

## ECCRINE CARCINOMA IN THE FOOT OF AN ASIAN ELEPHANT (*ELEPHAS MAXIMUS*)

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**Abstract:** A case of eccrine carcinoma of the interdigital foot glands in a 39-yr-old female Asian elephant (*Elephas maximus*) from Zagreb Zoo is described. The tumor between the toenails of the right forefoot was surgically removed 3 yr before postmortem examination (2003), and the histopathologic diagnosis was compound eccrine carcinoma characterized with glandular tubular and papillary proliferations, mild cellular pleomorphism, proliferation of the myoepithelial cells with mucoid secretions, and necrosis. Immunohistochemistry revealed strong immunoreactivity to S-100 protein, estrogen, and high-molecular weight cytokeratin. This elephant also had chronic renal fibrosis with uremia.

**Key words:** Adenocarcinoma, Asian elephant, eccrine interdigital glands, immunohistochemistry, kidney fibrosis.

### BRIEF COMMUNICATION

Despite the importance of Asian elephants as both working and zoo animals, limited information is available on the elephant's mortality or disease incidence. In particular, neoplasia in elephants is rarely reported.<sup>1,13,14</sup> Considering the lifespan of these animals, which is comparable to humans, more neoplasia would be expected.<sup>1</sup> However, foot problems are common in captive elephants. They can cause serious illness and death of the animal. Foot lesions are associated with quality of the enclosure, husbandry, quality and quantity of food, and veterinary care. The aim of this paper is to describe a rare foot tumor that could be overlooked easily or mistaken for more frequently reported inflammatory disease.

A female Asian elephant (*Elephas maximus*) arrived at the Zagreb Zoo (Croatia) in 1994. On arrival, the elephant was in poor body condition,

and weight loss was likely associated with transport. However, during the next several years, its condition had not changed and, along with foot problems, was deteriorating. Because of its poor general condition and the diagnosis of pododermatitis, a complicated foot surgery was performed by members of the Elephant Group in cooperation with staff from the Zagreb Zoo in 2003.

The elephant was premedicated with atropine (Phoenix; 200 mg/kg i.m.), and anesthesia was induced with ketamine (100 mg/ml, CP Pharma; 400 mg/kg i.m.) and xylazine (100 mg/ml, Rompun TS, Bayer AG; 150 mg/kg i.m. per nostril), and 30 min later, etorphine (10 mg/ml, Immobilon, Albrecht; 2 mg/kg i.m. per nostril).

During the anesthesia, systemic therapy was applied, which included betamethasone dihydrogen phosphate (12 mg/ml) and betamethasone acetate (3.995 mg/ml) (Celestovet, Essex Tierarznei, Essex Pharma GmbH; 15 ml i.m.), and prednisolone (25 mg/ml, prednisolone-acetate, CP-Pharma, 500 mg i.m.), benzathine benzylpenicillin (95.3 mg/ml), procaine benzylpenicillin (25 mg/ml), dihydrostreptomycin sulfate (155 mg/ml) (Tardomyocel comp. III, Bayer; 100 ml i.m.), methylprednisolone sodium succinate (Medrate soluble, Serumwerk Bernburg; 200 mg/kg i.v.), epinephrine (1 mg/ml, Synethenephrin, Phonix; 80 mg/kg i.m.), caffeine (Dicophedrin, Atarost; 60 ml i.m.),  $\alpha$ -tocopherol acetate (50 mg/ml) and sodium selenite (0.5 mg/ml) (Vitamin E-Selen, Serumwerk Bernburg; 80 ml i.m.), Vitamin C forte (0.2 g/ml, Chassout; 4 mg/kg i.m.), infusion solution with 5.5% glucose, electrolytes, and amino acids (Amynin, Merial; 500 ml i.m.), 4% succinylated gelatin plasma expander (Infucol M

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**Figure 1.** Foot. Elephant. Tumor between toenails.

