Pathological Study on Cutaneous Papillomatosis in a Japanese Serow (*Capricornis crispus*)

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Abstract. A case of general cutaneous papillomatosis in Japanese serow, a wild animal naturally reserved by law (a special natural monument), was reported. Histological observation revealed fibrous proliferation with papillary extension in the dermis, acanthosis with marked vacuolation of epithelial cells and hyperkeratosis. Electron microscopy disclosed vacuolated cytoplasm with scanty tonofibrils in some of the epithelial cells proliferated in the spinosum layer, and virus-like particles in the nucleus of degenerated cells in the corneal layer through the middle spinosum layer. The particles were spherical and measured about 30 nm in diameter, and occasionally arranged in a crystalline array. According to the findings obtained, they were possibly identical to a member of the papovavirus group. Discussion was made in comparison with papillomatosis in the other animal species reported previously. Besides, bundles of needle-like filamentous structures were detected in the nucleus in several spinosum layer cells. Lung worms, *Protostrongylus Shiozawai* (Ohbayashi et Ueno, 1974) were observed in pulmonary sections histologically.

Since cutaneous papillomatosis in cotton tail rabbit produced by a virus was reported by Shope [22] in 1933, a number of electron microscopic studies on papillomatosis in cattle [3, 9, 13, 26], deer [26], horses [10], dogs [6, 30, 31], rabbits [20, 21, 23], chimpanzees [11, 27], man [2, 4, 5, 12, 24] and bird [14] have been published, and indicated that causative agents of these diseases were viruses. These viruses have been named as papillomavirus and classified into Papovaviridae or papovavirus group. Crystalline arrangement of the virus particles in papilloma lesions has been demonstrated in the nucleus of the proliferated and degenerated epidermic cells, especially in the stratum spinosum and corneum, which was apparently corresponded to the intra-

nuclear inclusion body observed by light microscopy [2, 6, 10, 20, 21, 26, 30].

The present authors experienced a case of cutaneous papillomatosis in Japanese serow which had been designated a special natural monument. This report is concerned in pathologic changes observed in that case. Comparison to similar conditions of other animal species, especially of ruminants might help to understand problems of zoonoses cycle between man and animals or in the world of animals.

Materials and Methods

About twelve years old male Japanese serow was found in the forest area in Morioka City on June 4th, 1973. He was so emaciated and difficult to stand that he was protected and hospitalized in the Veterinary Hospital of Iwate University. A biopsy

ニホンカモシカ (*Capricornis crispus*) にみられた皮膚乳頭腫症に関する病理学的研究: 千早 豊・大島寛一・三浦定夫・沼宮内 茂 (岩手大学農学部家畜病理学教室)

material was taken from cutaneous lesion for histological and electron microscopic examinations. After 3 weeks of medical treatment he died and necropsy was performed.

Materials taken from lesions and organs as well as tissues of the whole body were fixed in 10% neutral formalin solution. Paraffin sections were made by the routine procedure, and stained with hematoxylin and eosin, and by Giemsa's method for histological observation. For electron microscopy, tiny blocks excised from biopsy materials were fixed in 3.5% glutaraldehyde solution at pH 7.4. Post-fixation was performed in 1% osmium tetroxide solution by Palade's method [19]. They were embedded in Epon 812 after dehydration by Luft's technique [15]. Thin sections were made by a Porter-Blum MT2-B ultramicrotome, and stained with 1% uranyl acetate solution and lead citrate solution by method of Venable and Coggeshall [29]. Hitachi HS-8 electron microscope was used. Sequential thick sections of 0.5 μ m were made, and stained with 0.5% toluidine blue solution for histologic comparison. Tiny blocks from formalin fixed materials followed by similar procedures were also used for observation of viral particles.

Results

Autopsy findings: The animal weighing 29 Kg of body weight was middle in size, but markedly emaciated with rough hair. Opacity and scab of red-bean in size was detected on the right cornea. Papilloma lesions were found all over the body surface; e.g. the cubituses, axillae and medial thighs of both sides, abdominal region, around the penis as well as the anus, periorcular areas, the lips and the muzzle, etc. The lesions were irregular, prickly, cauliflower-like, granular and pedunculated in shape and as large as red bean to top of the little finger in size, with hairless rough surface. Decubitus and subcutaneous phlegmone were observed at the cubituses.

