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Histopathological Features of Listerial Rhombencephalitis in Dairy Calves in Kazakhstan

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ABSTRACT

Listeriosis is an infectious disease with zoonotic potential and can cause high fatality rates in animals and humans. Although the available evidence demonstrates the significance of post-mortem histological evaluation in the appropriate diagnosis, there is no sufficient evidence addressing histopathological alterations observed in calves clinically suspected of listeriosis. The current study aimed to carry out a post-mortem analysis of the dairy calves with clinical suspicion of listeriosis and determine a condition that contributed to death using histopathological evaluation. The tissue samples were obtained from 16 dairy calves with the age range of one day to one month, which died presumably due to listeriosis on several livestock farms in the Almaty region of Kazakhstan. The calves had symptoms of neurologic dysfunction prior to death. Necropsy and collection of brain histological specimens were performed, followed by procedures of fixation, dehydration, paraffinization, sectioning, staining with hematoxylin, and eosin along with Levaditi's method, and the microscopic examination. The histopathology findings were consistent with infection by *Listeria monocytogenes* and localized to the brainstem leading to the diagnosis of listerial rhombencephalitis.

Keywords: Brain, Calves, Histopathology, *Listeria monocytogenes*, Listeriosis, Rhombencephalitis

INTRODUCTION

A facultative anaerobic bacterium *Listeria monocytogenes* is the causative organism of listeriosis (Todd and Notermans, 2011) and is disseminated through the oral route predominantly (Gelbíčová et al., 2016). Ruminants and monogastric animals may cause listeria infection in humans, constituting a particular hazard to individuals with various immune system vulnerabilities, including infants, organ transplantation patients, and others. The mortality rate for listeriosis is up to 75% in humans and up to 100% in animals. Animals under three years of age are more susceptible to the disease (Chlebicz and Śliżewska, 2018). Moreover, this intracellular pathogen is capable of permeating the placental barrier (David and Cossart, 2017), which poses substantial risks to human and animal fetuses. For instance, the transmission during pregnancy may lead to congenital infection (St Edmunds et al., 2008). As a reflection of this data, a survey of 1500 dairy farmers conducted by Erdogan et al. (2001) showed the highest within-herd incidence of clinical listeriosis in dairy calves and replacement heifers. According to a study performed on 20 dairy farms in the northern hemisphere, the average yearly prevalence of *L. monocytogenes* was more than twice as high among calves compared to cows (Bandelj et al., 2018). Concerning the nervous form of listeriosis affecting the central nervous system (CNS), such as rhombencephalitis, a retrospective cohort study revealed that the mean age of sheep and cattle with neurolisteriosis was 39 months (Giles et al., 2017).

The methods usually carried out to confirm the diagnosis of bovine listeriosis are histopathology and culture tests (Radostits et al., 2007). The advantages of histopathology include low cost and ability to observe the tissue reaction (Gupta et al., 2009). Inherent operator variability is among its limitations (Fernandez et al., 2005). *Listeria monocytogenes* can be detected by silver impregnation staining techniques (Topalovski et al., 1993; Luca et al., 2015; Wilson et al., 2015). Their advantage is high sensitivity (Rabilloud, 2012), whereas the imperfection is poor suitability for quantification (Weiss et al., 2009). As for the present study, brain tissues were stained by Levaditi's silver impregnation method. Molecular tests frequently used for the identification of pathogenic material are immunohistochemistry and polymerase chain reaction (PCR). Due to the quantitativeness of the latter, it is less sensitive to interobserver variability (Furrer et al., 2015). Immunohistochemistry allows the identification of specific antigens within tissue sections (Duraiyan et al., 2012). One of its drawbacks is the complexity of the interpretation of results owing to different specificity and sensitivity of commercial antibodies (Furrer et al., 2015). Besides, as Kim et al. (2016)

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has remarked, more sensitive methods spawn increased background signal aside from the target signal. Interestingly, Peters et al. (1995) stated that the clinical diagnosis of listeric encephalitis was confirmed in eight of eleven cerebrospinal fluid samples from ruminants by histological and/or bacteriological examination, whereas PCR allowed detecting *L. monocytogenes* in only one of the samples.

The authors point out a considerable role of postmortem inspections, including histological examination, in a correct diagnosis for listeriosis. At the same time, it must be recognized that no ample evidence addressing histopathological alterations observed in calves with clinical suspicion of listeriosis is available. Therefore, the aim of this cross-sectional study was to carry out a post-mortem analysis of the dairy calves' tissues and determine a condition that contributed to their death using histopathological evaluation.