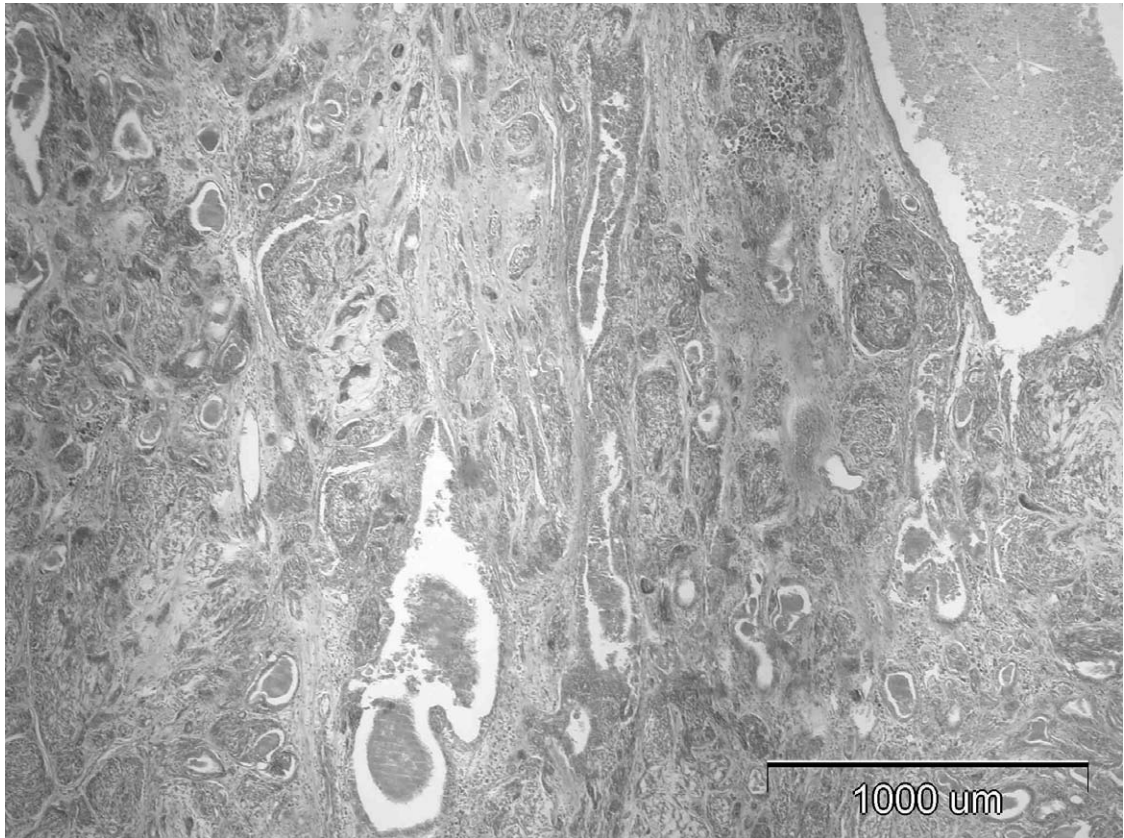
40, Serumwerk Bernburg; 250 ml s.c.), 10% injectable solution of butaphosphan with 0.005% cyanocobalamin (Catosal 10%, Bayer; 50 ml i.m.), and injection solution containing metabolic constituents (Biodyl, Albrecht; 30 ml i.m.).

After cleaning the surfaces of the feet and nails and the interdigital surfaces (abscesses), topical spray (Chassout) was placed on the purulent areas, and Jodophorm (Albrecht), Novaderm (WDT) Haemascon (Phoenix) 100 ml was applied. On the front right foot, an elevated, reddish mass was removed and sent for histopathologic analysis (Fig. 1). After the surgery, sedation was antagonized with diprenorphine (2.67 mg/1 mg, Revivon, Albrecht; 4 mg i.v. and 5.34 mg i.m.), Yohimbine hydrochloride (Caesar & Loretz GmbH; 400 mg i.v.), atipamezole (5 mg/ml, Antisedan, Pfizer; 150 mg i.m.), and Doxapram V (20 mg/ml, Albrecht; 600 mg i.v.). Five minutes after administering reversals, the elephant rose without difficulty and nibbled hay. The husbandry included intensive veterinary care and reconstruction of the enclosure. After treatment, the elephant received therapy per os, which included

