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MORPHOLOGICAL OBSERVATION ON TWO CASES OF ACARDIUS AMORPHUS IN HOLSTEIN-FRIESIAN CATTLE

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It is well-known that acardius amorphus, a special kind of double monster, is the most severe one among various monsters. In foreign countries, it has been reported that the anomaly is present both in humans and in farm animals. In the veterinary area acardius amorphus was first reported by GURLT (1832) to occur in cattle. Afterwards many investigators described this type of malformation in cattle^{1, 8, 11, 15, 21, 25}, horses^{4, 19, 24}, sheep²² and goats^{8, 21}, especially its occurrence being most frequent in cattle. In Japan, up to now, only 7 cases of acardius amorphus in cattle have been reported by YOSHIDA (1938), OHBAYASHI (1951), OYAMADA (1953), MOTOHASHI (1955), INOBUCHI (1955), HIGAKI & NISHIDA (1955) and MIYAGAKI & HARA (1959).

Morphologically, these monsters usually had skin, blood vessels, connective tissue, fat tissue, muscle, some kind of glands, cartilage, bone, nervous tissue, etc. Furthermore, in some cases, they had also an imperfect tongue, oral cavity, digestive organs or their vestiges, etc. The development of these organs or tissues varied from case to case, and they were characterized by having no heart or only a vestigial one.

In recent years, the present authors encountered two cases of this type of monster in Holstein-Friesian cattle. One of them was very interesting and such as has never been described in the literature. This short report outlines the morphological findings of these two cases.

METHODS

After having been fixed with 10 per cent formol solution, specimens were measured, radiographed and examined macroscopically. Some sections were made from them and stained with hematoxylin-eosin for microscopical examination.

DESCRIPTIONS

Case No. 1

1. Clinical history

This case was obtained at Sapporo, June 25, 1957. It was born together with a normal

female calf. The mother was a fourteen-year-old Holstein cow of which the previous breeding history was not known in detail but was probably almost normal. The duration of gestation was normal, 284 days.

At parturition, the co-twinning normal calf was helped by a veterinarian as the fetal position was slightly abnormal. When the normal calf's head came out, the monster was palpated in the maternal vagina as a jellyfish-like material completely enveloped in an amnion-like membrane. The fetal membrane of the normal calf was retained, so it was removed manually after 2 days. The post-parturient period passed normally and the estrus came again after nearly 2 months. She was conceived by the first insemination and delivered a normal male calf the next spring.

2. Macroscopical findings

1) General appearance and covering membrane The monster was completely covered with a highly edematous smooth amnion-like membrane. The whole appearance was discoid in shape and $24 \times 23 \times 4$ cm in size, weighing about 700 g. The covering membrane slightly protruded upward at the central portion in which the monster was located, having a slender umbilical cord-like structure of 30 cm in length.

When the covering membrane was cut, a small quantity of transparent fluid came out and the monster was found attached closely to the bottom of the covering membrane. The thickness of the membrane was 0.3 to 0.8 cm, and its inside surface was, in general, smooth but partially thickened, showing scab-like or moss-like appearances. In some parts, these structures had even a few hairs and were closely similar to the skin in appearance. The main blood vessel, only 0.5 cm in diameter, originated from the umbilical cord-like structure and penetrated through the monster, then extended over on the covering membrane with many small branches.

2) The monster Macroscopically the monster was slightly flattened and ovoid in shape, $7.5 \times 6.4 \times 3.8$ cm in size, 200 g in weight, closely attached to the bottom of the covering membrane. It was a mass of tissue with white hairy skin, giving an elastic or muscular sensation to the touch. The body had a small opening, highly resembling the oral structure, in which a tongue-like structure was seen.

