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Osteosarcoma in a male giraffe (*Giraffa camelopardalis reticulata*)

Andriy Marunchyn*
PhD in Veterinary Sciences, Head Doctor of Veterinary Clinic "UniVet"
02002, 6E Mykilsko-Slobidska Str., Kyiv, Ukraine
https://orcid.org/0009-0001-1035-457X

Beata Kwiatkowska-Palys
Veterinarian
Private Practice, Veterinary Office "AGAMA"
32-046, 181 Minoga Str., Minoga, Poland
https://orcid.org/0009-0000-4836-7627

Marcin Palys
Veterinarian
Private Practice, Veterinary Office "AGAMA"
32-046, 181 Minoga Str., Minoga, Poland
https://orcid.org/0009-0002-8359-6470

Anastasiia Kovalova
Head of Pathomorphological Department, Universal Clinic "Oberig"
03057, 3 Zoolohichna Str., Kyiv, Ukraine
https://orcid.org/0000-0002-9886-3685

Kateryna Tereshchenko
Veterinarian, Master's Degree
Veterinary Clinic "UniVet"
02002, 6E Mykilsko-Slobidska Str., Kyiv, Ukraine
https://orcid.org/0009-0009-8786-8163

Abstract. The relevance of this study lay in the limited information available regarding documented clinical cases of oncological diseases, specifically osteogenic sarcoma, in even-toed ungulates of the *Giraffidae* family. In domestic small animals, computed tomography, magnetic resonance imaging, and radiography are commonly employed to assess bone lesions and distant metastasis. However, applying such imaging techniques to wild animals presents significant

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*Corresponding author

challenges. This research aimed to verify the clinical diagnosis of osteogenic sarcoma in a giraffe. To achieve this, the animal was administered general non-inhalation anaesthesia, followed by diagnostic clinical and radiographic examinations of the joint, along with pathological verification of tumour tissue. Tissue samples obtained from the animal were subjected to histological analysis. Clinical examination revealed an increase in volume and thickening of the carpal joint in the left thoracic limb. Based on clinical signs, the pathological process extended through the cortical layer, distending the periosteum of the carpal joint. Additionally, characteristic symptoms of chronic pain and exhaustion were observed in the animal. Radiographic imaging revealed evidence of bone and cartilage involvement in the joint, exhibiting an aggressive and destructive pattern, along with osteolytic changes accompanied by minimal calcification. The cortical bone layer remained intact, with the lesions localised to the epiphyseal-metaphyseal region of the forelimb bones. Pathohistological analysis confirmed the presence of osteoblastic osteosarcoma. The findings of this comprehensive investigation provided novel insights into the progression of osteosarcoma in giraffes, particularly highlighting the pathological morphology of a malignant bone-forming tumour. These results contribute to the existing knowledge of the pathogenesis of bone oncopathology in wild animals, which holds significance for veterinary practice and scientific research. Therefore, the practical value of this study lies in enhancing the understanding of the pathological morphology of oncological diseases affecting the appendicular skeleton

Keywords: tumour osteogenesis; nuclear pleomorphism; atypical cells; osteoid; clinical diagnostic investigation; pathohistological examination

Introduction

Under current conditions, zoos play a crucial role in wildlife conservation, as anthropogenic interference has a negative impact on ecosystems. Four species of giraffe are kept in national parks and other organisations where artificial conditions are created. Osteosarcoma of the skeleton in these animals has been little studied and is not described in the professional literature regarding the morphology of oncopathology. Thanks to palaeo-oncological studies of the remains of humans and animals that existed at least 350 million years ago, objective data on the existence of oncological diseases (tumours) have been obtained. Modern scientific achievements have allowed for more accurate diagnosis of ancient pathologies in fossilised material. Confirmation is particularly strong for neoplasms of bone tissue, as biological samples that underwent study have been better preserved. The results obtained made it possible to establish a multifactorial aetiology of tumours, which includes the main factors: genetic, environmental, lifestyle, and living conditions.

