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ไวรัสฝีดาษกับการเกิดโรคไฟโบรมาโทซีสที่ผิวหนังในกระบือเผือก: รายงานสัตว์ป่วย

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บทคัดย่อ: ตัวอย่างชิ้นเนื้อผิวหนังจากกระบือเผือกเพศผู้อายุ 5 ปี ได้ถูกส่งเข้ารับการตรวจทางจุลพยาธิวิทยา จากประวัติการพบก้อนที่ผิวหนังหลายบริเวณร่วมกับเป็นแผลหลุมและสะเก็ดมานาน 1 ถึง 2 เดือน ผลการตรวจทางจุลพยาธิวิทยา พบการเกิดแผลหลุมร่วมกับการอักเสบบริเวณด้านบนของผิวหนัง ด้านล่างพบการเพิ่มจำนวนของเซลล์ไฟโบรบลาสท์โดยแทรกอยู่ระหว่างมัดของคอลลาเจน พบอินคลูชันบอดีในไซโทพลาซึมของไฟโบรบลาสท์ลักษณะกลมรีติดสีชมพู การตรวจทางอิมมูโนฮีสโตเคมีให้ผลบวกอย่างเด่นชัดต่อโปรตีนโครงร่างชนิดไวเมนติน แต่ให้ผลลบทางอิมมูโนฮีสโตเคมีต่อโปรตีนโครงร่างชนิดแพนไซโทเคอราทีน จากข้อมูลข้างต้นสรุปได้ว่าเกิดโรคไฟโบรมาโทซีสที่ผิวหนัง การตรวจทางกล้องจุลทรรศน์อิเลคตรอนชนิดลำแสงส่องผ่านโดยใช้เนื้อเยื่อที่ดองไว้ในฟอร์มาลิน พบเชื้อไวรัสที่ยังเจริญไม่เต็มที่ขนาดประมาณ 200 x 300 นาโนเมตร ลักษณะคล้ายพ๊อกไวรัสภายในบริเวณที่เป็นก้อนอินคลูชันบอดี โรคไฟโบรมาโทซีสที่มีการตรวจพบอินคลูชันบอดี มีรายงานพบน้อยในคนและสัตว์ สำหรับในสัตว์ พ๊อกไวรัสมีรายงานก่อโรคไฟโบรมาโทซีสและมึกโซมาโทซีสในกระต่ายบ้านและกระต่ายป่า จากการสืบค้นข้อมูล ไม่การรายงานว่าพ๊อกไวรัสเกี่ยวข้องกับการเกิดไฟโบรมาโทซีสชนิดที่พบอินคลูชันบอดีในไซโทพลาซึมในกระบือเผือกมาก่อน รายงานฉบับนี้ เป็นการรายงานรูปแบบที่ไม่พบบ่อยของการติดเชื้อพ๊อกไวรัส โดยที่พบลักษณะรอยโรคเป็นแบบไฟโบรมาโทซีสในกระบือเผือก สัตวแพทย์ที่ตรวจวินิจฉัยกระบือที่พบรอยโรคเป็นก้อนที่ผิวหนังและมีแผลหลุมหลายบริเวณโดยไม่ประสบผลสำเร็จในการรักษา ควรคำนึงถึงโอกาสเกิดโรคไฟโบรมาโทซีสจากการติดเชื้อพ๊อกไวรัส เช่นเดียวกับในกรณีศึกษานี้ด้วย

คำสำคัญ: กระบือ ผิวหนัง เผือก พยาธิวิทยา ไฟโบรมาโตซีส ไวรัสฝีดาษ

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Poxvirus Associated with Cutaneous Fibromatosis in an Albino Buffalo (*Bubalus bubalis*): A Case Report