Hyperemia of the parenchymatous organs, grayish white spots scattered on the liver surface as large as rice grain in size, miliary whitish spots on the mucosal membrane of the small intestine, emphysematous and atelectatic lesions as well as a

thumb-sized abscess in the right middle lobus of the lungs were observed.

Histological findings: Fibrous proliferation with vacuolization, hyperemia, focal hemorrhages, infiltration of neutrophils, histiocytes and plasma cells were observed in the dermis of the cutaneous lesions. The papilla extended into the dermis due to acanthosis with occasional mitotic cells (Fig. 1). Germinal and lower spinosum layer cells contained spherical or elliptic nuclei with a distinct nucleolus. The shape of nucleus was irregular and polymorphic, and cytoplasmic vacuolation was evident in the upper layer of the epidermis. Eosinophilic cytoplasmic inclusion bodies were observed in some of the spinosum layer cells (Fig. 2). Pale cells were scattered in the stratum granulosum which were characterized by poorly stained vacuolated cytoplasm with degenerated nucleus of eccentric location. No inclusion body was detected in the nucleus. Marked eosinophilia of the cytoplasm and hyperkeratosis were evident in the stratum corneum. A part of the cutaneous surface was covered with neutrophil and fibrinous exudation. Sections from the muzzle showed similar findings to those of the skin lesions.

Besides, multiple necrosis, pseudo-bile ducts with fibrous proliferation at the periportal area and focal fibrous thickening of the capsule were observed in the liver. In the lungs, a nodular abscess and adult lung worms sectioned transversely measured 50–70 μ m in diameter as well as the eggs and larvae with lymphocytic infiltration were observed. Emphysema and atelectasis were accompanied in the lesions. Moreover, a few coccidia in the mucosa of the small intestine and necrotic keratitis of the right cornea were observed. These seemed to have no relationships to the papillomatosis directly. Sinus catarrh and lymphadenitis

were observed in the lymph nodes of the body surface, but no tumoral metastasis was detected.

Electron microscopic findings: Plasma cells, histiocytes and fibroblasts were detected in the dermis. Electron dense degenerated fibroblastic cells were observed. They were located near a capillary and characterized by polymorphism of the nucleus, irregular indentations of the nuclear membrane, margination of chromatin and cytoplasmic vacuolation. Virus-like particles scattered and accumulated in the nucleus. They were spherical and measured 29–32 nm in diameter (Fig. 3). The germinal layer cells with oval nuclei were arranged vertically to the line of the basement membrane. Although the cytoplasmic projections of these cells extended into the dermis, the basement membrane was well maintained. The cytoplasm contained numerous tonofibrils and increased in its density. Intercellular spaces were dilated in the stratum spinosum. The cells in this stratum were elongated with numerous irregular projections. They attached one another with 20–30 of desmosomes. The cells were stained dark due to numerous ribosomes, tonofibrils attaching to the desmosomes and mitochondria in their cytoplasm, and contained nucleus with distinct nucleolus (Fig. 4).

From the middle to the upper layer of the stratum spinosum, cells, which have irregular nuclear indentations and an evident nucleolus, contained numerous tonofibrils in perinuclear region of the cytoplasm. Most of these cells were relatively dark. Pale cells which were characterized by irregular shape of the nucleus with indentations, margination of the nuclear chromatin, scarce tonofibrils and cytoplasmic vacuolation, were also located. Dilation of intercellular space with a few

desmosomes was obvious (Fig. 5). Further, bundles of filamentous structures of various sizes were observed in the nuclei with marked margination of the nuclear chromatin in the spinosum layer cells. The distance from center line to center line of the adjacent filaments was measured about 12 nm (Figs. 6, 7). Some of these filaments were embedded in the nuclear chromatin accumulated grossly. Numerous ribosomes and tonofibrils were contained in the cytoplasm of these cells. Scattered and accumulated virus-like particles measuring about 30 nm in diameter were observed in the degenerated and flat nucleus of the superficial spinosum layer cell (Fig. 8).

