VETERINARSKI ARHIV 80 (4), 539-547, 2010

Cerebral listeriosis in a she-camel at Qassim Region, Central Saudi Arabia - a case report

Abdul A. Al-Swailem[1], Musaad A. Al-Dubaib[2], Ghanim Al-Ghamdi[3], Essam Al-Yamani[1], Abdul M. Al-Naeem[3], Abdulaziz Al-Mejali[3], Maher Shehata[1], Mahmoud E. Hashad[2], Dia El-Lithy Aboelhassan[2], and Osama M. Mahmoud[2]*

[1]King Abdulaziz City for Science and Technology, Saudi Arabia

[2]College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia

[3]College of Veterinary Medicine and Animal Resources, King Faisal University, Saudi Arabia

AL-SWAILEM, A. A., M. A. AL-DUBAIB, G. AL-GHAMDI, E. AL-YAMANI, A. M. AL-NAEEM, A. AL-MEJALI, M. SHEHATA, M. E. HASHAD, D. E. ABOELHASSAN, O. M. MAHMOUD: Cerebral listeriosis in a she-camel at Qassim Region, Central Saudi Arabia. Vet. arhiv 80, 539-547, 2010.

ABSTRACT

A she-camel of about 6 years of age with neurological signs was admitted to the University Veterinary Teaching Hospital at Qassim, central Saudi Arabia, for diagnosis and treatment. Clinical examination showed lack of coordination of movements, Parkinson's-like tremors of the head and lower lip paralysis. No parasite was detected in the stained blood smear, and except for leukocytosis and monocytosis, the result of the complete blood count (CBC) was normal. The results of the liver and kidney function tests were normal. The animal was infused with 4 units of 5% dextrose saline and injected with vitamin B1 and selenium preparations for the nervous manifestations. However, its health deteriorated rapidly and it was on lateral recumbency by the second day of admission. It died after one more day and was necropsied to investigate the cause of death. Postmortem examination showed slight congestion of the liver and the kidneys. The heart, meninges and the brain were congested and haemorrhagic. Histopathological examination showed acute lymphocytic meningeoencephalomyelitis in the medulla oblongata and spinal cord. Micro-abscesses containing neutrophils were seen in the medulla oblongata. Colonies of *Listeria monocytogenes* were obtained when the brain tissue was cultured in a cold environment. Smears made from the colonies showed Gram positive cocco-bacilli. *Listeria monocytogenes* was confirmed by PCR on DNA extracted from brain tissue.

Key words: listeriosis, camel, Qassim, Saudi Arabia, encephalitis

*Corresponding author:

Prof. Osama M. Mahmoud, Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, P.O. Box 6622 Buraydah, Qassim, Saudi Arabia, Phone: +96 66380 0050 2386; Fax: +96 66380 1360; E-mail: oabdelbari@gmail.com

ISSN 0372-5480
Printed in Croatia

Introduction

Listeriosis, caused by *Listeria monocytogenes*, is a bacterial infection with a very wide host range of farm animals, poultry and man (JONES et al., 1997; FRANCIOSA et al., 1998; SMITH, 2002; KURAZONO et al., 2003). The disease has three clinical forms: neurological, septicaemic and abortive. The neurological form (circling disease) is usually seen in farm animals where the bacterium gains entrance to the body through cuts in the oral cavity. From there it is transported through the trigeminal nerve to the brain (CHARLTON and GRACIA, 1977; ANTAL et al., 2005; DONS et al., 2007). For this reason, most of the lesions caused by the infection are localized in the brain stem (mainly the medulla oblongata), and the spinal cord (KUMAR et al., 2007; ANTAL et al., 2005; ANONYM., 2005).

Listeria monocytogenes is found in soil, vegetables, sewage, genital secretions and the nasal mucous membrane of healthy animals. The organism is very resistant to dryness and might stay viable in dry soil and feces for up to 2 years (SEELIGER and JONES, 1986). Most infections are subclinical. Clinical infection developed in immuno-compromised or stressed animals (SEELIGER and JONES, 1986).

The clinical signs of animals affected with listeriosis included depression, fever, disorientation and indifference to the surroundings. The animals often separate themselves and crowd into corners and head press. They stumble and circle continuously (circling disease). Facial paralysis, characterized by drooping ears and lips and dilated nostrils, has been found to be common in infected animals (ANONYM., 2005). Progressive paralysis usually develops and in terminal stages the animal cannot stand. Once the animal becomes recumbent, coma and death usually follow (ANONYM., 2005).