digoxin (4 mg, Lanicor, Pliva, Zagreb); Karsivan 100 (Intervet; 15 tabs twice); Vitamin C (100 mg/ml, Veterina, Zagreb; 20 g/day); Hipoviton (30 g, Vetoquinol); and Betamag (30 g, Equistro). Also, bathing routines to remove dirt and excrement from skin and feet two times a day were established. After bathing, Betadine 10% (Alkaloid, Skoplje) and Dermo-spray (Lek, Ljubljana) were applied topically. Novaderma (WDT) paste was applied around the nails. Therapy was adopted to respond to frequent intermittent exacerbations. Also, the elephant was offered various food items five times a day, including green grass, hay, and branches, leaves, and bark.

In May 2006, this elephant died after a history of end-stage kidney disease and multisystemic organ failure unrelated to the previously mentioned foot neoplasm. No evidence of metastasis was found. At postmortem, severe kidney fibrosis, lung edema, hydropericardium, chronic and acute gastric ulcers, liver fibrosis, and atrophy of the lymph nodes and spleen were noted.

Tissue samples of the foot tumor were taken for histopathologic examination, fixed in neutral



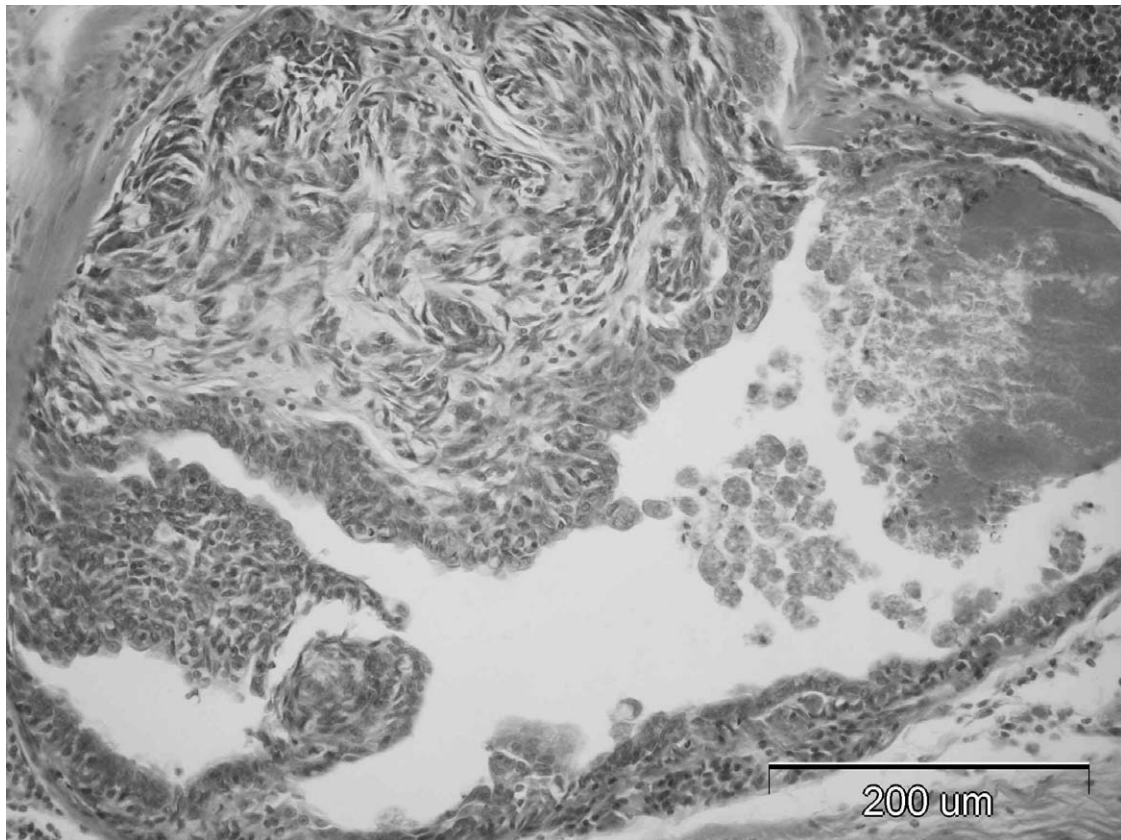
**Figure 2.** Elephant. Eccrine carcinoma. Tubular appearance of the epithelial cells within fibrous and myoepithelial stroma with cystic formations filled with amorphous eosinophilic substance. H&E.