The cross section of the body showed well-developed skin, 0.3 to 0.6 cm in thickness, at the outer layer. Abundant grayish white connective tissue accompanied by yellowish gray fat tissue mainly occupied the middle layer. The central portion of the monster was also composed of connective tissue and fat tissue, being dark brown or yellowish brown in color. In addition, a small piece of bone, $1.5 \times 1.2 \times 1.0$ cm in size, and two small cavities, $1.7 \times 1.7 \times 1.5$ cm and $2.1 \times 1.8 \times 1.7$ cm in size, respectively, were observed. These cavities containing a small quantity of yellowish brown colored fluid communicated with each other by a narrow pass. The inside surfaces of the cavities were similar to mucosal membrane. Especially, one of the cavities had many small folds and papilla-like structures, which covered the inside surface of the connective pass and extended to a part of the other cavities. On another cut surface of the monster, there were two chocolate colored round small tissue structures, 0.9 and 0.8 cm in diameter, respectively.

3. Radiological findings

The radiogram of the monster clearly showed some shadows corresponding to the small bone and the small cavities described above. The shadow of the bone showed that the tissue

was composed of several pieces of bones with different X-ray permeabilities.

4. Microscopical findings

1) The covering membrane Generally, the inside and outside surfaces of the covering membrane were covered with a simple layer of cuboidal or columnar epithelium. The stroma of the membrane consisted of highly edematous coarse connective tissue. Thus, the membrane was very similar to amnion. However, in some parts of the inside surface where the scab-like or moss-like appearances were observed macroscopically, almost normal skin structure developed.

2) The monster In the skin of the monster, the epidermis was stratified squamous epithelium, of which the external layer was cornified. The subcutaneous tissue was mainly occupied with well-developed connective tissue. There also existed many hair roots, sebaceous glands, fine pilomotor muscles and fat tissue.

The tongue-like structure possessed secondary papillae, but lacked taste buds. At the portion near the tongue well-developed glandular tissue was observed, but its origin could not be determined. The inside surface of the smaller cavity was covered with stratified epithelium and two or three layers of smooth muscle surrounding it. While the inside surface of the larger cavity had a single layer of columnar epithelium, ill-developed glandular tissue was noticed in its deep layer. Therefore, the histological findings of the larger cavity were closely similar to those of the small intestine. The sections from the chocolate-colored round tissue structures revealed hemangiomatic capillary network structures filled with blood. In the connective tissue surrounding these structures, there were many cellular foci mainly consisting of neutrophils and lymphocytes. But embryonal hematopoiesis was found in no parts of the monster. A number of striated muscular fibers and of small blood vessels were seen here and there in the connective and fat tissues. In some parts, several nerve bundles were noted. However, arrangement of these structures was very irregular.

The microscopical finding of the bone corresponded to that of the embryonal osseous development, showing irregular arrangement of fibrous bone trabeculae and cartilaginous elements without bone marrow. In addition, it seemed not to be a simple bone, but to be a complex of several small bones in different stages of development.

Case No. 2

1. Clinical history

This case was obtained at Eniwa in the vicinity of Sapporo, December 7, 1958. Its father was a normal three-year-old Holstein bull and its mother an eight-year-old Holstein cow which had never produced any kind of monsters in five previous parturitions. The period of gestation was normal, 283 days. This monster came out with a fetal membrane 5 hours after delivery of a co-twinning normal male calf.

2. Macroscopical findings

The monster was almost spherical, but slightly flattened in shape, 10.5×9.8×6.1 cm in size, weighing 455 g. It had an umbilical cord of 8 cm in length and of 1.0 cm or so in diameter. The monster was entirely covered by skin with almost black dense hairs of 1.0 to 3.3 cm in length, but a narrow stripe of white hair surrounded the body from the dorsal side to the ventral. The body had three small projections on its surface approximately from soya bean size to

thumb head size. Additionally, a hairless clitoris-like small structure and a very small oral-like opening were seen near the part of the umbilical attachment.

On the cut surface of the monster, the skin measured 0.7 to 1.2 cm in thickness, under which light gray colored connective tissue was mixed in light yellow colored fat tissue, giving somewhat a mosaic structure. These tissues abounded in small blood vessels. On the cross section of the umbilical attachment, the umbilical connective tissue penetrated into the central portion of the monster to a depth of nearly 1.3 cm. The clitoris-like projection contained two soya bean sized tissues of dark brown color. Light yellowish opaque hard tissue, 0.3 cm in diameter, which seemed to be osseous or cartilaginous tissue, was noted on the cut surface through the small opening.

