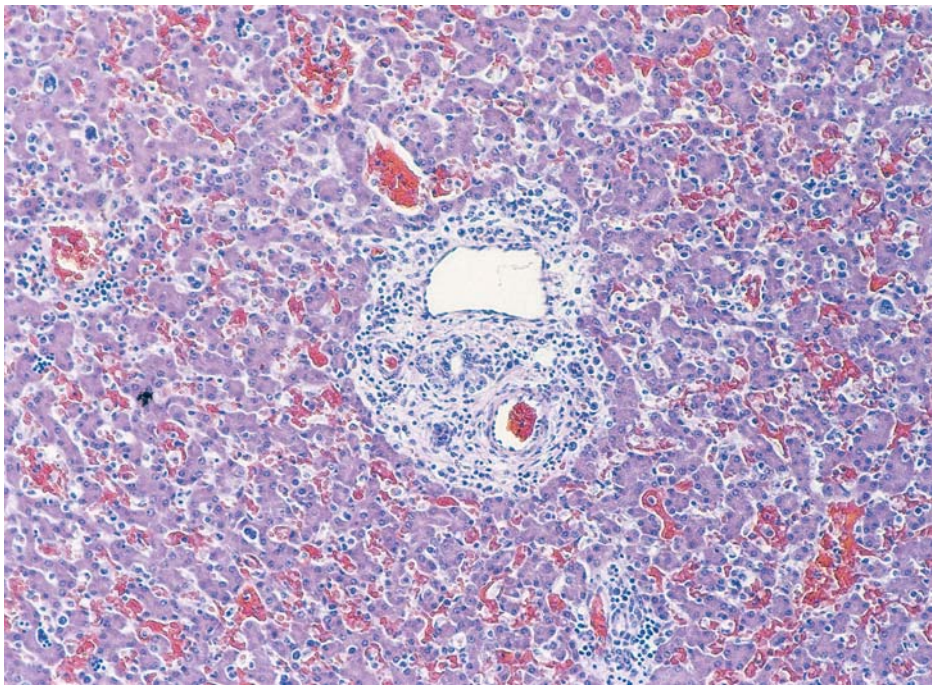

Figure 2: Liver. Many extramedullary haematopoietic foci and few macrophages located throughout the liver tissue and in portal areas. Haematoxylin & eosin, x 10

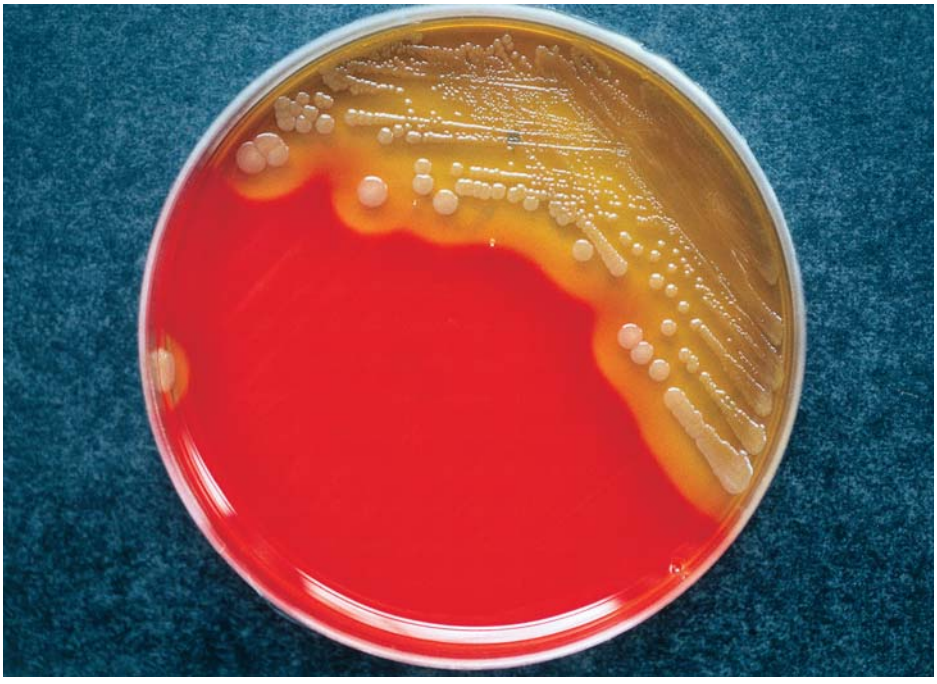

Figure 3: Culture of *Aeromonas hydrophila* with a large zone of haemolysis on 5 % ovine blood agar after 48 h incubation at 37 °C