S. Ekhtiari *et al.* (2020) and A. Garcês *et al.* (2024) confirmed the diagnosis of osteosarcoma in a dinosaur, the herbivorous ceratopsian (*Centrosaurus apertus*). The diagnosis was confirmed by radiographic and histological analysis by human medicine pathologists. To date, authors have focused on the problem of osteosarcoma morbidity in millions of people worldwide. The peak incidence occurs in the second decade of human life. At the same time, the causes, genetic changes, and oncogenic factors of tumour occurrence are insufficiently studied, which limits further progress in targeted therapy.

According to D. Meuten (2017), there is a certain predisposition for the prevalence of

bone neoplasms in large breeds of dogs with significant body weight. These include the Great Dane, Saint Bernard, Rottweiler, German Shepherd, Boxer, Doberman, Irish Setter, and Golden Retriever. Osteosarcoma is diagnosed in 7-8-year-old animals, although it can occur in slightly younger animals as well. Excess weight also provokes the development of onco-osteopathology. In terms of percentage, osteosarcoma in dogs is found in 1% of animals weighing no more than 10 kg, in 8% of animals with a body weight of 25-30 kg, and in 60% of animals weighing more than 35 kg. Males are more likely to get the disease than females. In felines, osteosarcoma is the most common type of bone tumour, accounting for about 75% of all primary tumours. The average age of onset was 8.03 years (± 4.01) with involvement of the appendicular skeleton and 10.41 years (± 3.06) with involvement of flat bones: skull, ribs, and scapula. In 10% of cases, extraskeletal osteosarcoma was diagnosed. Regarding genetic studies, most classical osteosarcomas had complex chromosomal aberrations, both quantitative and structural. The modal chromosome number of the pathological clone is highly variable. The variation in chromosomal aberrations from cell to cell reflects a high level of genetic instability and heterogeneity of osteosarcoma.

The general diagnostic strategy for detecting this disease involves radiographic and pathohistological examination, as outlined in the scientific studies of K. Dittmer & S. Pemberton (2021). The authors demonstrated that instrumental studies should begin with radiography of the appropriate segment. Radiographic signs of bone and cartilage damage vary from a picture similar to a benign process to a picture of a pronounced aggressive and destructive nature, which can lead to a pathological fracture. The radiographic picture typical for osteosarcoma is osteolysis with calcification, a pronounced periosteal and endosteal lytic reaction, which determines the degree of differentiation. Osteosarcoma is characterised by a high degree of malignancy, and accordingly, damage to the bone and periarticular tissues will be with osteolytic changes. Cortical layers are usually with a pronounced periosteal reaction and altered. According to statistical data obtained by scientists from Great Britain and Sweden, of the registered cases of bone tumours in dogs, 73% were malignant, 0% were benign, and 27% were unspecified.

Research into primary osteosarcoma remains highly relevant today, as this pathology, according to J. Beck *et al.* (2022), is the most common oncological disease in paediatrics. Rapid metastasis of the tumour is the main cause of death in patients. In 30% of clinical cases, metastases develop within 5 years of diagnosis. Therefore, collecting scientific information on the detection and progression of the disease in humans, dogs, and laboratory animals is essential. Experimental studies on laboratory mice and dogs have allowed for the creation of various models of oncopathology, which has enabled a deeper study of pathogenetic mechanisms and drawn parallels with osteosarcoma in human medicine. Thus, the presented generalised information on the prevalence of osteogenic osteosarcoma in veterinary and medical practice confirms the relevance of continuing diagnostic and therapeutic research. However, there is insufficient information in the literature about the development of osteogenic osteosarcoma in wild animals. Therefore, considering the above, this study aimed to conduct clinical and morphological studies of the features of osteosarcoma progression in giraffes.

Literature Review

H. Beird *et al.* (2022) focused on the prevalence of primary malignant osteosarcoma in individuals aged 18 to 60 years. They identified genetic predisposition syndrome factors as risk groups

for the disease. Therefore, modern diagnostic screening is specifically conducted on these patient groups. If osteosarcoma of the bone is suspected in a patient after radiographic examination, it is important to conduct a morphological study and, if necessary, a computed tomography scan to detect lung metastases. At the same time, reliable oncomarkers do not exist. Under current conditions, the treatment of osteosarcoma (chemotherapy, surgery) requires a team-based multidisciplinary approach involving specialised oncologists, orthopaedists, general surgeons, pathologists, and radiologists. Research is ongoing in the direction of selecting treatment methods for primary tumours and complicated cases (with the formation of metastases).

