Severe Jackknife-like Kyphosis Malformation in the Fetus of a Free-ranging Sika Deer (Cervus nippon)

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ABSTRACT

A malformed fetus was found in the uterus of a hunted wild sika deer (Cervus nippon) in Japan. Computed tomography, gross pathological and histopathological examinations revealed multiple congenital deformities, including severe jackknife-like kyphosis, exposure of the abdominal viscera, cleft palate, cleft muzzle, excess dorsiflexion of the digits in the right forelimb, third and lateral ventriculomegaly, aberrant lobulation of the lung, and imperforate anus. No pathogens associated with congenital malformation in ruminants were detected. This case report is the first to describe a severe congenital fetal malformation in a free-ranging sika deer; however, the cause of the malformation remains unknown.

Key words: fetus, malformation, sika deer

Congenital malformations have been reported mainly in domestic animals. Despite being widely distributed and hunted, there have been only a few reports of congenital malformations in cervids, including white-tailed deer (Odocoileus virginianus) [1-2] and red deer (Cervus elaphus) [3] but not sika deer (C. nippon). Deer are actively culled around the world, including in Japan, where sika deer are culled to control the population. In 2017, a total of 605,300 sika deer were culled and 158,900 were hunted [4]. However, most carcasses including pregnant does are wasted, and their utilization for scientific research is sporadic. Therefore, obtaining specimens of congenital malformation is extremely rare, making it difficult to characterize symptoms, calculate the prevalence and investigate possible causes. Outbreaks of congenital malformation are thought to be caused by several factors, including the spread of infectious diseases, radioactive pollution, and chemical pollution [5-7]. These factors can seriously impact the population dynamics of wildlife, so early detection of the cause is critical. Documenting the symptoms and determining possible causes of congenital malformation provides valuable information that can help investigators detect the cause of an outbreak when it occurs. This case report is the first to describe a severe congenital fetal malformation in a free-ranging sika deer.

In April 2018, a pregnant doe was hunted by a local hunter in Nagano Prefecture, Japan. The carcass was transported to a municipal facility for wildlife product commercialization in Komoro, Nagano, Japan. The head and uterus of the doe were frozen and transported to Central Region Agricultural Research Center of National Agriculture and Food Research Organization in Tsukuba, Ibaraki, Japan. The doe was estimated to be three years old using cementum age determination, a method known for its high accuracy in ruminants [8], including sika deer [9]. The fetus was covered by the fetal membrane. Upon extracting the fetus from the uterus, exposure of the abdominal viscera
Gross pathological examination revealed a cleft palate (Fig. 3A) and a cleft muzzle (Fig. 3B). Excess dorsiflexion of digits without ankylosis was present in the right forelimb (Fig. 3C), presumably due to the attitude. In the brain, remarkable third and lateral ventriculomegaly was observed (Fig. 3D). Although all internal organs were present, imperforate anus (Fig. 3E) and aberrant lobulation of the lung was observed (Fig. 3F). The lungs had hypoplasia with abnormal lobulation in both caudal lobes. The accessory lobe extended narrowly toward the caudal end.

For histopathological examination, we obtained tissue samples from the frontal, temporal, and occipital lobes of the cerebrum, mesencephalon, cerebellum, pons, medulla oblongata, cervical and lumbar intumescences of the spinal cord, and biceps brachii and quadriceps femoris muscles. The formalin fixed samples were routinely embedded in paraffin wax, sectioned at 4μm thickness, and stained with hematoxylin

was observed (Fig. 1A, B). There were congenital defects of the abdominal wall and hernia of the abdominal organs, which were covered by the peritoneum instead of skin. The fetus was male, weighed 1,350g, and had fur with white spots (Fig. 1A, B). Because body size of sika deer varies according to geography [10], we used a formula for estimating gestational age that was based on the deer population inhabiting Tochigi Prefecture [11]. According to this estimation, the gestational age was 174 days (normal gestation period: 216-260 days [12-13]). The fetus was frozen and transported to Nippon Veterinary and Life Science University in Tokyo, Japan, where we performed computed tomography (CT: Aquilion PRIME/Beyond Edition, Toshiba Medical Systems Corporation, Tokyo; 120 kV, 300 mA). The internal organs of the fetus were subjected to gross pathological examination after fixation in 10 % formalin.

CT revealed multiple skeletal deformities (Fig. 2), the most notable of which was severe jackknife-like kyphosis. To our knowledge, no such deformity has previously been reported in cervids. The spine was markedly bent below the sixth thoracic vertebra, and spina bifida occulta was diagnosed (Fig. 2A). Bone adhesion between the sixth and seventh thoracic vertebrae and malformation of the ribs were also observed (Fig. 2A). In addition, the skull was hypoplastic (Fig. 2B). Gross pathological examination revealed a cleft palate (Fig. 3A) and a cleft muzzle (Fig. 3B). Excess dorsiflexion of digits without ankylosis was present in the right forelimb (Fig. 3C), presumably due to the attitude. In the brain, remarkable third and lateral ventriculomegaly was observed (Fig. 3D). Although all internal organs were present, imperforate anus (Fig. 3E) and aberrant lobulation of the lung was observed (Fig. 3F). The lungs had hypoplasia with abnormal lobulation in both caudal lobes. The accessory lobe extended narrowly toward the caudal end.
Fetus malformation in sika deer