Listerial infection of farm animals is affected by many environmental and animal species factors. NIGHTINGALE et al. (2004; 2005) showed that the prevalence of *L. monocytogenes* in ruminant farms was seasonal and affected by farm management practices, and was higher in small ruminants (sheep and goats) compared to cattle.

Diagnosis of listeric encephalitis is based on clinical signs, histopathological lesions in the brain, as well as isolation and identification of the causative organism from infected tissues (JOHNSON et al., 1995; LOEB, 2004). Demonstration of the bacterium in tissues by Gram stain might be difficult, and LOEB (2004) showed that proper diagnosis of listeriosis in tissues could be achieved by immunohistochemistry. Culture was only 28.5% sensitive, Gram stain was 47.6% sensitive, while immunohistochemistry was 80.9% sensitive (LOEB, 2004). In contrast to conventional diagnostic tests, polymerase chain reaction (PCR) assays are not only less time consuming, but are also less likely to be influenced by external factors that alter the growth and metabolism of bacteria. The assays detected species differences at a genetic level (BATT, 1997). Transcriptional regulators are specialized DNA-binding proteins that play an essential role in directing gene expression within bacteria for their adaptation and survival in different environmental

conditions. Since different bacterial species and subspecies are able to adapt to different and sometimes highly specialized environmental niches, unique transcriptional regulators would be required for each group of bacteria. Therefore, it is likely that transcriptional regulators may be genus-, species-, or subspecies-specific with potential for diagnostic applications. A specific gene (*Imo*0733) that encodes a protein similar to a transcriptional regulator has been identified by for *Listeria monocytogenes* (GLASER et al., 2001; LIU et al., 2004). The *Imo*0733 gene was a target for PCR assay.

Listerial infection in farm animals in Saudi Arabia is poorly documented in the literature, and only one outbreak of the disease has been reported in a sheep farm in the eastern region of the country (AL-DUGHAYM et al., 2001). In this outbreak morbidity of 7.1% and mortality of 2.4% were reported. In this study we report the clinical signs, pathological lesions and diagnosis of an acute case of cerebral listeriosis in an adult she-camel.

Materials and methods

A she-camel, at about 6 years of age, suffering from central nervous system signs, was brought to the University Veterinary Clinic for diagnosis and management. Blood was obtained from the jugular vein for haematological studies and to obtain serum for biochemical analysis. A blood smear was made and stained with Giemsa stain for parasitological examination. Complete blood count (CBC) was performed by manual methods. The activity of the enzyme aspartate amino transferase (AST) and the concentrations of bilrubin and ctreatinine in the serum were determined by an automatic analyzer (Reflotron, Switzerland) using commercial kit-test sets.

The she-camel died and was autopsied to investigate the cause of death. Pieces of tissues from the liver, kidneys, spleen and brain were divided into 2 portions. One portion was sent to the microbiological laboratory and the other was further sampled into smaller pieces and fixed in 10% formol saline. These were later processed in wax, sectioned, dewaxed and stained with haematoxylin and eosin for routine histopathology.

The liver, kidneys, spleen and brain were sliced open and impression smears were made from them. These were stained with Gram stain for demonstration of bacteria (SEELIGER and JONES, 1986; HIRSH and ZEE, 1999).

Brain and cerebrospinal fluid samples were cultured by cold enrichment at 4 °C in tryptic soy broth (TSB-Oxoid) followed by subsequent plating on blood and oxford agar plates (Unipath Ltd. UK), and examined once every week for 6 weeks. *Listeria monocytogenes* identification was carried out according to the colonial morphology, haemolysis, aesculin hydrolysis and fermentation of xylose and rhamnose (LOVETT, 1990).

For PCR, DNA was extracted from the brain and liver tissue samples using a MaNA Pure LC kit (Roche Diagnostics GmbH, Germany) as described in the manufacturer's leaflet. The *Listeria monocytogenes* specific gene (*Imo*0733) was selected as the PCR target. The gene is located between nucleotide sequences 123783 and 124307, and encodes a 269 amino acid protein similar to a transcriptional regulator (GLASER et al., 2001). Two *L. monocytogenes*-specific oligonucleotide primers (*Imo*0733F: 5'CGCAAGAAGAAATTGCCATC-3' and *Imo*0733R: 5'TCCGCGTTAGAAAAATTCCA-3' were designed from the coding sequence of this gene. These primers correspond to the *Imo*0733 gene sequences at nucleotide positions 123844-123863 and 124277-124269 respectively and allow amplification of a 453-bp DNA fragment by PCR (LIU et al., 2004).