A study by A. Mazurkevich *et al*. (2023) demonstrated that in dogs, the metaphyses of limb bones (75%) are most commonly affected by tumours – the pelvic limb (femur, tibia, and fibula); the thoracic limb (humerus, ulna, and radius); less frequently – the jaws, skull, ribs, scapula, and pelvic bones (25%). Extraskeletal osteosarcoma may also be recorded. The authors provided a classification of osteosarcoma, structured according to the modified TNM system, which assesses two parameters: T – primary lesion, presence or absence of distant metastases (M0 and M1); T0 – the tumour is not identified; T1 – identified involvement of the periosteum and bone; T2 – the process extends beyond the periosteum. The authors provided information on characteristic cytological studies of tumour aspirates: isolated, and sometimes densely grouped (up to 50 cells) tumour elements are revealed. Some cell groups may resemble papillary structures. Based on the size and polymorphism of cells, monomorphic and polymorphic forms are distinguished. In the latter, cells of varying sizes from large to giant predominate. The nuclei are oval or round, located eccentrically, with the presence of 1 to 3 hypertrophic nucleoli. Satellite nuclei are typical. The cytoplasm has an irregular shape, is basophilic, usually vacuolated, and may contain small granules. Numerous mitotic figures are often found.

A clinical case of femoral osteosarcoma in a 27-year-old Arabian pony (gelding) was described by R. Pfeifle *et al.* (2021). Clinical signs of the disease were accompanied by weight loss, swelling of the pelvic region on the left side, and a pronounced lameness in the left pelvic limb. Haematological studies revealed neutropenia and thrombocytopenia. Radiographic examination established destructive-lytic changes in the bone tissue of the proximal part of the femur. A biopsy confirmed osteoblastic osteosarcoma. The animal was treated with radiation therapy, which proved ineffective, and the animal was euthanised.

G. Doden *et al.* (2021) discovered that neoplastic pathology in giraffes is poorly described in scientific literature, with a lack of collected clinical material, despite the widespread breeding and zookeeping of these animals. A clinical diagnosis of neoplasia is primarily established during post-mortem examinations of deceased animals. The authors focused on the study of 30 deceased giraffes from 22 zoos in the United States of America. During autopsies, age-related changes in the body and the presence of tumours that led to death were established in 13 animals. At the same time, 15 animals with chronic conditions were euthanised for clinical reasons, and tumours were found at necropsy. Two animals underwent antemortem tumour biopsies for morphological examination. Pathohistological examination confirmed the presence of various types of tumours: leiomyoma (7), adenoma (4), luteoma (4), lymphoma (4), pheochromocytoma (3), squamous cell carcinoma (3), adenocarcinoma (2), ameloblastic fibroma (1), carcinomatosis of undetermined cell lineage (1), cavernous haemangioma (1), cystic granulosa cell tumour (1), dysgerminoma (1), fibrosarcoma (1), leukaemia (1), lipoma (1),

pituitary nerve sheath tumour (1), rhabdomyosarcoma (1), and teratoma (1). In most cases, neoplasms were detected during autopsy. The published material confirms the importance of further studying pathology in this species.

M. Esmaili Nejad *et al.* (2019) described chondroblastic osteosarcoma in dogs and its aggressive course. They noted that the appendicular skeleton is most frequently affected by this tumour. The article provides detailed data on the aggressive course of the disease with a high degree of metastasis in a large, mixed-breed dog. *In vivo*, radiographic examination and computed tomography of the patient demonstrated significant lytic changes in the area of the maxilla with spread to the orbital region. Metastatic lesions of the lungs were found in the form of a large number of nodules of various sizes. Cytological examination revealed pleomorphic mesenchymal cells of oval to spindle shape with signs of malignancy. Histomorphological evaluation of bone tissue revealed areas of bone differentiation containing osteoid foci with extensive fields of cartilaginous differentiation with chondroblasts in lacunae. Based on the studies conducted, a diagnosis of chondroblastic osteosarcoma with diffuse lung metastases was made. The animal was euthanised.