3. Radiological findings

The radiogram of this case showed a distinct shadow corresponding to the above mentioned osseous or cartilaginous tissue.

4. Microscopical findings

Histologically, the monster was covered with cornified, stratified squamous epithelium. The well-developed submucosa contained many hair roots and hair sheaths. The stroma of the deep layer was mainly composed of abundant coarse connective tissue and well-developed collagenous fibers and in some places ill-developed fat tissue and many capillaries were observed here and there. The microscopical examination of the dark brown colored small tissues showed lymph-node-like structures which were observed separated into several lobules by the connective tissue. Each lobule contained a number of cells which had undefined cell membranes and various sized nuclei with abundant chromatin. At the clitoris-like structure, under the cornified epidermic layer there was the corium composed of well-developed coarse connective tissue, where a number of hair follicles with ill-developed sebaceous glands and pilomotor muscles were seen. At the oral-like structure, there was no remarkable finding except that the stratified squamous epithelium contained melanin pigments. In the section preparation of the umbilical cord, no remarkable abnormality was observed. The histological examination of the light yellowish opaque hard tissue revealed cartilage.

DISCUSSION

Etiologically, there are two principal theories which have been discussed about the development of an acardius amorphus; one is the germ defect theory which explains that the monster would probably be due to a defective ovum or abnormal division of a normally fertilized ovum (MECKEL, HEIJL and KRÜGER), the other is the blood vessel anastomosis theory in monozygotic twins, in which it is believed that in monozygotic twin pregnancy the umbilical vessels of the two embryos sometimes make anastomosis with each other and a weak embryo of the twin will be incompletely nourished by the blood of the umbilical artery of the other embryo, so that the heart of the former would be disturbed in development and caused to degenerate in the very early embryonic stage (CLAUDIUS, SCHATZ and CRAIG). In addition to the anastomosis theory, OHBAYASHI

and MOTOHASHI separately stressed that disturbance of circulation through the ill-developed slender funiculus might also be an important factor eliciting the development of the monster.

On the other hand, YOSHIDA reported an interesting case of acardius amorphus which developed under the condition of superfetation. According to his description, a Holstein cow was mated with a Jersey bull and then after a week she was coupled again with a Holstein bull. At the term, she delivered a normal female calf of mixed breed with dark brown hair and at the same time an acardiac monster with the black and white hair coat characteristic of the Holstein breed. This fact has led to the assumption that an acardiac monster would be able to develop also from dizygotic twins. TIEGEL also described a bovine triplet case which consisted of one normal calf and two acardiac monsters, the hair coats of which were different in color from one another. He cited additionally WEBER's similar case in which a male acardiac monster was delivered together with two intersex calves. On the basis of these facts, TIEGEL suggested that acardiac monsters would also be able to develop in the case of fraternal twins.

According to ARTHUR, an acardiac monster has usually afferent and efferent blood vessels, connecting with the allantois of the normal fetus, and it probably attaches to the inside surface of the fetal membrane of the normal fetus, which is to be liberated from the membrane at birth. In the present authors' cases, just the same as in those of many other authors, no direct proof of connection with the normal fetus could be obtained. However, it must be emphasized at least that in the present case No. 1 the monster was covered completely with a highly edematous membrane resembling the amnion. Such a case has never been reported in the literature except some reports in which adhesions between fetal membrane and umbilical area were described by MÜLLER and TIEGEL, respectively.

In addition, it is very interesting that in case No. 1, metaplasia occurred on some parts of the inside surface of the amnion-like covering membrane, where almost normal skin structure existed even with some hairs. A similar description can only be found in MÜLLER's report in which he wrote that the external layer of the epithelium of an amnion-like membrane was cornified.

Acardiac monsters are various in form, but in general, they are spherical or ovoid in shape. Their weights have also a wide variation as shown in TIEGEL's 21 cases ranging from 160 to 2,440 g and in an exceptional large case of ALBRECHT weighing 5,000 g. Most of the cases in our country including those of the present authors were comparatively small ones as shown in the table.