formalin, embedded in paraffin, cut in 5- $\mu$ m-thick sections, and routinely stained with hematoxylin and eosin stain. Histopathologically, the tumor comprised two components: epithelial elements embedded within a myxoid and fibrous stroma (Fig. 2). The epithelial cells formed tubular structures, cysts, and solid cords. Focally, there were single cells in an abundant myxoid stroma. The tubular structures were lined by two layers of cells: a luminal layer of cuboidal cells and a peripheral layer of flattened myoepithelial cells. Necrosis, papillary intra-acinar proliferations, and multilayer epithelial and myoepithelial cell proliferation were seen (Fig. 3). The tubular lumina contained amorphous eosinophilic material. The epithelial cells showed some pleomorphism and mitotic activity (6 mitoses/10 high-power fields).

Immunohistochemical stains using monoclonal antibodies to high-molecular weight (CK HMW) and low-molecular weight cytokeratin (CK LMW), cytokeratin 7 (CK7) and 20 (CK20), estrogen (ER) and progesterone receptors (PR),

S-100 protein, carcinoembryonic antigen (CEA), and epithelial membrane antigen (EMA) were performed (all antibodies were from DAKO, Glostrup, Denmark). For immunostaining, a standard streptavidin-biotin method was performed according to manufacturer specification. Immunohistochemical analysis revealed positive staining of the inner layer of epithelial cells with CK HMW and ER (Fig. 4) and staining of peripheral layer with S-100 protein. Staining with CK LMW, CK7, CK20, PR, CEA, and EMA was negative (Table 1). Histopathologic and immunohistochemical findings were consistent with the diagnosis of a malignant mixed tumor of eccrine glands described in human pathology.<sup>4,8,12</sup>

Although differential macroscopic diagnosis of the foot tumor should include squamous cell carcinoma (i.e., nail bed carcinoma) or proliferative granulation tissue, the histopathologic diagnosis of the tumor described in this case was facilitated by the fact that the planocellular and eccrine carcinomas are quite different histologically and that eccrine glands were already de-



**Figure 3.** Elephant. Eccrine carcinoma. Multilayer appearance, papillomatous proliferation and myoepithelial cells. H&E.