R. Weinschenk *et al.* (2021) demonstrated that chondrosarcoma is the second most common primary bone tumour. Most conventional (classic) chondrosarcomas are locally aggressive (originating in the medullary portion of the bone), low-grade malignant, and non-metastasising tumours compared to high-grade chondrosarcomas. Conventional chondrosarcomas can sometimes dedifferentiate into a very aggressive high-grade sarcoma with a poor prognosis – these are socalled dedifferentiated chondrosarcomas. Grade I lesions rarely metastasise or recur and have a 10-year survival rate of 80%. In contrast, grade III lesions have a poor prognosis and the highest recurrence rate, with a 50% incidence of metastases and a 10-year survival rate of 30%.

H. Fattahian *et al.* (2023) highlighted the importance of differential diagnosis in bone neoplasms. They investigated and described a clinical case of a 4-year-old spayed female Spitz dog with an osteoma. The authors noted the overall prevalence of bone neoplasms in dogs and cats. Clinical examination revealed characteristic symptoms such as lameness in the left pelvic limb, pain on manual palpation, and general ataxia during walking. A radiographic examination revealed a periosteal reaction and osteolysis of the head of the right femur. Osteobiopsy and histological examination revealed trabecular formations of irregular shape with varying degrees of mineralisation, surrounded by a single layer of osteoblasts and osteoclasts. The fibrovascular stroma of the tumour was adjacent to areas of bone tissue with pronounced signs of sclerosis, and no mitotic figures were found in the material. Thus, the clinical diagnosis of periosteal osteoid osteoma was confirmed.

B. Carneiro *et al.* (2021) and K. Tepelenis *et al.* (2021) indicated that osteoid osteoma is a fairly common bone tumour in young people. The typical clinical picture is a pain syndrome at night, which decreases with the use of non-steroidal anti-inflammatory drugs. Radiographic and computed tomography images show lytic changes in the tumour focus, signs of demineralisation, possible cortical thickening, and sclerotic changes in the bone. However, the tumour may manifest as an intra-articular osteoid osteoma or have an epiphyseal localisation, multicentric foci, and even atypical radiographic manifestations. Therefore, a morphological study can establish an adequate diagnosis and prevent an unfavourable prognosis.

In a scientific study, L. Leonardi (2022) presented data on the morphological differentiation of common oncologic tumours comparing dogs and humans, specifically central osteosarcoma

(conventional or classic osteosarcoma). Microscopic characteristics of classic osteosarcoma are reflected in a variety of histotypes. General features are characterised by a population of neoplastic cells, which are represented in varying proportions and have a spindle, ovoid, plasmacytoid, and epithelioid structure, as well as multinucleated osteoclast-like giant cells. In all cases, there are signs of the formation of osteoid matrix produced by malignant cells. The degree of cellular atypia is high, with variable-sized eosinophilic cytoplasm of neoplastic cells. The nuclei in the cells are located centrally or eccentrically, with pronounced signs of hyperchromasia, formed nucleoli, and a large number of atypical mitotic figures. At the same time, the following histological types of osteosarcoma were distinguished based on their structure: undifferentiated osteosarcoma, consisting mainly of mesenchymal cells with a high degree of anaplasia and pleomorphism; osteoblastic osteosarcoma with abundant but varying amounts of osteoid deposition (productive and nonproductive forms); chondroblastic osteosarcoma with a predominance of the cartilaginous cell component; fibroblastic (a subtype in which spindle-shaped cells predominate); telangiectatic osteosarcoma – a highly aggressive sarcoma characterised by the presence of blood-filled cavities lined by tumour cells, rather than endothelium as in hemangiosarcoma, with signs of osteoid production; giant cell osteosarcoma (in this form, osteoclast-like giant cells predominate).

