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A Case of Vertebral Malformation in a Slow Loris

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Abstract The serious malformations of the thoracic vertebrae and ribs were found in a hybrid slow loris (NSMT-M 31600) born in a zoo. Hemicentrums, incompletely divided centrums, and fused centrums were found. The vertebral distortion resulted in rib fusions, scoliosis, and ankylosis. These abnormalities closely resemble the typical symptoms of complex vertebral malformation (CVM), which is caused by a genetic defect and which occurs in association with extensive interbreeding. This is a rare case in that the vertebral malformation resembling CVM occurred in a slow loris born in a zoo in which extensive inbreeding was artificially avoided.

Key words: slow loris, complex vertebral malformation, thoracic vertebrae, rib, hemicentrum, scoliosis, ankylosiss.

Introduction

Complex vertebral malformation (CVM) is known to occur in domestic animals, and various cases such as agenesia of caudal vertebrae, hemicentrums, butterfly vertebrae, scoliosis, kyphosis, lordosis, and torticollis, has been reported (Oksanen, 1972; Boyd and McNeil, 1987; Leipold *et al.*, 1970, 1993; Greene *et al.*, 1973a, 1973b, 1974; Vaughan *et al.*, 2000). Concomitant malformation of the internal organs often occurs in Holstein cattle (Agerholm *et al.*, 2001). Although most studies of CVM have involved cattle (Bovidae), the syndrome seems to occur in various mammalian taxa. For example, hemicentrums have been reported in dogs (Carnivora) (Grenn and Lindo, 1969), foals (Perissodactyla) (Kirberger and Gottschalk, 1989), and goats (Bovidae) (Rowe, 1979). Additionally, congenital caudal vertebral malformation has been reported in mice (Rodentia) (Murakami and Kameyama, 1964) and alpaca (Vaughan *et al.*, 2000).

CVM syndrome is correlated with a failure in notochord, somite, or neural tube formation (Theiler, 1988; Burbidge *et al.*, 1995) caused by a genetic defect (Bendixen *et al.*, 2002; Nielsen *et al.*, 2003). Bendixen *et al.* (2002) and Nielsen *et al.* (2003) demonstrated that the vertebral malformation is inherited recessively. Agerholm *et al.* (2001) also demonstrated that CVM syndrome occurred when extensive interbreeding was performed.

A substantial amount data on CVM syndrome in domestic animals is available, however, there are few reports involving wild and zoo animals. Agerholm *et al.* (2001) suggested that the appearance of CVM was expected in cattle and other domestic animals because of widespread use of semen from elite sires. In contrast to domestic animals, serious interbreeding rarely occurs in wild populations. Furthermore, the syndrome is difficult to identify even when it occurs in wild populations. Animals kept in zoos are under breeding control, thus, the syndrome is not expected to appear. We herein describe a case of the vertebral malformation in a slow loris resembling CVM that had been born in a zoo. We also discussed the background of the appearance of the syndrome.

Materials and methods

The vertebrae and ribs of a male slow loris (NSMT-M 36100) were examined. The loris was born in Ueno Zoo, Tokyo, Japan, on 3 April 2008 and died of pneumonia on 14 May 2008. Its body length and weight were 110 mm and 63.5 g, respectively, at the time of death. This loris was a hybrid between a male Sunda slow loris, *Nycticebus coucang* (Boddaert, 1785) and a female Bornean slow loris, *Nycticebus menagensis* Trouessart, 1898. Because Bornean and Sunda slow lorises were historically regarded as the same species (Groves, 2001), these two species had been kept in the same cage at Ueno Zoo and their breeding resulted in the birth of the above-mentioned hybrid loris. Recently, they were determined to be as distinct species (Nekaris and Munds, 2010).

The loris was dissected for necropsy at Ueno Zoo. The dissected body without the inner organs was donated to the National Museum of Nature and Science (Tokyo, Japan). The specimen was prepared using common hide beetles (*Dermestes maculatus*) because soft tissue removal can be controlled by adding or excluding the beetles. The preparation was finished before the centrum and neural arch of the vertebrae were separated from each other. The skull, four limbs, and four ribs on the right side were separated from the vertebral column, and the malformations of the vertebrae and ribs were discovered.