MATERIALS AND METHODS

This study was carried out at the Department of Biological Safety at Kazakh National Agrarian University, Kazakhstan between 2018 and 2019. The test materials were tissue samples obtained from 16 dairy calves ranging in age from one day to one month, which died presumably due to listeriosis, displaying symptoms of neurologic dysfunction prior to death on several livestock farms in Almaty region of Kazakhstan. The provisional diagnosis was made by rural veterinarians with higher education in the veterinary field. Clinical manifestations included throwing head back, muscle tremors, circling, gait abnormalities, and recumbency. On the day of the calves' death, in order to establish its cause, they were delivered to the department by farm workers. The above cranial neuropathies allowed narrowing the diagnosis to rhombencephalitis (Bradshaw and Venkatesan, 2016). Necropsy and collection of brain histological specimens were performed. The pathological specimens were routinely fixed in 10% neutral buffered formalin and Carnoy's solution. Paraffin and cryostat 5-10 µm sections were stained with hematoxylin-eosin. Levaditi's technique was used to detect *L. monocytogenes* in the samples (Drury et al., 1967). The slices were examined under Zeiss Axiostar plus microscope (Zeiss Inc., Göttingen, Germany) and micrographs were captured by means of a microscope-mounted Leica digital camera (Leica Camera AG, Wetzlar, Germany), at magnifications of 150x and 300x.

Ethical approval

All procedures involving the animals were carried out in conformity with Directive 2010/63/EU and were approved by the ethics committee of Kazakh National Agrarian University (Kazakhstan).

RESULTS

According to the results of the histopathological assessment, in the medulla oblongata and pons Varolii, accumulations of lymphocytes, histiocytes, and neutrophils were observed. The blood vessels were hyperemic and thickened 5 to 319 times (16.5 - 1052.8 µm). Thus, in the region of the medulla oblongata adjacent to the fourth cerebral ventricle, we noted necrotic foci, lymphoneutrophilic pleocytosis, neuronal damage (chromatolysis, acute swelling, vacuolization, pycnosis, translucent cell formation), diapedesis, inflammatory cell infiltrates around blood vessels, perivascular and pericellular edema. The damaged neurons were surrounded by glial cells (Figure 1).

Lymphoneutrophilic pleocytosis, edema, and necrotic foci of approximately 105 µm (the largest foci were up to 1300 µm) were found underneath the ependyma of the fourth ventricle as well. In the necrotic foci, *Listeria* were Levaditi-stained black (Figure 2). The disease was confirmed by Levaditi's staining in all sixteen calves.

In the cerebellum, many Purkinje cells were edematous, their nuclei shriveled or poorly stained, the vessels were congested, and hemorrhages were found, along with cellular infiltrates comprised of lymphocytes, neutrophils, and histiocytes. These histopathologic changes are concordant with infection by *L. monocytogenes* and localized to the brainstem, which therefore led to the diagnosis of listerial rhombencephalitis.

DISCUSSION

The predilection of *L. monocytogenes* for brainstem is widely acknowledged (Xu et al., 2019). In the current study, histopathological examination of specimens from dairy calves with neurological manifestations revealed cerebral tissue inflammation enveloping hindbrain structures, which is characteristic of rhombencephalitis (Oevermann et al., 2010). Cell populations involved in the intracerebral immune response against *L. monocytogenes*-induced lesions overlap those reported in animals (Haligur et al., 2019). In addition to the already asserted axonal pathway of *L. monocytogenes* into the CNS, the transneuronal spread was hypothesized for this infectious agent (Henke et al., 2015). The observed neuronal necrosis phenomenon is a common sequel of encephalitis, although is not yet explained explicitly. It is alleged to be caused by metabolites of infiltrating neutrophils or neuronal infection engendered by *L. monocytogenes* (Precht et al., 2018).