T. Guim *et al.* (2019) described extraosseous osteosarcoma, a malignant mesenchymal tumour that develops outside of bone tissue and sometimes adjacent to the periosteum. The tumour is built on the phenotype of an osteoblast and produces atypical bone tissue. The tumour occurs spontaneously, and no breed predisposition has been observed in animals. The tumour was most frequently recorded in the mammary glands (80.6%), subcutaneous tissue (5.6%), liver (5.6%), spleen (2.8%), omentum (2.8%), and kidneys (2.8%). Histological examination of tumours confirmed the presence of various types: osteoblastic (61.1%), chondroblastic (33.3%), fibroblastic (2.8%), and giant cell osteosarcomas (2.8%). Metastases to the lungs were more frequently observed with mammary gland osteosarcoma. At the same time, L. Leonardi (2022) proved that this type of sarcoma is a primitive osteosarcoma that arises in tissues other than bone, without primary involvement of the skeleton. Extraosseous osteosarcomas have been described in several animal species, with the mammary glands being most commonly affected in dogs and cats, followed by other organs and tissues such as the spleen, subcutaneous tissue, gastrointestinal tract, skin, muscles, liver, urinary system, thyroid gland, and salivary glands, regardless of the animal breed. Typically, extraosseous osteosarcoma presents as an infiltrative mass with rapid growth and irregular internal areas of ossification, mixed with haemorrhage and necrosis. In all tissues, especially in the mammary glands, the tumour can arise from metaplastic bone foci or malignant transformation of myoepithelial cells. Extraosseous osteosarcoma is microscopically similar to other bone osteosarcomas and is always highly malignant and metastatic.

D. Barrantes Murillo & T. Negrão Watanabe (2023) described an osteoblastic osteosarcoma of the left clavicle in a 33-year-old female red-bellied lemur (*Eulemur rubriventer*). L. Moreira *et al.* (2020) investigated osteoblastic osteosarcoma in a scarlet macaw (*Ara macao*). The tumour is considered a rare disease in birds. The clinical picture was characterised by significant swelling in the distal part of the femur and proximal part of the tibia. At the same time, ulcerative pododermatitis and cachexia were observed. After euthanasia, a post-mortem examination and histopathological study established osteoblastic osteosarcoma with metastases

to the liver and lungs. Thus, research into the detection of osteosarcoma in humans and animals continues. Certain clinical and diagnostic successes have been achieved in verifying the clinical diagnosis of osteogenic tumours. A final diagnosis is established after a radiographic examination and the results of an osteobiopsy. This pathology is insufficiently described and studied in wild animals kept in artificial conditions, particularly in giraffes.

Materials and Methods

The study was conducted between 2022 and 2024 at the Krakow Zoo (Ogród Zoologiczny w Krakowie) and the pathology department of the medical centre Universal Clinic "Oberig" in Kyiv. The article presents a clinical case of a tumour pathology of the carpal joint in a giraffe. All experimental procedures involving the animal were performed in adherence to the fundamental principles of bioethics. These principles align with Article 26 of the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (2006), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (European Convention, 1986), and the "General Ethical Principles for Animal Experiments" adopted by the First National Congress on Bioethics (2012).

A clinical and instrumental examination was conducted on a reticulated giraffe (*Giraffa camelopardalis reticulate*), aged 7.8 years, male, named Malik, weighing approximately 550 kg. For 6 months, the animal exhibited lameness in the left thoracic limb, swelling and thickening of the left carpal joint, which gradually increased. There was limited flexion during walking, which affected its motor activity (Fig. 1). Overall weight loss of the animal was noted, especially in comparison with two other males of the same age. Therefore, to achieve the goal, in this case, an anaesthesia protocol was used to induce the animal into a state of anaesthesia. The animal was fasted for 24 hours, and water was withheld for 12 hours before the diagnostic procedures. Anaesthetic agents were injected remotely at a distance of 3-4 meters from the animal intramuscularly using a Telinject pneumatic gun (Germany). The injection with a "flying" plastic syringe was performed smoothly, not explosively, which was perceived calmly by the animal (Fig. 2).

Figure 1. Neoplasm of the carpal joint
Note: *thickening and enlargement of the left carpal joint*
Source: *authors' photo*

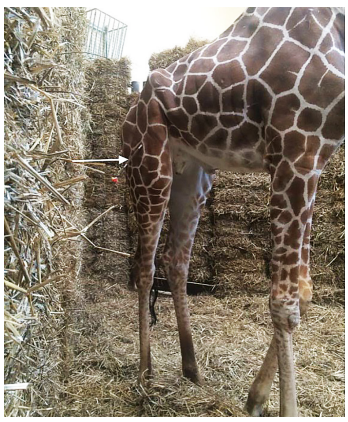

Figure 2. Administration of medetomidine using a flying syringe
Note: *injection site of the anaesthetic*
Source: *authors' photo*