rinous bronchopneumonia with multifocal areas of coagulative necrosis and acute fibrinous pleuritis. Several bronchi and bronchiole had necrotic walls and were filled with desquamated epithelial cells, numerous macrophages and neutrophils. The alveoli were distended and filled with large quantities of fibrin and inflammatory cells; mostly macrophages and some neutrophils. There were also large areas of multifocal coagulative necrosis. In many parts of the lung, we found numerous small rod-shaped gram-negative bacteria. Bacterial colonies of gram-negative rods were especially numerous and large in necrotic areas and were growing towards the periphery of such areas. In one puppy there were also large areas of multifocal haemorrhages. The interstitium around blood vessels was distended by oedema and contained a few macrophages, monocytes and neutrophils. Grocott's and PAS staining of the lungs and spleen established that they were negative for mycotic infectious agents. Goodpasture staining also established that the

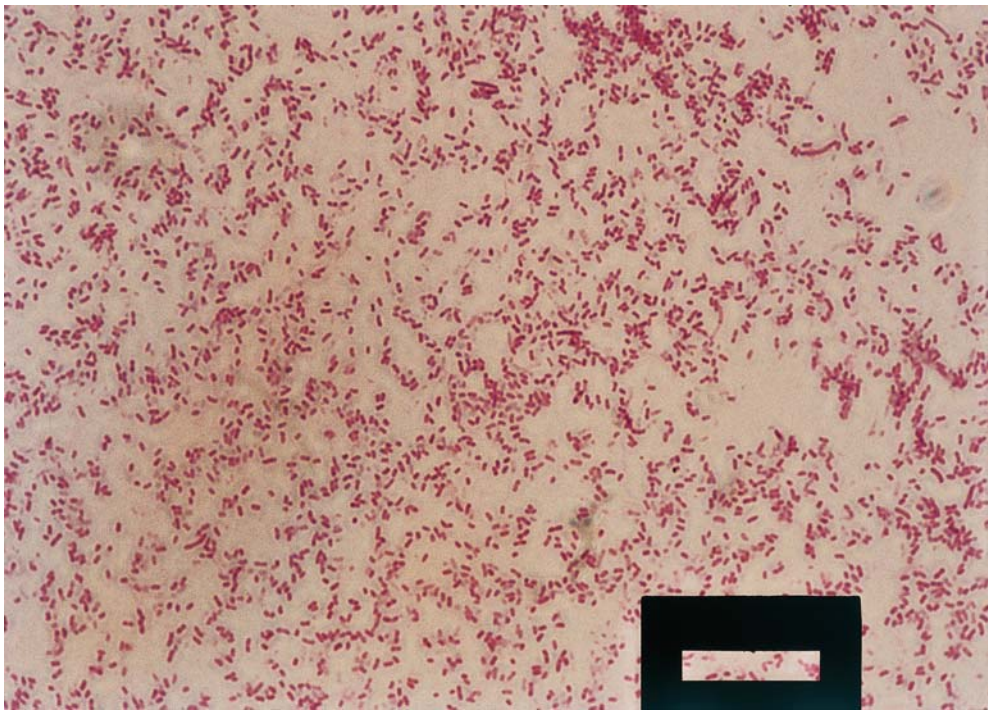

Figure 4: Gram stain of Aeromonas hydrophila ilustrating gram-negative rods

spleen was negative. The portal areas of the liver and interstitium around hepatic veins were oedematous, lymph vessels in the portal areas were distended, and portal tissues were infiltrated with numerous macrophages and a few eosinophils. Macrophages were also found scattered within the sinusoids. There were swollen hepatocytes as well as some disseminated necrotic hepatocytes, and karyolysis and cholestasis were also present. The Kupffer cells were swollen and many of them were in mitosis. There were many extramedullary haematopoietic foci with erythropoietic cells and megakaryocytes located throughout the liver tissue (Fig. 2). The kidneys were underdeveloped for the age of animals. They had foetal cortices with numerous mitoses of epithelial cells in the nephrons, and small, hyperchromatic glomerular cells forming palisades. The tubular cells were vacuolated and many revealed small accumulations of calcium salts within their cytoplasm due to tubular degeneration. The interstitium was distended by oedema. Erythrocytes in small vessels formed hyalinized cylindrical structures without obvious boundaries between cells, which was diagnosed as disseminated intravascular coagulation. The white and the red pulp of the spleen were not clearly separated. Lymphopenia was evident and a few necrotic lymphocytes were found in some follicles. The red pulp was highly cellular with diffuse extramedullary haematopoiesis, and the red pulp was congested. The epithelial lining of the small and large intestine was largely desquamated and the ratio between the intestinal villa and crypts in jejunum was approximately 4:1. Between the epithelial cells and the lamina propria in non-desquamated areas of mucosa there was a vacuolated layer of severe oedema; the propria was also oedematous and infiltrated with a small number of eosinophils. The intestinal lumen contained desquamated cells, an amorphous mass with several haemosiderin granules, and many bacteria forming colonies (mostly short rods and small cocci). In one puppy, the intestinal mucosa was strongly congested with evidence of haemorrhaging into the intestinal lumen. Lesions in other tissues were mostly congestion. Imprints of the pulmonary surface contained numerous short rod-shaped bacteria, degenerate macrophages – some of them containing bacteria in their cytoplasm, and a few neutrophilic granulocytes, erythrocytes and mesothelial cells (acute septic pleuritis).