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Abstract: Skin biopsy of a five-year-old male albino buffalo (*Bubalus bubalis*) was submitted for histopathological diagnosis. The buffalo had a history of multiple skin nodules, ulceration and scab formation at the trunk for about two months. Histopathological examination revealed superficial ulceration with inflammatory reaction and underlying proliferation of fibroblasts among collagen bundles within the dermis. The proliferated fibroblasts showed large oval to round eosinophilic intracytoplasmic inclusion bodies. Immunohistochemically (IHC), the proliferated cells were strongly positive to vimentin. Immunohistochemical reaction to pancytokeratin was negative. The diagnosis of cutaneous fibromatosis was made. For transmission electron microscopic examination from the formalin fixed skin samples, some immature virions, about 200x300 nm. in size and morphologic consistent with poxvirus, were detected within cytoplasmic inclusion body. Fibromatosis with intracytoplasmic inclusion body was a rare condition reported in human and animals. In animals, poxvirus associated with fibromatosis or myxomatosis was well documented in Lagomorphs such as rabbits and hares. The presence of poxvirus associated with fibromatosis with eosinophilic intracytoplasmic inclusion body in an albino buffalo has never been documented in the literature. This article demonstrates atypical form of poxvirus infection with histopathologically appearance as fibromatosis in an albino buffalo. Veterinarians deal with nodular and ulcerative skin lesions without responsive to treatment in buffalo should keep in mind of a possible fibromatosis, poxviral associated lesions, as observed in this study.

Keywords: Albino, Buffalo, Fibromatosis, Pathology, Poxvirus, Skin

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Introduction

Cutaneous fibromatosis is a condition with multiple nodules of fibroblastic proliferation has been found on skin. However, fibromatosis with intracytoplasmic inclusion body is a rare condition reported in human and animals. Poxvirus is one of the most common causes of fibroblastic proliferation in animals. The shape of Poxviral virions is oval or brick-shape of about 200 to 400 nm in length, with axial ratios of 1.2 to 1.7. There are two forms of the nonenveloped virion namely the intact M (mulberry) form and the C (capsule) form (Mark and Palumbo, 1991). A well-known Leporipoxvirus induced fibroma and fibromatosis is the rabbit (shope) fibroma virus and squirrel fibroma virus (Bangari *et al*., 2009). This virus causes fibroma on the legs, feet, and ears and persists for several months. Intracytoplasmic inclusion bodies can be histologically detected in fibroblasts. Other example of virus induced fibromatosis is papillomavirus infection in roe deer and other animals of family *Cervidae* such as white-tailed deer, red deer, mule deer, European elk and reindeer (Kureljušić *et al*., 2012; Sunderberg and Nielsen, 1981). Equine sarcoid is an example of locally aggressive, fibroblastic skin tumor of animals in family Equidae such as horses, mules, donkeys and zebra (Jangir *et al*., 2013). Bovine papillomavirus (BPV-1 and BPV-2) has been reported to be associated with equine sarcoid pathogenesis (Chambers *et al*., 2003; Bogaert *et al*., 2008; Kumar *et al*., 2013). Although papillomaviruses have been described as highly species–specific, however, inter-species infection occurs as in equine sarcoid (Jangir *et al*., 2013). Buffalopox virus infection is a proliferative viral skin disease occurs in buffalos, cows and human. In a recent outbreak of this virus in India did not demonstrate any fibroblastic proliferation (Venkatesan *et al.,* 2009).

The aim of this study is to describe histopathological, immunohistochemical and transmission electron microscopical findings of a rare condition of cutaneous fibromatosis in an albino buffalo (*Bubalus bubalis*).

Materials and methods

***Case history*:** A five-year-old male albino buffalo (*Bubalus bubalis*) with a history of multiple skin nodules, ulceration and scab formation at trunk for about two months without satisfactory treatment result. Skin biopsy was performed and the specimen was submitted in 10 % phosphate-buffered formalin to Mahanakorn Veterinary Diagnostic Center for histopathological investigation.

***Histopathology*:** Tissue slides for microscopic examination were prepared using routine

paraffin embedding technique. Briefly, the tissues were processed and embedded in paraffin, sectioned at 4 microns and stained with hematoxylin and eosin (H&E).

***Immunohistochemistry (IHC)*:** For immunohistochemistry, the polymer detection system (NovoLink Cat. No. RE7140-K, Chemo Science) was applied. Primary antibodies to pancytokeratin (dilution 1:400; AE1/AE3, Nichirei) and vimentin (dilution 1:100: Clone V9, Nichirei) were used with 3-3'-diaminobenzidine (DAB, Dako) as chromogen as previously described (Ramos-Vara *et al.,* 2003; Mamom, 2012). Briefly, the tissue sections were deparaffinized and hydrated. Antigen retrieval was performed by boiling tissue sections in citrate buffer using microwave. Inhibition of endogenous peroxidase was done using hydrogenperoxide. Other steps including the use of blocking protein, primary antibodies, secondary antibody, peroxidase enzyme, substrate/chromogen and hematoxylin counterstain were done according to the product information (NovoLink Cat. No. RE7140-K, Chemo Science). The slides were evaluated under light microscope.