PCR reactions were performed in final volumes of 50 μl in 0.5 mL tubes containing 100 ng (1 μL) of DNA, 1X reaction buffer (50 mM KCl, 10 mM tris HCl pH 9, 0.2 mg BSA), 1.5 Mm $MgCl_2$, 0.2 mM (1 μL) dNTPs, 10 pm of each primer (1 μL) and 1.25 U (0.25 μL) of *Taq* polymerase (Pharmacia Biotech, Germany).

Amplification reactions were run in a thermocyler (Flexigene 384, Techne, Flexigene, Cambridge, UK) under the following conditions: first denaturation for 5 minutes at 95 °C, 40 cycles for one minute at 95 °C, 30 seconds at 55 °C, 30 seconds at 72 °C. A final extension step was carried out at 72 °C for 10 minutes. The amplification products were separated on 1.3% agarose gel in 1X tris-borate EDTA (TBE) buffer (pH 8.4) containing ethidium bromide (0.5 μg/mL) and visualized by a UV gel documentation system (Bio-Rad Laboratories, CA, USA).

Results

The clinical signs were mainly nervous, manifested as weakness of the muscles of the legs, Parkinson-like tremors on the head, and lower lip paralysis. Clinical examination showed no respiratory, cardiovascular or digestive manifestations. Blood was negative for parasites. Complete blood count result was normal (Hb: 12.2 g/dL, PCV: 30%) except for leukocytosis (total leukocyte count was 15.3×10^3 cmm) and monocytosis (monocyte differential was 18%). Serum analysis results showed normal liver (aspartate aminotransaminase activity of 21 IU/L, and bilirubin concentrations of 0.3 mg/dL) and kidney functions (creatinine concentration of 0.9 mL/dL).

Vitamin B1 and selenium preparations were injected for the nervous manifestations, and 2 liters of 5% dextrose saline were infused intravenously. The condition of the animal deteriorated quickly and it was on lateral recumbency by the second day of admission. It died with violent paddling movements on the third day of admission and was autopsied to investigate the cause of death.

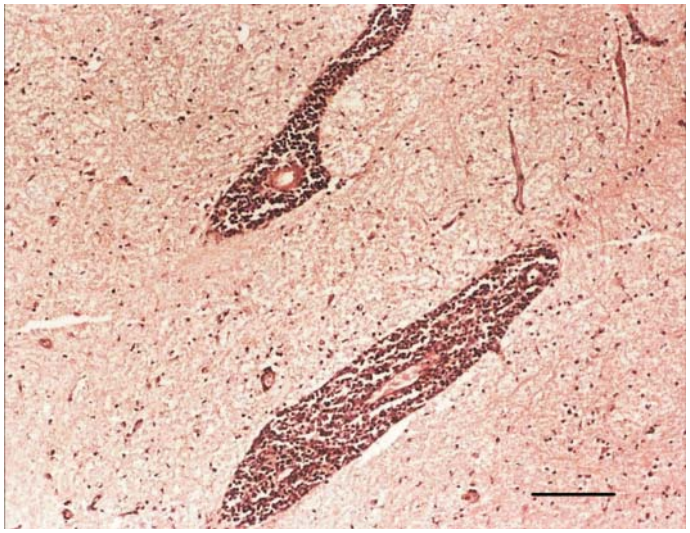

Fig. 1. Lymphocytic encephalitis with perivascular cuffing of brain blood vessels. H&E; scale bar = 100 μm.

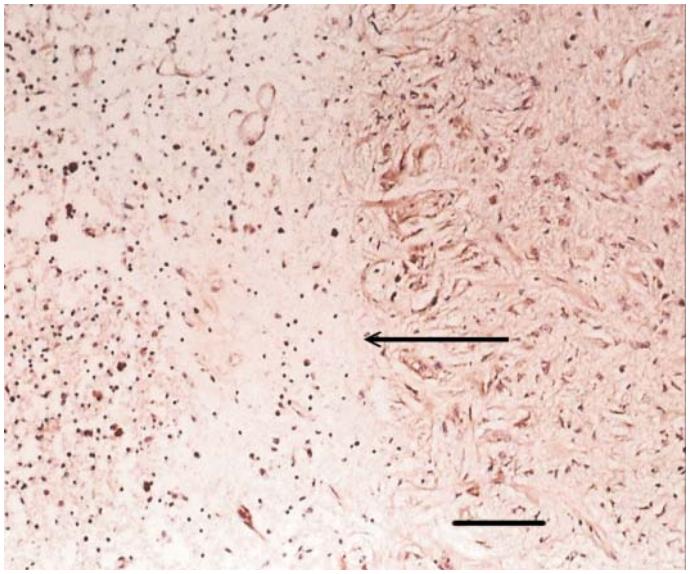

Fig. 2. A microabscess with neutrophils (arrow) in the brain. H&E; scale bar = 50 μm.