Bacteriological cultures of the organs yielded abundant growth of large colonies (2-3 mm) that were flat, greyish, circular and convex with an entire margin and surrounded by a large zone of beta-haemolysis (Fig. 3). Gram-stained cultures demonstrated gram-negative rod-shaped bacteria (Fig. 4). A presumptive diagnosis of an *Aeromonas*

species was based initially on a positive oxidase reaction and additionally on the fermentation of carbohydrates. Our isolate produced both acid and gas from glucose and acid from arabinose, manitol, sucrose and maltose, but not from inositol or lactose. The numerical profile of the biochemical reactions in the Api 20NE system was 7577754. *Aeromonas hydrophila* was grown in an entirely pure culture, except with some rare colonies of non-haemolytic Escherichia coli in the intestines.

Bacteriological examinations of all the samples taken from the dam were negative for *A. hydrophila*. The vaginal swab yielded only a few colonies of *Staphylococcus haemolyticus*, which belongs to the group of coagulase-negative staphylococci. The bacteriological culture of the dam's rectal swab yielded non-haemolytic *Escherichia coli* and alpha-haemolytic streptococci.

Discussion

The sudden death of seven, out of eight, newborn puppies in such a short period after parturition can have many causes but an intensive infection with *Aeromonas hydrophila* was probably the fatal one. The gross pathology, the histological determination of numerous short rod-shaped bacteria in many tissues and the isolation of this bacterium in a pure culture indicated an acute septic condition.

Aeromonas hydrophila is a part of the normal flora of freshwater fish and is commonly present in fish ponds and tanks (10). Occasionally it can cause infections in humans, which range from soft-tissue infections, pneumonia, endocarditis and gastroenteritis to septicaemia (4, 11, 12). Cases of hospital infections with *A. hydrophila* were reported in humans as well (13). In dogs, *A. hydrophila* was demonstrated in a few cases as an aetiologic agent of disease, usually in young adults where it was considered an opportunistic pathogen (8, 9). That means that some other stressful factor should have been present at first. Animals can be faecal carriers of *Aeromonas* spp. (14). Ghenghes and others presented an interesting study on the presence of the *Aeromonas* species in domestic dogs and cats. They found that *Aeromonas* are not uncommon in healthy dogs and cats. Furthermore, they found this organism occurred in the Doberman breed to a higher degree than in other breeds. They emphasized that haemolysin-producing *Aeromonas* species in the faeces of healthy domestic dogs and cats may present a public health problem for humans who came into contact with them.

Numerous opportunistic bacteria can cause septicaemia in susceptible neonates. There are a few reports of neonatal *A. hydrophila* septicaemia in children that are comparable to our case. In these reported cases, the children were born at term and no signs of immaturity were present (15, 16). The aetiopathogenesis of infection in our case allows some speculation. Which factor facilitated the development of the extensive pneumonia and sudden death involving a microorganism of inherently low pathogenicity for mammals? The owner initially reported that the puppies were born at term. According to the structure of the kidney cortex and the diffuse, extensive extramedullary haemopoiesis in the spleen and liver at the age of seven days, we estimated that they were underdeveloped. Therefore immaturity could be a possible factor contributing to susceptibility for the infection and the fatal exit (17). Common canine viral infections, which can be an underlying factor for the secondary infection were ruled out as a predisposing factor because there were no morphological or histological lesions characteristic of parvoviral, distemper or herpes virus infections, and besides that the dam was vaccinated against them on a regular basis.