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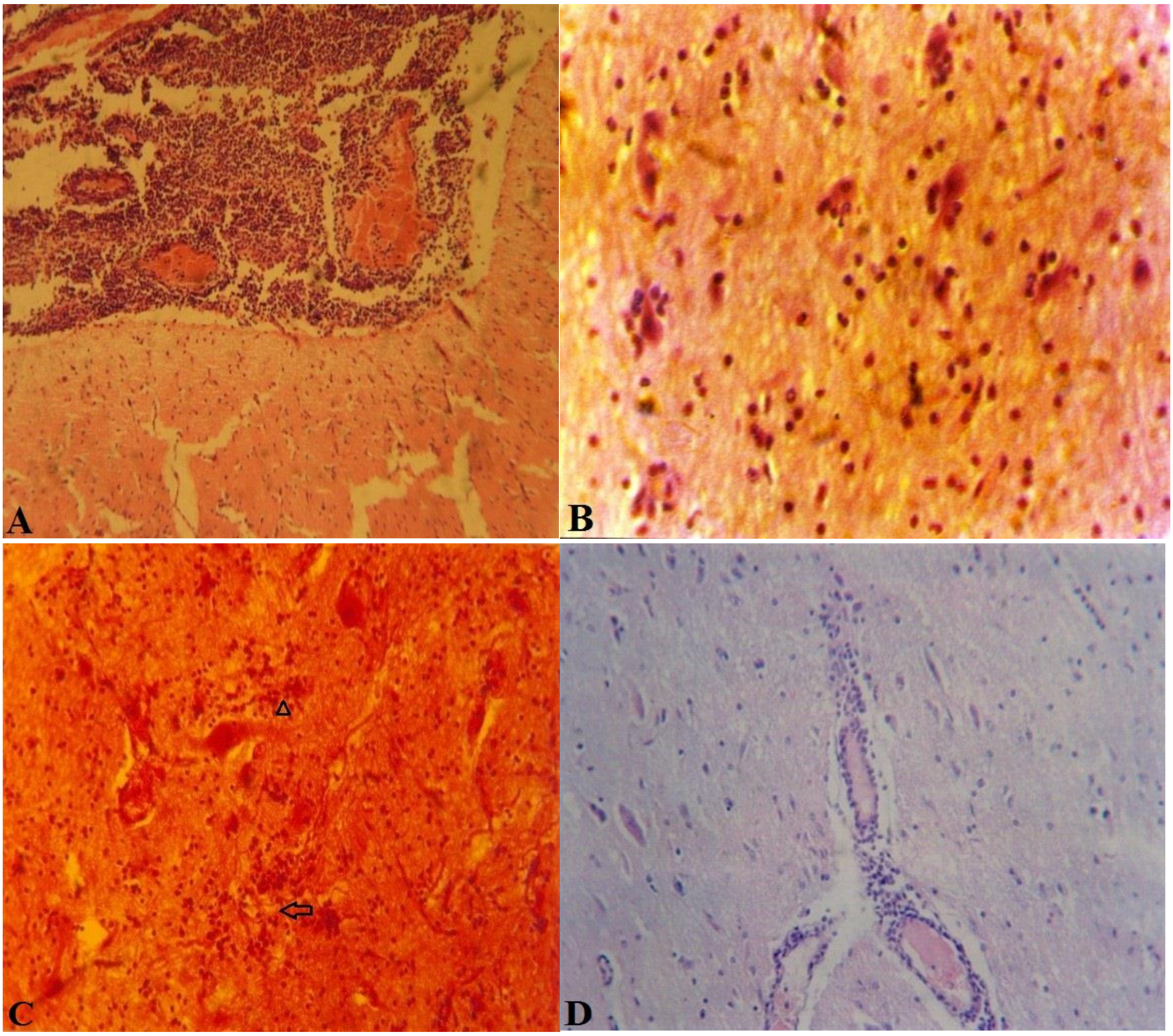

Figure 1. Histological features of listerial rhombencephalitis in the medulla oblongata of calves' brain that died with neurological signs. **A:** multifocal and coalescing foci of necrosis and hemorrhage, with lymphocytes, neutrophils and histiocytes. Hematoxylin and Eosin (H&E) stain, 150×; **B:** neuronophagia. H&E, 300×; **C:** neuronal dystrophy (arrow) and glial cell proliferation (arrowhead). H&E, 150×; **D:** inflammatory perivascular cuffs. H&E, 150×