Fig. 3 Several abnormalities are observed in the fetus.
(A) Ventral view of the skull. The arrows show the cleft palate. (B) Cleft muzzle. (C) The arrow shows excess dorsiflexion of digits without ankylosis in the right forelimb, presumably due to the attitude. (D) Remarkable ventriculomegaly of third (the upper figure) and lateral ventricles (the lower figure) of the brain. Samples were collected for pathogen detection from the points indicated by the arrows. (E) The inguinal region showing an imperforate anus. The arrow shows the original location of the anus, which is covered with skin. (F) Ventral views of the lung showing aberrant lobulation. The arrows show hypoplasia with abnormal lobulation in both caudal lobes. The arrowheads show the accessory lobe extended narrowly toward the caudal end. The ruler is calibrated in centimeters.

and eosin. Microscopically, enlargement of the cerebral ventricles and focal fat displacement in the biceps brachii muscle were observed.

Next, we looked for the presence of pathogens by molecular biology to determine whether they might have caused congenital malformation. Although such pathogenicity is not known in deer, fetal malformation in ruminants could be caused by infection with Akabane virus (Peribunyaviridae orthobunyavirus), Aino virus (Peribunyaviridae orthobunyavirus) [14], Chuzan (Kasba) virus (Reoviridae orbivirus) [15], bluetongue virus (Reoviridae orbivirus) [16], bovine viral diarrhea virus (BVDV) (Flaviviridae pestivirus) [17-18], and Neospora caninum [19]. We collected tissue samples from the cerebrum, cerebellum, thoracic spinal cord, and back and right hind thigh muscles of the fetus which was fixed with 10% formalin. Then, 0.2 g of each samples in 1 mL of PBS were treated with proteinase K (Qiagen, Hilden, Germany), homogenized using TissueLyser LT (Qiagen), and centrifuged. For the detection of Akabane virus, Aino virus, Chuzan virus, bluetongue virus, and BVDV, RNA was extracted using ISOGEN II (Nippon Gene, Tokyo, Japan). The RNA was reverse transcribed with random primer oligonucleotides using ReverTra Ace (Toyobo, Osaka, Japan) and amplified using KOD One PCR Master Mix (Toyobo). For the detection of Neospora, DNA was extracted using DNeasy Blood and Tissue Kit (Qiagen). The DNA was amplified using KOD One PCR Master Mix. The PCR primers used were previously described [20-23]. The presence of amplified products was confirmed by gel electrophoresis.

We did not detect any pathogens, so a pathogenic cause for the congenital malformation could not be confirmed. In histopathological examination, there were also no other findings associated with virus infection such as loss of motor neurons of the ventral horns in the spinal cord. Therefore, it is possible that the congenital malformation occurred due to a genetic mutation, but we could not rule out the possibility of a pathogenic cause for the following two reasons. First, damage to nucleic acid resulting from formalin fixation might have interfered with genomic analysis. Second, it might not have been possible to detect the pathogen because some pathogens do not remain in the body for a long time. Although we did not
find evidence of the infection in this case, wild deer are at risk of infection by pathogens that cause congenital malformation in ruminants. Invasion of sown grasslands by wild deer has been well documented [24-25]. Because the pathogen we assessed in this study is frequently detected in grazing cattle [26], the deer using sown grasslands may have a higher risk of infection. Further investigation is needed to elucidate the potential risk of infectious disease in wild deer populations.

It is notable that such a severely malformed fetus continued to develop in late gestation. Finding severe congenital malformations in free-ranging wildlife is extremely rare because most such fetuses are aborted, still-born, or die immediately after birth. Therefore, it is difficult to identify the cause and the prevalence of such malformations in free-ranging wildlife. As mentioned above, deer populations are now actively being culled all over the world, including Japan. Therefore, it is easier to collect the carcasses of deer compared with other wild mammals. Constructing a system to monitor culled pregnant does would enable the collection of data on congenital malformations in wild deer. Further investigation will aid our understanding of congenital malformation in free-ranging wildlife. Moreover, the accumulation of reports should also aid the early detection of the causes of outbreak of congenital malformations when they occur in the future.

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症例報告 病理学

野生下における重度のジャックナイフ様脊柱後弯を伴うニホンジカ（Cervus nippon）奇形胎仔の一例

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要約

野生ニホンジカ（Cervus nippon）の胎内から奇形胎仔を発見した。コンピュータ断層撮影・解剖・病理検査を行った結果、重度のジャックナイフ様脊柱後弯等の骨格異常、白線の離開と腹部臓器の腹壁外への脱出、口蓋裂、鼻裂、右前肢指骨の過剰背屈、第3および側脳室の拡大、肺の分葉異常、鎖肛がみられた。反芻動物で胎仔奇形を引き起こす病原体は検出されなかった。本研究は野生ニホンジカで重度の奇形胎仔を記録した初めての例である。

キーワード：胎仔、奇形、ニホンジカ

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