The pathomorphological lesions in both puppies were predominantly located in lungs. The diagnoses of pneumonia in children with *A. hydrophila* septicaemia were made after clinical and x-ray examinations only. There is no information on pathomorphological and histopathological changes in the lungs of children with septicaemia (15, 16). Pneumonia is frequently diagnosed in cases of *A. hydrophila* septicaemia (11, 18). In a study of fifteen cases of *A. hydrophila* septicaemia, extensive bilateral pulmonary lesions were found in more than half of the patients (13). The pneumonia in our case is, according to the histopathological lesions, similar to *A. hydrophila* pneumonia described in man (11). No pulmonary lesions were noted in dogs with *A. hydrophila* septicaemia (8, 9). Histopathological lesions in the lung can be partly the consequence of the aspiration of milk that occurs during supplemental feeding. But in our case that happened after the puppies devel-

oped clinical signs and was just a factor contributing to the quicker course of the disease.

Infections of the neonate may be acquired from a vaginal flora during parturition, through penetrated skin, a contaminated umbilicus or from the environment. No skin or umbilical lesions were found in any of puppies. Due to the *A. hydrophila*-negative results from all the samples taken from the dam, we don't believe that the dam was a carrier of this bacterium. On the other hand, it should be noted that the dam was on a prolonged antibiotic therapy, which could have changed intestinal bacterial flora by the time the rectal swabs were taken for bacteriology. This could explain why the *A. hydrophila* cultural examinations were unable to determine the presence of the bacterium.

Looking for other sources of infection we checked for the presence of other animals in their household that are known as carriers of *A. hydrophila*. The owners had no other animals or reptiles at all. Contaminated water or food, or even the hospital environment might have been the source of infection in this case but unfortunately we were not able to check into these possibilities. *Aeromonas hydrophila* has been implicated as a cause of gastroenteritis in humans and ingestion of contaminated water is another possible point of access for *A. hydrophila* into the intestine. The source of the puppies infection seems unlikely to be determined now since the material, taken from the dam, was negative to *A. hydrophila*. But the reason for the negative results could also be the preventative antibiotic treatment of the bitch after the caesarean section. The dam (but not the puppies) was treated with a combination of amoxycillin + clavulanic acid and gentamicin, the combination that is usually successfully used when dealing with *A. hydrophila* in fish (19). But in the present case the rapid progress of the disease was unfortunately fatal for all the puppies. According to the owner's data the dam had no further problems with infection. Six months after parturition, another rectal swab was taken and was also negative for *Aeromonas*.

To the best of our knowledge this is the first well document case of severe pneumonia caused by *Aeromonas hydrophila* in newborn puppies and their consequential sudden death with almost no chance of a successful treatment. Infection or contamination of domestic animals with *A. hydrophila* can be considered as a health risk for animals as well as humans, especially those in the early (neonatal and perinatal) and most-sensitive periods of life, particularly underdeveloped or premature individuals, and those with an impaired immune system.

References

1. Bergey's manual of systematic bacteriology. Vol 1. Baltimore: Williams & Wilkins, 1984: 546-8.

2. Pearson TA, Mitchell CA, Hughes WT. *Aeromonas hydrophila* Septicemia. Amer J Dis Chil 1972; 123: 579-82.

3. Austin B, Allen-Austin D. Bacterial pathogens of fish. J Appl Bacteriol 1985; 58: 483-506.

4. Gold WL, Salit IE. *Aeromonas hydrophila* infections of skin and soft tissue: report of 11 cases and review. Clin Infect Dis 1993; 16: 69-74.

5. Paniagua C, Arguello-Villares JL, Arias MA, Herreros M. *Aeromonas hydrophila* associated with a severe outbreak of infection in farmed rabbits. Zentralbl Hyg Umweltmed 1998; 201: 423-30.

6. Deodhar LP, Saraswathi K, Vardukar A. *Aeromonas* spp. and their association with human diarrhoeral disease. J Clin Microbiol 1991; 29: 853-6.

7. San Joaquin V, Pickett DA. *Aeromonas* associated gastroenteritis in children. Pediat Infect Dis J 1988; 7: 53-7.

8. Pierce RL, Daley CA, Gates CE, Wohlgemuth K. *Aeromonas hydrophila* Septicemia in a Dog. J Am Vet Med Assoc 1973; 6: 469.

9. Andre-Fontaine G, Monfort P, Buggin-Daubie M, Filloneau C, Ganiere JP. Fatal disease mimicking leptospirosis in a dog, caused by *Aeromonas hydrophila*. Comp Immun Microbiol Infect Dis 1995; 18: 69-72.

10. Monfort P, Baleux B. Haemolysin occurrence among *Aeromonas hydrophila*, *Aeromonas caviae*, and *Aeromonas sabria* strains isolated from different aquatic ecosystems. Res Microb 1991; 142: 95-102.