Intercellular spaces were narrow in the granular layer. Organelles decreased in number or disappeared in the vacuolated bright cytoplasm. Virus-like particles were detected in the nucleus located in vacuolated cytoplasm (Fig. 9).

The corneal layer cells were flat and electron dense without distinct nucleus. Decrease and disappearance of tonofibrils and of cytoplasmic projection were observed. Intercellular spaces were extremely narrow and interdigitations were frequently observed. The particles with crystalline arrangement scattered in the nucleus-like structure surrounded by a bright halo. Of these particles, distance from center to center was measured about 31 nm (Fig. 10).

Discussion

Results obtained by light and electron microscopic observations of a general cutaneous papillomatosis in a Japanese serow were described. The spherical virus-like particles measuring about 30 nm in diameter were demonstrated. They were scattered in random fashion or arranged in a crystalline array in the nuclei of the degenerated epithelial cells. The distance from

center to center of the adjacent particles in crystalline arrays was about 31 nm. Usually, the size of virus particles in a thin section is smaller than that of negatively stained ones [20, 21, 26]. The distribution of the particles in the present case is in agreement with that previously reported in papillomatosis of the other mammalian species. Though the size of the particles measured in the present work was a little smaller than 35 nm [26] and 40 nm [3, 9] reported in cattle, 34 nm [26] in deer, 33 nm [5] and 38 nm [4] in man, the particles described here might possibly be viral particles of the papovavirus group.

Regarding the occurrence and distribution of the virus particles in papilloma lesions, many workers [2-6, 9, 10, 13, 14, 20, 21, 23, 24, 26, 30, 31] investigated electron microscopically and reported that the virus particles were recognized in relation to the nucleolus of prickle cells, and that the crystalline arrangement of the particles was detected in the cell nuclei in the superficial layer of the stratum granulosum and the stratum corneum of the epidermis. The present authors detected sporadic and aggregated virus-like particles in the nucleus of a degenerated fibroblastic cell in the dermis. The findings were similar to those reported by Fujimoto and Olson [9]. The sporadic ones were various in size and might be perichromatin granules and the aggregated ones might be virus particles, though some problems to be examined would remain in the future. The lesions of the present case were in progressed stage and appeared to correspond to the 5th and 6th stages of classification made by Fujimoto and Olson [9]. While evident nucleoli and frequent mitosis were observed in the lower layer of epidermis, a consecutive process of cellular degeneration such as marked degenerative changes in the super-

ficial layers of the epidermis, and occurrence of the pale cells [9, 10], were observed electron microscopically.

The intranuclear inclusion bodies which are observed by light microscopy have close relationships with the virus particles detected by electron microscopy, that is, numerous virus particles are usually recognized in the nucleus where inclusion bodies are observed [2, 6, 10, 20, 21, 24, 26, 30]. Difficulty to demonstrate intranuclear inclusion bodies in the present material by light microscopy might be explained by assuming that only a limited number of virus particles was present in the nuclei. The eosinophilic cytoplasmic inclusion bodies observed would be keratohyaline granules as described by Tajima et al. [26] and Fulton et al. [10].

Virus particles in the cytoplasm released from the nucleus after their maturation were reported in the epithelial cells [3, 5, 21]. Numerous virus particles were observed in a degenerated nucleus-like structure in the corneal layer in the present case. Concerning the relationships between the virus and the proliferation of the epithelial cells, Cheville and Olson [6], Fulton [10] and Stone et al. [23] expressed their opinion respectively, but there are still many obscure problems. Intranuclear crystalline arrangement of the virus particles which is characteristic of papilloma virus was demonstrated in many investigators [2, 5, 6, 10, 13, 14, 20, 21, 24, 26, 27, 30, 31]. The occurrence of the arrangement of the particles is based on the species of virus, nature of the host cell and stages of the papilloma lesions. It is most likely to relate with the stage of the papilloma lesions from the results obtained in the present animal.