***Transmission Electronmicroscopy (TEM)*:** Specimen preparation for transmission electron microscopic examination using formalin fixed tissue was done at Pathology Section, the National Institute of Animal Health (NIAH) according to the previous study (Mamom *et al*., 2010). Briefly, the tissue samples were embedded in epoxy resin (Epon). The ultrathin sections were cut and stained with 5% uranyl acetate and lead citrate. The samples were examined under transmission electron microscope (JEM-1200EX, JEOL, Japan).

Results

Histopathological findings:

Histopathologic examination of hematoxylin & eosin (H&E) stained slides revealed fibroblastic proliferation among pre-existing dermal collagen fibers (Fig. 1A) with expansive growth to superficial dermis and extended deep into subcutis. The proliferated fibroblasts showed oval nuclei with less heterochromatin and prominent nucleoli. Large oval to round shaped eosinophilic intracytoplasmic inclusion bodies (Fig. 1B) were found in many fibroblasts. Some cells underwent cytoplasmic vacuolar change. Mitotic figures were rare and ranged from 0 to 2 per high power field. Areas of necrosis and hemorrhages were seen especially at the center of nodules. The superficial epidermis revealed necrosis, ulceration and hemorrhage with inflammatory cells infiltration especially

neutrophils. No epithelial cell proliferation and ballooning degeneration were observed from the submitted samples.

Immunohistochemical findings:

Vimentin and pancytokeratin are cytoskeletal proteins normally observed in cytoplasm of the cells of mesenchymal and epithelial origin respectively. The proliferated fibroblasts in this case expressed vimentin (Fig. 1C). Immunoreactivity to pancytokeratin was negative. This result confirmed the non-epithelial origin of proliferative cells. The intracytoplasmic inclusion bodies were also strongly positive for vimentin as well.

Transmission electron microscopic (TEM) findings:

Transmission electron microscopic (TEM) findings results showed some immature virions of about 200 to 300 nm. in size (Fig. 1D) in the area of cytoplasmic inclusion bodies. The virions contained dumbell-shaped nucleocapsid characteristic for poxvirus.

Discussion

The diagnosis of fibromatosis in this case was done on the basis of gross and microscopic lesions and immuno histochemical results. The presence of poxvirus within the cytoplasmic inclusion bodies by transmission electron microscopic examination confirmed the cause of fibroblastic proliferation in this case (Mark and Palumbo, 1991; Fenner and Nakano, 1988; Westwood *et al*., 1964). The absence of epithelial lesions, especially ballooning degeneration, is possibly due to the small biopsy sample which was collected from ulcerated lesion. The presence of immature virions in intracytoplasmic inclusion body verified the role of poxvirus in inducing fibroblastic proliferation in buffalo which is similar to a condition in rabbit caused by poxvirus infection, rabbit (shope) fibroma virus. Whether the albino status of this buffalo plays an important role as a predisposing factor on the tumor development is unclear because of the lack of information concerning the tumorigenesis in albino animal caused by poxvirus infection. Unfortunately, the animal die and the owner did not allowed to use the carcass for further studies. The type of poxvirus in this case could not be able to identify and requires additional molecular study.

In conclusions, we reported the presence of poxvirus in association with fibromatosis in an albino buffalo (*Bubalus bubalis*), which has never been documented in the literatures. This article demonstrated atypical form of poxvirus infection with histopathological appearance as fibromatosis. Veterinarians who deal with nodular and