The external shape of the vertebrae and ribs was macroscopically observed. The internal structure of these bones was observed using a microcomputed tomography scanner (LA Theta LTC-100; Hitachi-Aloka Medical, Ltd., Tokyo, Japan). The slices were taken at 0.06-mm intervals, and constructed in three dimensions using OsiriX ver. 4.1.2 software (Pixmeo Bernex, Switzerland). Cross-sectional images were created from the three-dimensional images using the same software.

Results

External appearance of the vertebrae and ribs

Malformations were found in the ribs and thoracic vertebrae (Fig. 1a). Some neural arches of

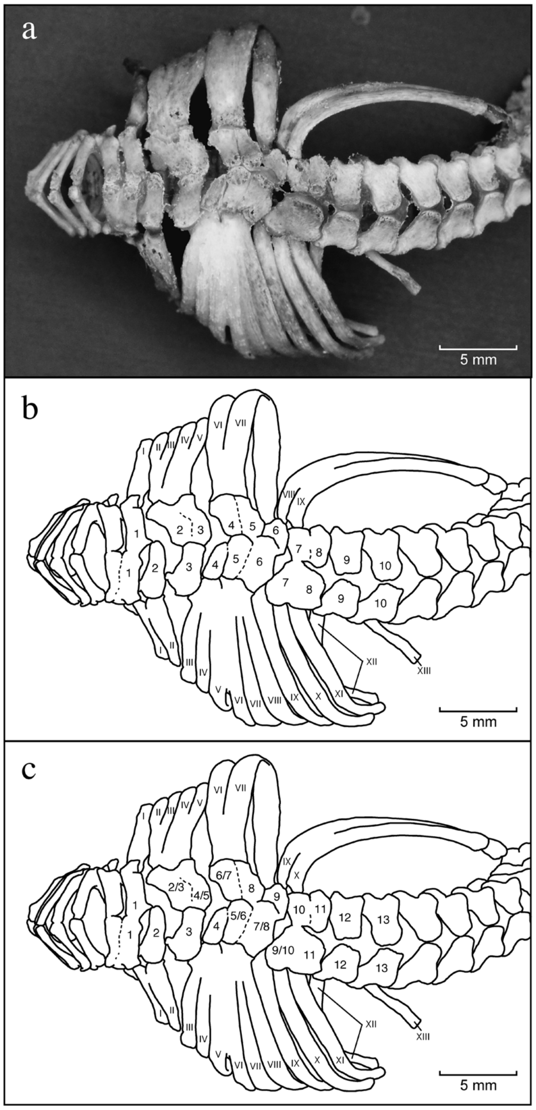

Fig. 1. Dorsal view of the vertebral column and ribs of the slow loris (NSMT-M 36100). Figures 1b and 1c show the tracing of the vertebrae and ribs in 1a. In 1b, the thoracic vertebrae and ribs are numbered from cranial to caudal. In 1c, each thoracic vertebra was given a number corresponding to the rib number. Arabic numeral, rib number; Roman numeral, thoracic vertebra number.

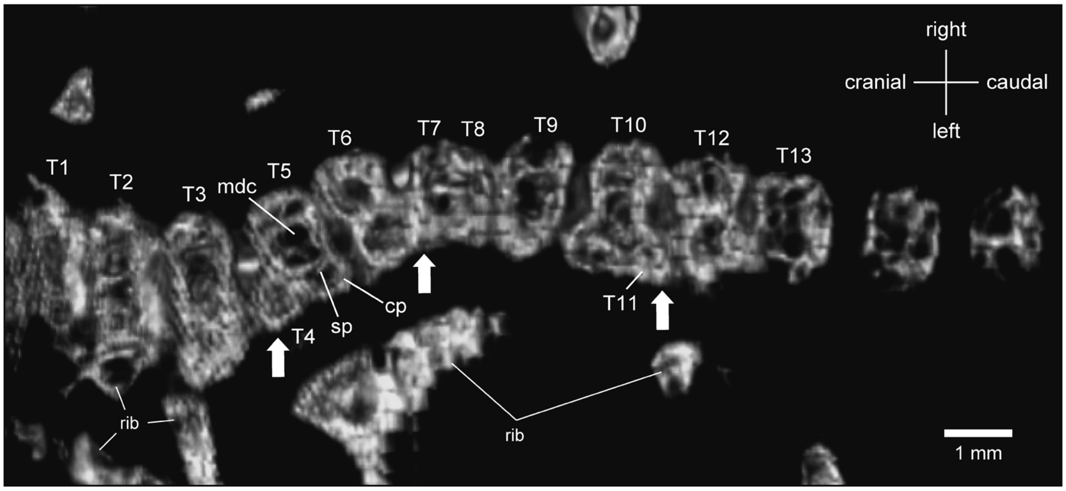

Fig. 2. Horizontal section of centrums of the thoracic vertebrae. Arrows show the hemicentrum. Abbreviations: cp, compact bone; mdc, medullary cavity; sp, spongy bone.