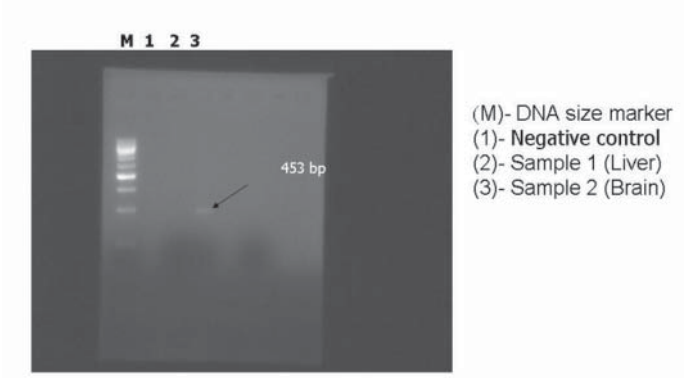

Fig. 3. A 453-bp fragment of *Listeria monocytogenes* from brain tissue

The gross pathological lesions seen in the autopsied animal were congestion and haemorrhages in the meninges, brain and endocardium, The liver and kidneys were only slightly congested. Histopathological examination of the brain showed the lesions were confined to the meninges, medulla and spinal cord. There was acute lymphocytic meningeoencephalomyelitis with intense lymphocytic perivascular cuffing of the blood vessels of the brain and the spinal cord (Fig. 1). Microabscesses were very few and limited to the medulla oblongata. They contain neutrophils, some of which were degenerate (Fig. 2).

Listeria monocytogenes-like organisms were detected in brain impression smears stained with Gram stain. Dark colonies, consistent with those of *Listeria monocytogenes,* were obtained from cultured brain tissue. Gram positive cocco-bacilli were demonstrated on smears made from the cultured colonies.

Using *L. monocytogenes*-specific primers, the predicted 453-bp fragment was amplified from DNA extracted from the brain tissue. In contrast, no amplification product was obtained from liver-extracted DNA.

Discussion

The authors are not aware of any previous report describing listerial infection of the brain of a one-humped camel (*Camelus dromedarius*). However, BUTT et al., (1991) reported encephalitic listeriosis in two adult llamas (*camelid*) and found that the clinical signs and pathological lesions were similar to those reported for other farm animals infected with the disease. Listeriosis is a food-borne infection occurring mainly in humid environments (NIGHTINGALE et al., 2004) and it is not expected to be seen in camels in

Saudi Arabia because of the nomadic nature of these animals and the extreme heat and dryness of the desert environment in which they live.

The clinical signs, as well as the results of the microbiological investigations of the above she-camel were diagnostic for encephalitic listeriosis. The location of lesions in the medulla oblongata and spinal cord, and the presence of microabscesses in the brain tissue, as well as the perivascular cuffing of the brain and spinal cord vessels seen in this case, were consistent with findings reported in other animals with cerebral listeriosis (BUTT et al., 1991; JONES et al., 1997; JOHNSON et al., 1995; KUMAR et al., 2007).

The demonstration of Gram positive cocco-bacilli in impression smears from the brain of the investigated she-camel has been reported by many authors studying animals with encephalitic Listeriosis (BUTT et al., 1991; KUMAR et al., 2007). Cultural and biochemical characteristics of the bacteria investigated in this study were similar to those described for *L. monocytogenes* (SEELIGER and JONES, 1986). The demonstration of *L. monocytogenes* in the brain tissue by PCR was confirmatory of the diagnosis (LIU et al., 2004; BORDER et al., 1990).

Acknowledgements

This work is part of a research project investigating neurological diseases of camels, which is financed by the King Abdulaziz City for Science and Technology, KACST, Saudi Arabia. The help of the administration of the air-force base at Hafr Albatin in the eastern region of the Kingdom of Saudi Arabia, is gratefully acknowledged.