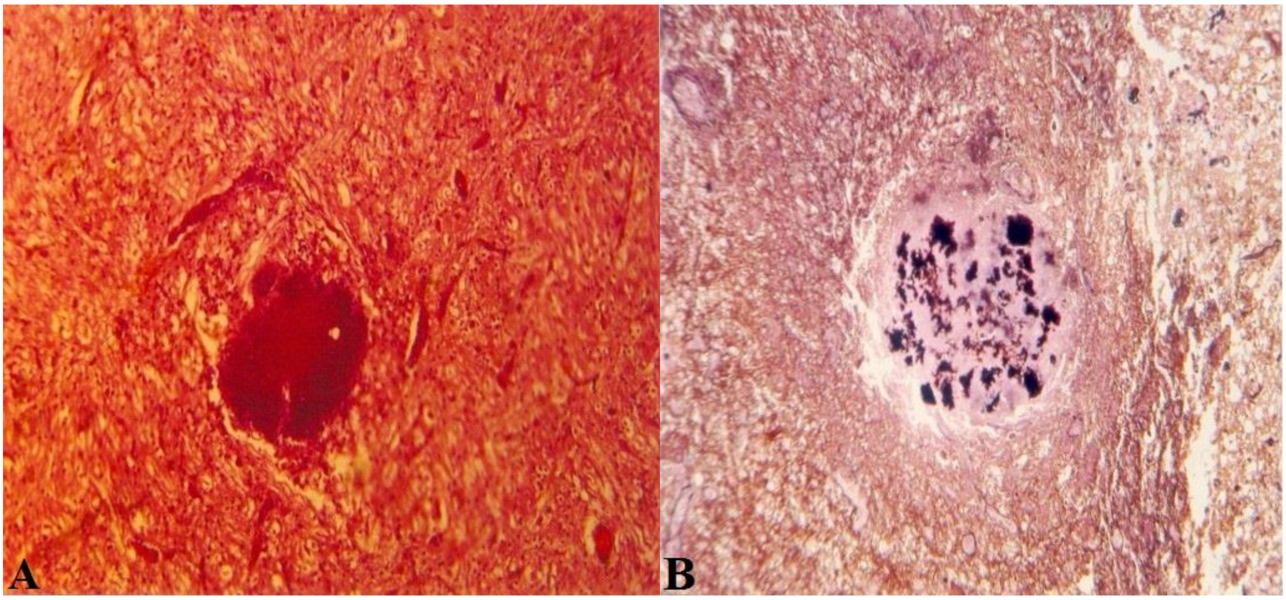

Figure 2. Histological features of listerial rhombencephalitis in the fourth cerebral ventricle of calves that died with neurological signs: **A:** necrotic focus. H&E, 150×; **B:** *Listeria monocytogenes* (black) in necrotic focus. Levaditi stain, 150×

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Another complication associated with *L. monocytogenes* infection is ventriculitis. In our case, the fourth ventricle was found edematous. However, we have not managed to retrieve any research publication containing information on similar alterations in the fourth ventricle of animals with listeriosis. Inter alia, dilatation of the fourth ventricle, as well as inflammation of its choroid plexus and ependyma have been previously described by other authors in human listeriosis of various forms (Engelen-Lee et al., 2018; Liang et al., 2019).

Perivascular infiltration of inflammatory cells was detected in the medulla sections from the calves' brain, since neuroinvasive *L. monocytogenes* infection triggers the inflammatory cascade. In particular, it recruits miRNA-155 which increases inflammation of the cerebral tissue via a variety of inflammatory cells, such as interferon γ-secreting lymphocytes, invading the brain and activating microglia during the adaptive immune response (Zhang et al., 2018).

The evidence confirms the extension of the brainstem encephalitis to the cerebellum (Coombs, 2017), which is in compliance with the data obtained in the current research. Nonetheless, this phenomenon is rarely reported in cattle, but, as revealed by Guldimann et al. (2015), *L. monocytogenes* can propagate in all microglia, including even those of the hippocampus. An experiment on ruminant rhombencephalitis (Rocha et al., 2013) indicated the presence of microabscesses and mononuclear perivascular cuffing in the white matter of bovine cerebellum.

The limitation of the current study is in the lack of an immunoassay or PCR analysis. Despite the prevalence of these diagnostic techniques, they also have a number of disadvantages, such as cross-reactivity (Favrot, 2015; Sakamoto et al., 2018; Ahmed et al., 2020). Furthermore, histopathological assessment is pathognomonic and is still exploited in some researches for confirmation of listeriosis (Teixeira et al., 2011; Precht et al., 2014).

CONCLUSION

This research provided an opportunity to investigate histopathological manifestations of listerial rhombencephalitis in dairy calves. The results indicate that *L. monocytogenes* produced destructive changes in parenchymal cells and brain microvascular system of the investigated animals. Based on the characteristic clinical signs and the obtained findings during histopathological examination, severe multifocal rhombencephalitis associated with *L. monocytogenes* infection was diagnosed.

DECLARATIONS

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Authors’ contribution

A. Ibazhanova and Zh. Kenzhebekova performed the histopathological examination (including necropsy and sample collection) and manuscript writing. B. Nurgazy supervised the laboratory procedures and participated in interpretation of results. A. Namet and K. Orynkhanov provided a critical review. D. Khussainov and A. Alimov prompted a number of literature sources for introduction and discussion sections.

Competing interests

The authors have not declared any conflict of interest.

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