the thoracic vertebrae were severely fused to one another. Therefore, some boundaries between two or more vertebrae were not discernable. The seventh cervical and the first thoracic vertebrae were also fused along the left half of their neural arches. Some ribs were severely fused. Fusion was noted from the head to the middle portion of the right 2nd to 5th ribs, right 6th to 7th ribs, and left 3rd to 8th ribs (Fig. 1a). The left 1st to 2nd ribs, left 9th to 11th ribs, and right 8th to 9th ribs were fused in the neck region (Fig. 1a).

The total numbers of ribs and thoracic vertebrae were inconsistent (Fig. 1b, c). Numbers were sequentially assigned to the ribs and thoracic vertebrae from the cranial region, resulting in thirteen ribs and ten thoracic vertebrae on the left side (Fig. 1b). On the right side, however, only nine ribs were connected to the vertebral column, and the remaining four ribs had fallen out during specimen preparation. In total, therefore, thirteen ribs and ten thoracic vertebrae were present on the right side. When the thoracic vertebrae were assigned numbers consistent with the rib numbers, there were a few gaps between the left- and right-half portions of the vertebrae (Fig. 1c). The left 2nd and 4th vertebrae had no paired right vertebrae. The left 3rd vertebra was paired with the 2nd to 5th vertebrae on the right side. The right 10th vertebra was connected to the left 9th and 10th vertebrae.

Internal structure of the thoracic vertebrae and ribs

A horizontal section of the centrums of the thoracic vertebrae is shown in Fig. 2. The thoracic vertebrae exhibited distorted shapes. The third centrum exhibited elongation of compact bone in the lateral direction. The 5th and 10th centrums were Y-shaped, due to the fusion of a hemicentrum and a normally shaped centrum. Each centrum constructing the 5th centrum contained the basic inner structure of a normal centrum, including compact bone, spongy bone, and a medullary cavity, therefore, the two centrums were easily distinguished from each other. On the other hand, the 10th centrum was unclearly divided into two parts. The 7–8th centrum also seemed to be incompletely divided into two parts. The part constructing the anterior half of the 7–8th centrum was a hemicentrum, while the caudal half was normal. Fusions also occurred in the 6th to 9th centrums and 10th to 12th centrums. These fusions resulted in vertebral ankylosis, and scoliosis occurred secondary to the distorted centrum and ankylosis.

The total number of vertebrae, which was

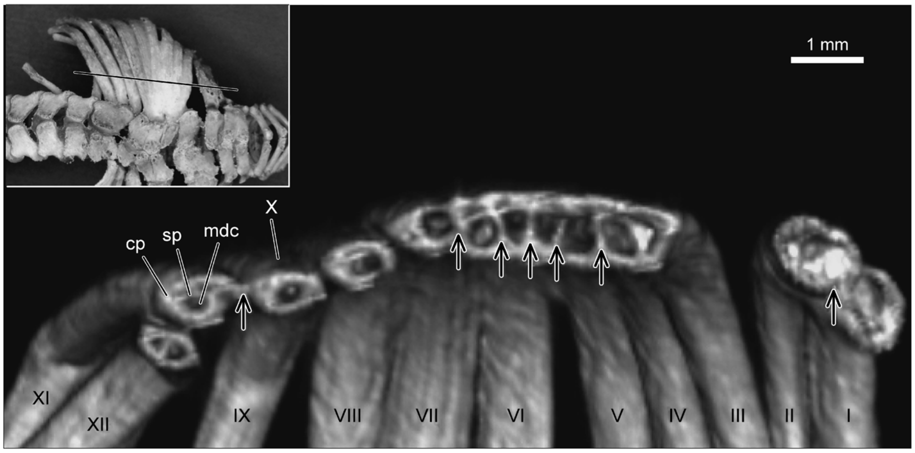

Fig. 3. Vertical section of ribs on the left side. Arrows show compact bone. Roman numerals indicate the thoracic vertebra number. The line shown in an upper left picture indicates the position of the section. Abbreviations: cp, compact bone; mdc, medullary cavity; sp, spongy bone.

uncertain according to the external appearance, was actually 13 when the hemicentrum was counted as a centrum.