References

AL-DUGHAYM, A. M., A. F. ELMULA, E. E. MOHAMED, A. A. HEGAZY, Y. A. RADWAN, F. M. HOUSAWI, A. A. GAMEEL (2001): First report of an outbreak of ovine septicaemic listeriosis in Saudi Arabia. Rev. Sci. Tech. 20, 777-783.

ANONYMOUS (2005): Listeriosis. In: The Merck Veterinary Manual, 50th edition. Merck and Co. Wiley Publishing Inc. USA.

ANTAL, E. A., E. M. LOBERG, E. DIETRICHS, J. MAEHLEN (2005): Neuropathological findings in 9 cases of *Listeria monocytogenes* brain stem encephalitis. Brain Pathol. 15, 187-191.

BATT, C. A. (1997): Molecular diagnosis for dairy-borne pathogens. J. Dairy Sci. 80, 229-330.

BORDER, P. M., J. J. HOWARD, G. S. PLASTOW, K. W. SIGGENS (1990): Detection of *Listeria* species and *Listeria monocytogenes* using polymerase chain reaction. Lett. Appl. Microbiol. 11, 158-162.

BUTT, M. T., A. WELDON, D. STEP, A. DE LAHUNTA, C. R. HUXTABLE, (1991): Encephalitic listeriosis in two adult llamas (*Lama glama*): clinical presentation, lesions and immunoflourescence of *Listeria monocytogenes*. Corn. Vet. 81, 251-158.

CHARLTON, K. M., M. M. GARCIA (1977): Spontaneous listeric encephalitis and neuritis in sheep. Light microscope studies. Vet. Pathol. 14, 297-313.

DONS, L., Y. JIN, K. KRISTENSSON, M. E. ROTTENBERG (2007): Axonal transport of *Listeria monocytogenes* and nerve cell-induced bacterial killing. J. Neurosci. Res. 85, 2529-2537.

FRANCIOSA, G., M. POURSHABAN, M. GIANFRANCESCHI, P. AURELI, (1998): Genetic typing of human and food isolates of *Listeria monocytogenes* from episodes of listeriosis. Euro. J. Epidemiol. 14, 205-210.

GLASER, P., L. FRANGEUL, BUCHRIESER, C. RUSNIOK, A. AMEND, F. BAQUERO, P. BERCHE, H. BLOECKER, P. BRANDT, T. CHAKRABORTY, A. CHARBIT, F. CHETOUANI, E. COUVÉ, A. DE DARUVAR, P. DEHOUX, E. DOMANN, G. DOMÍNGUEZ-BERNAL, E. DUCHAUD, L. DURANT, O. DUSSURGET, K.-D. ENTIAN, H. FSIHI, F. GARCIA-DEL PORTILLO, P. GARRIDO, L. GAUTIER, W. GOEBEL, N. GÓMEZ-LÓPEZ, T. HAIN, J. HAUF, D. JACKSON, L.-M. JONES, U. KAERST, J. KREFT, M. KUHN, F. KUNST, G. KURAPKAT, E. MADUEÑO, A. MAITOURNAM, J. MATA VICENTE, E. NG, H. NEDJARI, G. NORDSIEK, S. NOVELLA, B. DE PABLOS, J.-C. PÉREZ-DIAZ, R. PURCELL, B. REMMEL, M. ROSE, T. SCHLUETER, N. SIMOES, A. TIERREZ, J.-A. VÁZQUEZ-BOLAND, H. VOSS, J. WEHLAND, P. COSSART (2001): Comparative genomics of listerial species. Science 294, 849-852.

HIRSH, D. C., Y. C ZEE (1999): Veterinary Microbiology. Blackwell Scientific Publication, London, Philadelphia.

JONES, T. C., R. D. HUNT, N. W. KING (1997): Viral Diseases. In: Veterinary Pathology, 6th ed. Williams and Wilkins. Baltimore, Philadelphia.

JOHNSON, G. C., W. H. FALES, C. W. MADDOX, J. A. RAMOS-VARA (1995): Evaluation of laboratory tests for confirming the diagnosis of encephalitic listeriosis in ruminants. J. Vet. Diag. Invest. 7, 223-238.

KUMAR, H., B. B. SINGH, M. S. BAL, K. KAUR, R. SINGH, P. K. SIDHU, K. S. SANDHU (2007): Pathological and epidemiological investigations into listerial encephalitis in sheep. Small Rumin. Res. 71, 293-297.