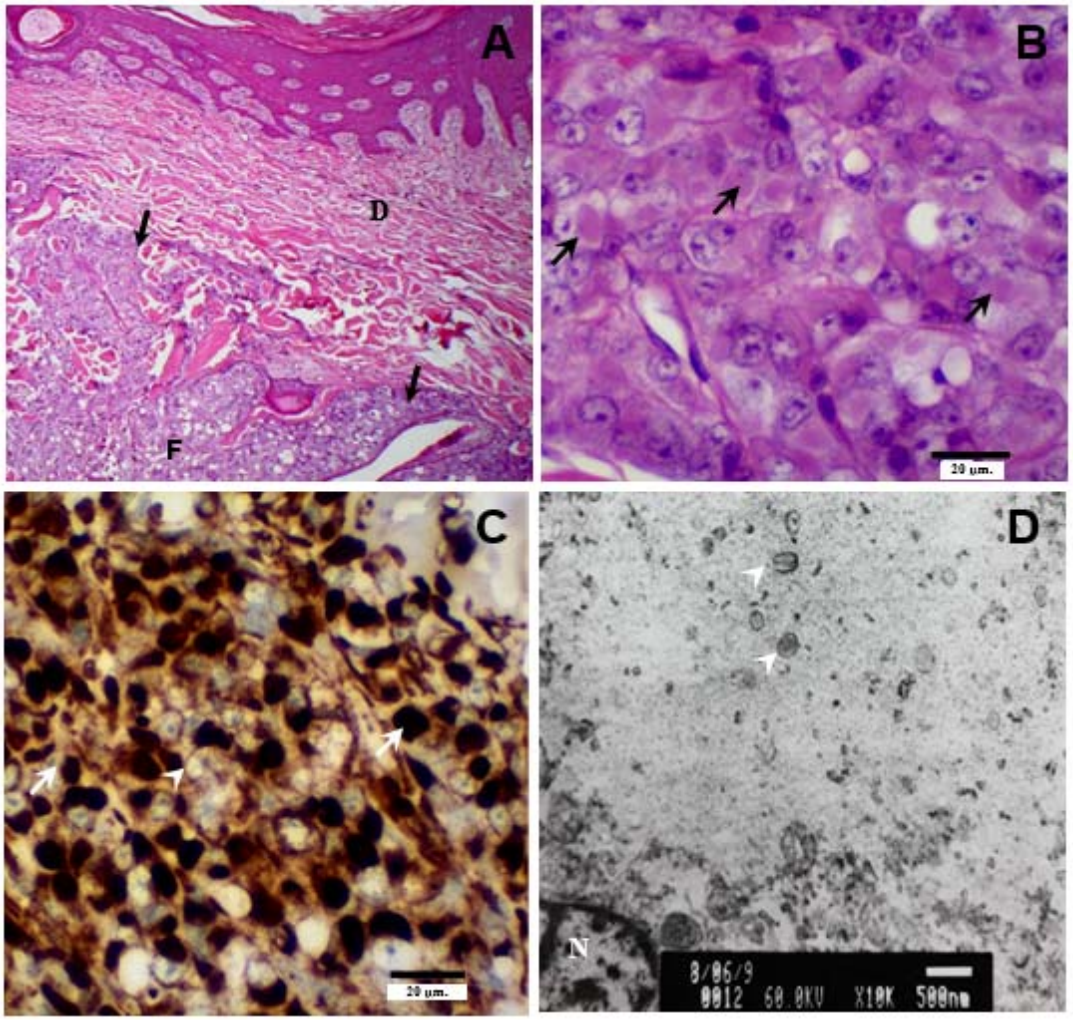

Fig. 1 A-D: Histopathological, immunohistochemical and transmission electron microscopic findings of skin lesion. **1A:** Outline (black arrows) of fibroblastic proliferation (F) within dermis (D). **1B:** Higher magnification of fibroblastic proliferation. Large round eosinophilic intracytoplasmic inclusion bodies (black arrows) were detected in some proliferated fibroblasts. **1C:** The proliferated fibroblasts showed positive IHC to vimentin shown as brown staining area in cytoplasm. Very strong immunoreactivity was found at cytoplasmic inclusion bodies (white arrows); arrow head = nuclei of fibroblast; DAB with hematoxylin counter stain. **1D:** Transmission electron microscopic picture of immature virions (white arrow heads), about 200x300 nm. in size, morphologic consistent with poxvirus. N = nucleus. Bar = 500 nm.

ulcerative skin lesions without response to treatment in buffalo should keep in mind of a possible fibromatosis, poxviral associated lesions, as observed in this study.

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