11. Goncalves JR, Brum G, Fernandes A, Biscaia I, Correia MJS, Bastardo J. *Aeromonas hydrophila* fulminant pneumonia in a fit young man. Thorax 1991; 47: 482-3.

12. Krovacek K, Conte M, Galderisi P, Morelli G, Postiglione A, Dumontet S. Fatal septicaemia caused by *Aeromonas hydrophila* in a patient with cirrhosis. Comp Immunol Microbiol Infect Dis 1993; 16: 267-72.

13. Picard B, Arlet G, Goullet P. *Aeromonas hydrophila* septicemia. Epidemiologic aspects: 15 cases. Presse Med 1984; 19: 1203-5.

14. Ghenghesh KS, Abeid SS, Jaber MM, Ben-Taher SA. Isolation and haemolytic activity of *Aeromonas* species from domestic dogs and cats. Comp Immunol Microbiol Infect Dis 1995; 22: 175-9.

15. Gupta P, Ramachandran VG, Seth A. Early onset neonatal septicemia caused by *Aeromonas hydrophila*. Indian Pediatr 1996; 33: 703-4.

16. Verghese SL, Raju BB, Ramanath A, Raghu MB, Siyakumar A. Septicaemia caused by *Aeromonas hydrophila* in a neonate. J Assoc Phys India 1994; 42: 909-10.

17. Quinn PJ, Donnelly WJC, Carter ME, Markey BKJ, Torgeson PR, Bteathnach RMS. Male and female reproductive systems. In: Microbial and parasitic diseases of the dog and cat. London: WB Saunders Company, 1997: 207-12.

18. Takano Y, Asao Y, Kohri Y, Oida K, Imanaka T. Fulminant pneumonia and sepsis due to *Aeromonas hydrophila* in an alcohol abuser. Intern Med 1996; 35: 410-2.

19. Quinn PJ, Carter ME, Markey B, Carter GR. *Aeromonas*, *Plesiomonas* and *Vibrio* species. In: Clinical veterinary microbiology. London:Mosby-Year Book Europe limited, 1994: 243-7.

VLOGA BAKTERIJE *AEROMONAS HYDROPHILA* KOT POVZROČITELJICE SEPTIKEMIJE PRI PSIH

I. Zdovc, T. Švara, P. Juntes, T. Kotnik, M. Ocepek, M. Černe, M. Pogačnik

Povzetek: *Aeromonas hydrophila* je mikroorganizem, ki ga običajno najdemo v vodi, predvsem v toplejših obdobjih, zelo redko pa ga izoliramo kot povzročitelja bolezni pri domačih živalih in ljudeh. V prispevku je prvič natančno predstavljen primer patogenega delovanja bakterije *Aeromonas hydrophila* pri pasjih mladičih. Opisani so klinični potek septikemije, spremembe na notranjih organih in diagnostični postopki v vseh fazah preiskave. Osem novorojenih mladičev, ki so bili po porodu klinično zdravi, je v naslednjih desetih dneh postopoma poginilo z enakimi kliničnimi znaki bolezni. Poginule živali smo pregledali patoanatomsko in patohistološko, notranje organe pa tudi bakteriološko. Ugotovlili smo difuzno akutno fibrinozno in nekrotično bronhopneumonijo. V notranjih organih (jetrih, vranici, pljučih in črevesju) smo z mikroskopsko preiskavo ugotovlili številne identične gramsko negativne paličke, z gojiščno preiskavo pa je bila izolirana bakterija *Aeromonas hydrophila*. Izolacija čiste kulture, difuzna rast iz vseh pregledanih notranjih organov ter ujemanje izolata s histološkimi in kliničnimi spremembami pomeni potrditev diagnoze, da je bila bakterija povzročiteljica septikemije in posledičnega pogina mladičev. Zaradi ugotavljanja vira okužbe smo pregledali še vaginalni in rekatalni bris ter vzorec mleka psice. V nobenem vzorcu nismo ugotovili bakterije *Aeromonas hydrophila*, kar pripisujemo dejstvu, da je bila psica po porodu zdravljena z antibiotikom, za katerega je bila bakterija zelo dobro občutljiva. Najbolj verjeten vzrok za razvoj sepse pri mladičih je bila njihova nerazvitost ob porodu in velika dovzetnost za okužbo v zgodnjem obdobju, ko imunski sistem še ni opravljal svoje vloge.

Ključne besede: pes; septikemija; bakterijska pljučnica; *Aeromonas hydrophila*