KURAZONO, M., K. NAKAMURA, M. YAMADA, T. YONEMARU, T. SAKODA (2003): Pathology of listerial encephalitis of chicken in Japan. Avian Dis. 47, 1496-1502.

LIU, D., A. J. AINSWORTH, F. W. AUSTIN, M. L. LAWRENCE (2004): Use of PCR primers derived from a putative transcriptional regulator gene for species -specific determination of *Listeria monocytogenes*. Int. J. Food Microbiol. 19, 297-304.

LOEB, E. (2004): Encephalitic listeriosis in ruminants: immunohistochemistry as a diagnostic tool. J. Vet. Med. A: Physiol., Pathol. Clin. Med. 51, 453-455.

LOVETT, J. (1990): Taxonomy and general characteristics of *Listeria* spp. In: Foodborne Listeriosis. (Millar, A. J., J. L. Smith, G. A. Somkuti, Eds.). New York, Elsevier, pp. 9-12.

NIGHTINGALE, K. K., E. D. FORTES, A. J. HO, Y. H. SCHUKKEN, Y. T. GROHN, M. WIEDMANN (2005): Evaluation of farm management practices as risk factors for clinical

listeriosis and fecal shedding of *Listeria monocytogenes* in ruminants. J. Am. Vet. Med. Ass. 227, 1808-1814.

NIGHTINGALE, K. K., Y. H. SCHUKKEN, C. R. NIGHTINGALE, E. D. FORTES, J. A. HO, Z. HER, Y. T. GROHN, P. L. McDONOUGH, M. WEIDMANN (2004): Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. Appl. Environ. Microbiol. 70, 4458-4467.

SEELIGER, H. P. R., D. JONES (1986): Genus *Listeria*. In: Bergey's Manual of Systematic Bacteriology. (Kandler O., N. Weiss, Eds.). 2nd ed., Baltimore, Williams and Wilkins, pp. 1235-1245.

SMITH, B. P. (2002): Large Animal Internal Medicine, 3rd ed., pp. 946-949.

Received: 21 April 2009
Accepted: 29 January 2010

AL-SWAILEM, A. A., M. A. AL-DUBAIB, G. AL-GHAMDI, E. AL-YAMANI, A. M. AL-NAEEM, A. AL-MEJALI, M. SHEHATA, M. E. HASHAD, D. E. ABOELHASSAN, O. M. MAHMOUD: Cerebralna listerioza u deve na području Qassim u Središnjoj Saudijskoj Arabiji - prikaz slučaja. Vet. arhiv 80, 539-547, 2010.

SAŽETAK

Deva u dobi od šest godina s neurološkim znakovima bila je radi dijagnoze i liječenja primljena na kliniku Veterinarskog Sveučilišta u Qassimu, Središnja Saudijska Arabija. Kliničkom pretragom ustanovljena je nekoordiniranost pokreta, tremor glave i paraliza donje usne slično kao kod Parkinsonove bolesti. U obojenim razmascima krvi nisu bili ustanovljeni paraziti, a hematološki nalaz bio je normalan osim što je ustanovljena leukocitoza i monocitoza. Nalazi funkcije jetara i bubrega bili su u fiziološkim granicama. Životinja je dobila infuziju 4 jedinice 5% fiziološke otopine dekstroze s vitaminom B1 i preparatima selena zbog živčanih znakova. Ipak se njezino zdravstveno stanje naglo pogoršavalo te je drugoga dana bespomoćno ležala na boku. Uginula je trećega dana od primitka na kliniku te je razuđena da bi se otkrio uzrok uginuća. Postmortem pretraga pokazala je blagu kongestiju jetara i bubrega. Ustanovljeni su kongestija i krvarenja u srcu, moždanim ovojnicama i mozgu. Patohistološka pretraga pokazala je akutni limfocitni meningoencefalomijelitis u produženoj i kralježničnoj moždini. U produženoj moždini nalazili su se mikroapscesi koji su sadržavali neutrofile. *Listeria monocytogenes* bila je izdvojena iz moždanoga tkiva uzgojem u hladnim uvjetima. U razmascima kolonija dokazani su gram-pozitivni kokobacili. Nalaz bakterije *Listeria monocytogenes* bio je povrđen lančanom reakcijom polimerazom u DNA ekstrahiranoj iz moždanoga tkiva.

Ključne riječi: listerioza, deva, Qassim, Saudijska Arabija, encefalitis