Likewise, the internal morphology of acardius amorphus cases is never identical, and is not always parallel to the external one; each element composing

TABLE Outline of *Acardius Amorphus* Cases in Japan

	BREED OF PARENTS	SEX OF CO-TWINNED CALF	SIZE AND FORM	WEIGHT	HAIR COLOR	OPENING AND TONGUE	MUSCULAR TISSUE	OSSEOUS TISSUE	BLOOD VESSELS	NERVOUS TISSUE	OTHER TISSUES OR VESTIGES
YOSHIDA (1938)	F: { Jersey Holstein M: Holstein	♀	7.4×7.9×4.5 cm ovoid globe	g	black and white	anus-like opening, tongue-like structure	+	+	+		vestige of lung, canal structure containing desquamated epithelial cells
OHYAYASHI (1951)	F: Holstein M: "		12×9×5.5 ovoid		black and white		striated M. smooth M.	with bone marrow	+	nerve bundle, ganglion	hematopoiesis
OYAMADA (1953)	F: Holstein M: "	♂	11×14×4.4 flattened globe		black and white		+	+	+		
MOTOHASHI (1955)	F: Japanese Black Polled Cattle M: "	♂	13.7×10×7 flattened ovoid	360	black	oral-cavity-like opening	striated M. smooth M.	with bone marrow	+	nerve bundle, ganglion	hematopoiesis
INOBUCHI (1955)	F: Holstein M: "	♀	14×6×5		black and white		+	+	+		
HIGAKI & NISHIDA (1955)	F: Holstein M: "	♀	11×6	390	black and white		+	with bone marrow	+		
MIYAGAKI & HARA (1959)	F: Japanese Black Cattle M: "	♂	9×6.5×5 flattened ovoid	144	light brown black	opening	striated M.	+	+	-	hematopoiesis
KOJIMA & KAWATA No. 1	F: Holstein M: "	♀	7.5×6.4×3.8	200	white	oral-cavity-like opening with tongue-like structure	striated M. smooth M.	embryonic ossification without bone marrow	+	nerve bundle	small-intestine-like cavities, glandular tissue, capillary nets formation
No. 2	F: Holstein M: "	♂	10.5×9.8×6.1 flattened sphere	455	black and white	oral-cavity-like opening	+	cartilage	+	-	clitoris-like projection, lymph-node-like tissue

NOTES: F: Father
M: Mother

the monster varies quantitatively and qualitatively, especially in the developmental stage, as has been pointed out by OHBAYASHI. Generally, the surface of the body is covered with thick skin with hairs, sebaceous glands and pilomotor muscles. In OHBAYASHI's case, no sebaceous glands nor pilomotor muscles could be found. As for supporting tissues, there are usually bones, cartilages, muscles, collagenous fibers and elastic fibers, etc., and in most cases fat tissues, too. However, existence or developmental degree of these supporting tissues was generally various. For example, in not a few cases the osseous tissues were not observed as was reported by TIEGEL, CURSON and MIYAGAKI & HARA.

Radiologically, SCHMINKE examined bones in 15 cases of acardiac monsters including 10 cases of acardi amorphii in domestic animals, and he discussed the origin of these bones. As for acardius amorphus, however, it seems to be very difficult to distinguish the origin of the osseous tissues, because the development of these tissues remained in very early stages. Though TIEGEL reported a rare case having cerebrospinal tissue, this case seems to be exceptional; most of all acardiac monsters have generally not so highly developed nervous system except nerve bundles or ganglia.

As for the circulatory system, lack of the heart is characteristic of these monsters, but blood vessels and capillaries are generally abundant. In some cases lymph nodes or hemolymph nodes are observed. The digestive system including oral cavity, teeth, stomach, intestine, tongue, etc. is commonly encountered, but its degree of development is various. In one of the present authors' cases, a comparatively well-developed tongue, an oral-like structure with an associated gland and small cavities resembling the small intestine were observed. The respiratory, urinary, genital or endocrine systems seem to be uncommon.