Bundles of filamentous structures observed by the present authors in the

nucleus of spinosum layer cells have not been described in papillomatosis of other mammals. Similar intranuclear structures were observed in the normal cells of canine pancreas [28], in tumor cells of man [1, 16, 25] and in cells of pig [7] and cattle [18] infected with poxviruses. Unuma et al. [28] reported intranuclear filaments in pancreatic cells in a dog, and expressed their opinion that the structure would be albuminous substance and in the process of accelerated molecule synthesis. Conroy and Meyer [7] recognized an intranuclear filamentous network in cells infected with swine poxvirus. According to their opinion, the network resulted from an alteration of the metabolic function in the cells and not from viral replication. The bundles of filamentous structure observed in the present study presented morphological similarity to the filamentous structure described by Mitsuhashi et al. [16] in myeloma cells. The structures might be produced due to some metabolic disorder of the host cells. However, there are still many problems to be clarified in the future.

As a concurrent lesion, verminous pneumonia was observed. The lung worm was identified as *Protostrongylus shiozawai*, Ohbayashi et Ueno, 1974 [17], that had been detected in Japanese serow in Nagano Prefecture. Coccidium infection in the small intestine, parasitic lesions in the liver and necrotic keratitis were also recognized. These findings seemed to indicate an importance of a vector role of wild animals in the diseases of domestic animals.

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Explanation of Figures

- Fig. 1. Fibrous proliferation with vascularization and cell infiltration in the dermis, irregularly extended papillae due to acanthosis and hyperkeratosis in the epidermis are observed. H-E. $\times 40$.
- Fig. 2. Nucleoli are evident in the germinal and spinosum layer cells. Eosinophilic inclusion body (arrow) and vacuolation in the cytoplasm of the prickle cell are shown. H-E. $\times 420$.
- Fig. 3. Degenerated fibroblastic dark cell in the dermis. Irregularly shaped nucleus with indentations, margination of the nuclear chromatin and vacuolation of the cytoplasm are observed. The surrounding shows degenerated changes. Formalin fixation. $\times 6,800$.
Inset shows intranuclear scattered and accumulated virus-like particles measured 29–32 nm in diameter. $\times 52,800$.
- Fig. 4. Dilated intercellular space, and elongated cells vertically to the line of the basement membrane with numerous irregular cytoplasmic projections attaching to the adjacent cells are shown in the stratum spinosum. The nucleus is ellipsoid and has a distinct nucleolus. The cytoplasm is dark due to the numerous ribosomes and tonofibrils connecting with desmosomes. $\times 5,200$.
- Fig. 5. Degenerated so-called "pale cell" in the stratum spinosum. Spherical and bright nucleus, irregular indentations of the nuclear membrane and slight margination of chromatin, decrease of tonofibrils in the vacuolated cytoplasm are recognized. The intercellular spaces have been dilated and a few desmosomes are presented. $\times 5,200$.
- Fig. 6. A bundle of the needle-like filamentous structures in the nucleus of a prickle cell with marked aggregation of nuclear chromatin is presented. $\times 25,300$.
- Fig. 7. Intranuclear filamentous structures are shown at higher magnification. They measured about 12 nm in distance from center line to center line between the two filaments. $\times 49,600$.
- Fig. 8. Virus-like particles in a flat degenerated cell with margination of nuclear chromatin and vacuolated cytoplasm in the superficial spinosum layer cell. $\times 24,500$.
- Fig. 9. Markedly vacuolated cytoplasm with decreased tonofibrils, and swelling of the nucleus with chromatin margination and virus-like particles are observed in a granular layer cell. Formalin fixation. $\times 8,400$.
Inset shows magnified virus-like particles measured about 30 nm in diameter. $\times 38,500$.
- Fig. 10. Virus-like particles either scattered or arranged in a crystalline array are observed in nucleus-like structure with bright halo in the stratum corneum. $\times 49,600$.
Inset shows virus particles at higher magnification. The distance from center to center of the adjacent particles measured about 31 nm. $\times 160,000$.