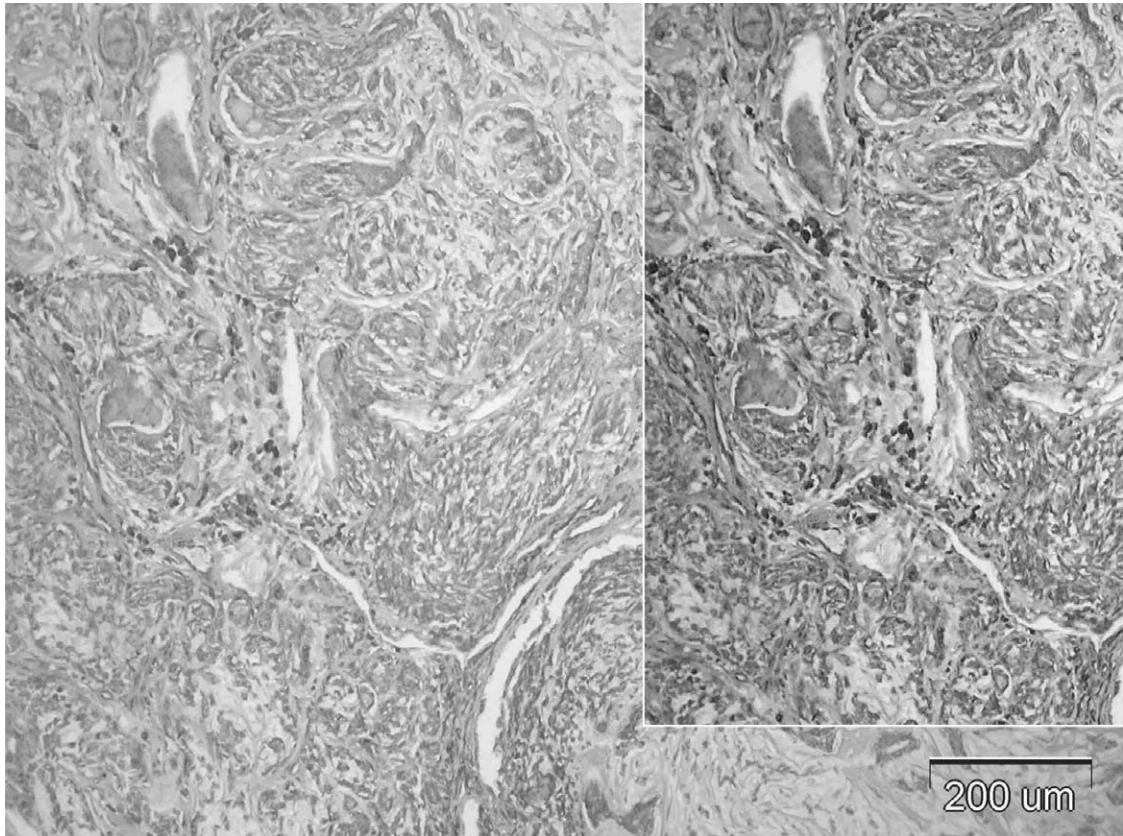
scribed in the foot in Asian elephants<sup>5</sup> with a similar hormonal reactivity as eccrine glands in humans. In both species, the immunoreactivity to estrogen, androgen, and progesterone was noted.<sup>7</sup> Differentiation between apocrine and eccrine tumors is also often dependent on the tumor site,

**Table 1.** Tumor immunoreactivity.

Antibody <sup>a</sup>	Eccrine carcinoma
CK HMW	+++
CK LMW	–
CK 7	–
CK 20	–
ER	++
PR	–
S-100 protein	+++
CEA	–
EMA	–

<sup>a</sup> CK HMW, high-molecular weight cytokeratin; CK LMW, low-molecular weight cytokeratin; CK7, cytokeratin 7; CK20, cytokeratin 20; ER, estrogen; PR, progesterone; CEA, carcinoembryonic antigen; EMA, epithelial membrane antigen.

which in this case confirms the diagnosis.<sup>2</sup> In this case, only estrogen immunoreactivity remained in the tumor, likely a consequence of neoplastic progression. It is well known that in some other glandular tumors, such as mammary gland neoplasms, more malignant tumors lose hormonal receptors.<sup>6</sup> CEA was also negative despite typical eccrine gland positive immunoreactivity to this antibody.<sup>11</sup> The same could be stated for EMA, but this negative reaction has already been described in human eccrine tumors.<sup>10</sup> In human pathology, there are several types of eccrine gland tumors—such as poroma, syringoma, and mixed tumor—that are relatively frequent.<sup>9</sup> In veterinary pathology, there are rare cases of eccrine carcinomas, predominately on the footpads of cats and dogs. However, a compound variant was described in carcinomas of the apocrine glands.<sup>3</sup> Compound adenoma as a diagnosis was excluded because tumor necrosis and pleomorphism found in the present tumor are characteristic of carcinomas.



**Figure 4.** Elephant. Eccrine carcinoma. Immunohistochemistry. Notable positivity of the epithelial cells to CK HMW. Streptavidin-biotin method.