It is interesting that several cases of acardius amorphus in Holstein-Friesian breed reported by YOSHIDA, OHBAYASHI, OYAMADA, HIGAKI & NISHIDA, INOGUCHI and CURSON had black and white hair, similarly to the present case No. 2, while case No. 1 was covered with only white hair. From the standpoint of heredity, MOTOHASHI made a detailed investigation on the hair-slopes and the location of hair whirls of the monster, and compared with those of the twin-born normal calf, but he could not observe any distinct similarity. In the present authors' cases, there existed a hair whirl and 2 hair-slopes in case No. 1, and 5 whirls in case No. 2, but unfortunately comparison to the co-twinning calves could not be made.

On the other hand, among 8 cases listed in the table, no tendency was noted in the sex ratio of the co-twinning normal calves, but this should not be stressed as a conclusion because of the inadequate number of cases observed.

In conclusion, various different opinions have been offered for classification

of acardiac monsters; classification is founded on the general appearance of the monster, and on the arrested development of the heart or of the bone. OGATA set forth a definite classification on the basis of the development of bone structure, in which acardiac monsters were classified roughly into three groups, acardius holosomus, acardius hemisomus and acardius amorphus. Acardius amorphus was subdivided into acardius amorphus externus and acardius amorphus totalis; the former is one with a comparatively well-developed bone structure, and the latter is one without well-developed bone or with only microscopically detectable osseous tissue. According to OGATA's classification which has been widely cited in Japan, the present authors' cases belong to the category of acardius amorphus totalis, just the same as the cases of CURSON, MÜLLER and many Japanese authors listed in the table.

SUMMARY

Two cases of acardiac monsters obtained from Holstein-Friesian cows were examined radiologically, macroscopically and microscopically. These monsters were each co-twinning with a normal calf. They were approximately spherical or ovoid in shape, weighing 200 g and 455 g, respectively, and covered with hairy skin. One of them was an interesting rare case which had never been reported in the literature: This case was covered completely with highly edematous amnion-like membrane, in some parts of the inside surface of which metaplasia had occurred, showing there almost normal skin structure. In addition, this case possessed an oral-like opening with comparatively well-developed tongue-like structure, a small bone and two small cavities resembling rudimentary digestive organs. Also, in the other case, a very small oral-like opening and a small cartilage were observed.

Histologically, both cases were composed mainly of connective tissue, fat tissue and striated or smooth muscles, but the degree of development in these tissues and organs remained at the embryonal stage, and their arrangement was irregular. According to OGATA's classification of acardiac monster, these two cases belong to the category of acardius amorphus totalis.

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EXPLANATION OF PLATES

PLATE I. Case No. 1

- Fig. 1. General appearance: Amnion-like edematous membrane entirely covers the monster, and slender umbilical cord attaches to membrane (scale: cm).
- Fig. 2. Dorsal view of monster and metaplasia occurring at inside surface of membrane (scale: cm).
- Fig. 3. Oral cavity and tongue.
- Fig. 4. Cross section through intestine-like cavities (a & b) and bone tissue (c) (scale: cm).
- Fig. 5. Cross section through hemangiomatous capillary network structures (scale: cm).
- Fig. 6. Radiogram shows clearly intestine-like cavities (a & b) and bone tissue (c).

PLATE II. Case No. 1

- Fig. 7. Microscopical figure of metaplastic part of covering membrane: $\times 150$.
- Fig. 8. Microscopical figure of non-metaplastic part of covering membrane: $\times 60$.
- Fig. 9. A part of wall of intestine-like cavity, resembling that of small intestine: $\times 60$.
- Fig. 10. Several nerve bundles seen in connective tissue and fat tissue: $\times 60$.
- Fig. 11. Glandular tissue neighboring oral cavity: $\times 60$.
- Fig. 12. Microscopical figure of bone tissue: $\times 42$.

PLATE III. Case No. 2

- Fig. 13. Dorsal view of monster (scale: cm).
- Fig. 14. Ventral view of monster, showing clitoris-like projection and umbilical cord.
- Fig. 15. Cross section through cartilage and lymph-node-like tissue.
- Fig. 16. Radiogram shows small shadow of cartilage (arrow).
- Fig. 17. Microscopical figure of cartilage: $\times 42$.
- Fig. 18. Lymph-node-like tissue showing some lobules separated by connective tissue: $\times 42$.