The ribs were also sectioned vertically (Fig. 2). Grossly, although the ribs were tightly fused to one another, they were distinguishable from one another. Each rib possessed the basic inner structure of a normal rib (compact bone, spongy bone, and a medullary cavity). Fusions only occurred in compact bone.

Discussion

Here, we described a slow loris (NSMT-M 31600) that exhibited serious malformations in the thoracic vertebrae, including hemicentrums, incompletely divided centrums, and fused centrums. These distorted vertebrae caused scoliosis and ankylosis. In addition, the total number of 13 thoracic vertebrae found in this specimen is uncommon because the slow loris (*Nycticebus* species) normally has 16 thoracic vertebrae (Ankel-Simons, 2007). According to Zelop *et al.* (1993) and Morimoto *et al.* (1995), hemicentrum formation is related to failed notochord formation during the early fetal period. Vaughan *et al.* (2000) also stated that fused vertebrae caused by segmentation failure leads to scoliosis. Agerholm *et al.* (2001) determined vertebral malformation caused by failed segmentation to be CVM. Therefore, it is likely that the vertebral malformation found in NSMT-M 31600 originated from a failure in notochord or somite formation and was associated with the typical symptoms of CVM caused by a genetic defect (Theiler, 1988; Burbidge *et al.*, 1995; Agerholm *et al.*, 2001, 2004). On the other hand, serious fusions of several ribs may not be caused by a genetic defect. In the present case, the fusions only involved the surfaces of the ribs, and all ribs retained their basic internal structure. Therefore, such fusions seem to occur secondarily due to a lack of space for rib growth in CVM syndrome. Agerholm *et al.* (2001) also reported that in some cattle with CVM, the fusions of the proximal and medial portions of the ribs coincided with each other.

It is known that CVM is recessively inherited (Bendixen *et al.*, 2002; Nielsen *et al.*, 2003). This syndrome has been reported in inbred domestic and experimental animals (Murakami and Kameyama, 1964; Grenn and Lindo, 1969; Oksanen, 1972; Greene *et al.*, 1974; Rowe, 1979; Vaughan *et al.*, 2000; Agerholm *et al.*, 2004). Agerholm *et al.* (2001) also demonstrated that CVM in Holstein appeared in the 5th to 7th generation when extensive interbreeding was performed. Therefore, the present case is unusual in that the vertebral malformation resembling CVM occurred in a slow loris born in a zoo in which extensive inbreeding was artificially avoided. Furthermore, the loris was a hybrid between a wild Sunda slow loris and a wild Bornean slow loris, which are genetically distant relatives. These facts imply that certain numbers of carriers of the recessive gene that causes CVM are present in wild populations of Sunda and Bornean slow lorises.

According to IUCN reports (Nekaris and Streicher 2008a, b), the populations of Sunda and Bornean slow lorises are decreasing. The Sunda slow loris is distributed in Indonesia (Sumatra, Batam and North Natuna Islands), Malaysia (on the peninsula and island of Pulau Tioman), Singapore, and southern peninsular Thailand (Groves, 2001). However, its presence is patchy through Peninsular Malaysia (Nekaris and Streicher, 2008a), where this species occurs at very low densities (Barret, 1981; Rudd and Stevens, 1994). The Bornean slow loris is distributed in Brunei, Indonesia (Kalimantan Borneo, Belitung, and Banka), Malaysia (Sabah and Sarawak, Borneo), and the Philippines (Fooden, 1991; Timm and Birney, 1992). In the Philipinnes, however, this species has a very limited distribution (Dagosto and Gebo, 1995; Heaney *et al.*, 1998). In Kinabalu Nation Park, Malaysia, it is rarely seen (Wells *et al.*, 2004). Nekaris *et al.* (2008) also found that *Nycticebus* species are abundant in some areas but genuinely rare in others. Although few data on genetic diversity in the Sunda and Bornean slow lorises are available, it is considered that these species may be prone to cause some genetic biases because of their patchy habitats. Therefore, it is supposed that the herein-described loris, which was born in a zoo and was disconnected from the natural environment, may reflect the genetic poor diversity in wild populations.

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