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#### LITERATURE CITED

1. Fowler, M. E., and S. K. Mikota (eds.). 2006. *Biology, Medicine and Surgery of Elephants*, 1st ed. Blackwell, Iowa State Univ. Press, Ames, Iowa.
2. Ginn, E. P., J. E. K. L. Mansell, and P. M. Rakich. 2007. Skin and appendages. *In: Maxie, M. G. (ed.). Pathology of Domestic Animals*, 5th ed. Saunders Elsevier, Edinburgh, London, New York, Oxford, Philadelphia, St. Louis, Sydney, Toronto. Pp. 757–759.
3. Goldschmidt, M. H., and M. J. Hendrick. 2002. Tumors of the skin and soft tissues. *In: Meuten, D. J. (ed.). Tumors in Domestic Animals*, 4th ed. Iowa State Press, Ames, Iowa. Pp. 45–118.
4. Klein, W., E. Chan, and J. T. Seykora. 2005. Tumors of the epidermal appendages. *In: Elder, D. E., and E. T. Lever (eds.). Histopathology of the Skin*. Lippincott Williams & Wilkins, Philadelphia, Pennsylvania. Pp. 867–926.
5. Lamps, L. W., B. R. Smoller, T. E. Goodwin, and L. E. Rasmussen. 2004. Hormone receptor expression in interdigital glands of the Asian elephant (*Elephas maximus*). *Zoo Biol.* 23: 463–469.
6. Lamps, L. W., B. R. Smoller, L. E. Rasmussen, B. E. Slade, G. Fritsch, and T. E. Goodwin. 2001. Characterization of interdigital glands in the Asian elephant (*Elephas maximus*). *Res. Vet. Sci.* 71: 197–200.
7. Misdorp, W. 2002. Tumors of the mammary gland. *In: Meuten, D. J. (ed.). Tumors in Domestic Animals*, 4th ed. Iowa State Press, Ames, Iowa. Pp. 575–606.
8. Murphy, G. F., and D. E. Elder. 1990. *Atlas of Tumor Pathology. Non-Melanocytic Tumors of the Skin*. AFIP, Washington, D.C.
9. Murphy, G. F., K. Sellheyer, and M. C. Mihm. 2005. The skin. *In: Kumar, V., A. A. Abas, and N. Fausto (eds.). Robins and Cotran Pathologic Basis of Disease*, 7th ed. Elsevier Saunders, Philadelphia, Pennsylvania. Pp. 1227–1272.
10. Obi, M., T. Satoh, H. Yokozeki, and K. Nishioka. 2004. Eccrine porocarcinoma with bowenoid changes: epithelial membrane antigen is not a useful

marker for malignant tumours arising from eccrine gland structures. *Acta Derm. Venereol.* 84: 142–144.

11. Penneys, N. S., R. Kott-Blumenkranz, and B. E. Buck. 1984. Carcinoembryonic antigen in fetal eccrine glands: an immunohistochemical study. *Pediatr. Dermatol.* 1: 281–282.

12. Requena, L., H. Kutzner, M. A. Hurt, D. J. Santacrus, D.A. Mehregan, D. R. Mehregan, Y. M. Mengesha, S. Kohler, Z. B. Argeny, Y. McNiff, P. Rudolph, and O. P. Sanguenza. 2006. Malignant tumors with apocrine and eccrine differentiation. *In: LeBoit, P. E., G. Burg, D. Weedon, and A. Sarasin. (eds.). World*

*Health Organization Classification of Tumours. Pathology and Genetics. Skin Tumors.* IARC Press, Lyon, France. Pp. 125–148.

13. Robinson, P. T., and J. E. Meier. 1977. Surgical removal of a tumor from an Asian elephant. *Vet. Med. Small Anim. Clin.* 72: 1638–1640.

14. Sundberg, J. P., W. C. Russell, and W. Lancaster. 1981. Papillomatosis in Indian elephants. *J. Am. Vet. Med. Assoc.* 179: 1247–1248.

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