Following the administration of Medetomidine HCL 10 mg/mL (NexGen Pharmaceuticals, USA) at a dose of 0.05 mg/kg body weight, the effects of the anaesthetic were recorded over the next 20 minutes. The giraffe's movements in the enclosure slowed down, skeletal muscle tremors appeared, and subsequently, the giraffe stopped, lowered its head and neck forward, and slightly abducted its forelimbs. At this level of anaesthesia, Bioketan 100 mg/mL (Vetoquinol, France) was additionally injected intramuscularly at a dose of 3 mg/kg body weight. After 15 minutes, the giraffe lay on its left side, and a surgical level of general anaesthesia was recorded. Monitoring of the course of general anaesthesia was carried out by counting the respiratory rate, auscultation of heart sounds, thermometry, observing the corneal reflex and pupil size, and subjectively assessing peripheral vascular tone by pressing a finger on the gums until they turned white, and recording the time to restore a light pink colour. Additionally, to study the cardiovascular system (heart rate and arterial blood oxygen saturation), an Innomed Heart Screen 60G VET electrocardiograph with a pulse oximeter (Hungary) was used.

Subsequently, a clinical and instrumental examination was conducted using manual joint examination and a digital radiographic unit Gierth TR 90/30 (Germany) in two projections, anteroposterior and lateral. The results of the radiographic examination of the joint revealed a diffuse destructive osteolytic process with clear signs of neoplasia. After the euthanasia of the animal, to resolve the stated goal and morphological verification of the clinical diagnosis, a necropsy was performed and a histological examination was carried out. Tissue samples were taken from the pathologically altered areas of the carpal joint of the animal and placed in a labelled container. Tissue samples were fixed by complete immersion in 10% buffered neutral formalin (pH 7.2, exposure 6-72 hours).

The tissue was processed for histotechnology in an STP120 Microm/Thermo scientific (Germany) carousel-type tissue processing apparatus, undergoing dehydration in a battery of ascending concentrations of alcohol and fixation in paraffin. Subsequently, paraffin blocks were prepared using an EC350 Microm/Thermo scientific (Germany) tissue embedding centre. Serial microtomy with a section thickness of 3-5 μm was performed on the obtained blocks using an HM340E Microm/Thermo scientific (Germany) rotary microtome, additionally equipped with an STS Microm/Thermo scientific (Germany) section transfer system. The specimens were placed on standard glass slides followed by deparaffinisation and histological staining in an HMS 740 Microm (Germany) tissue staining automaton. Hematoxylin (Harris Hematoxylin Accified series S, Epredia) and eosin (Eosin Y Alcoholic series R/ Epredia, Spain) were used for staining. After completion of the staining process, the tissue specimens were mounted under a coverslip using a Micromount / DiaPath (Italy) mounting medium. The tissue microscopic preparations were subsequently examined by light microscopy using an Olympus BX 46 (Japan) microscope equipped with an Olympus SC 50 (Japan) camera, with sequential examination at magnifications of x4, x10, x20, and x40 (with a x10 ocular lens).

Results and Discussion

General anaesthesia in giraffes has always been considered a complex anaesthetic procedure with a typically unpredictable course. Various factors influence this. The species-specific characteristics of the animal, namely: significant body mass, height, untamed nature, fearfulness, and sensitivity to stress, do not allow for diagnostic studies (palpation, biopsy, radiographic examination) without the use of general anaesthesia. Therefore, the preparation included: a specially equipped enclosure measuring

15x15 meters, the floor of which was covered with a thick layer of straw, and the walls of the enclosure were padded. These measures were necessary to prevent injury to the animal during the induction of anaesthesia and recovery.

When a deep level of general anaesthesia was achieved, the animal's body was positioned so that the head and neck were higher than the torso to prevent disturbances in hemodynamics and the occurrence of cerebral hypertension. Several specialists, during the course of the examination, constantly manually massaged the neck muscles, as spastic contractions that may occur leading to stress myopathy (Fig. 3).

Figure 3. Giraffe under anaesthesia
Note: *performing a neck muscle massage*
Source: *authors' photo*

Clinical palpation of the carpal joint revealed: an increase in volume over the entire area, absence of local temperature changes, hard tissue consistency, limited range of motion during flexion and extension of the limb, and the presence of indistinct crepitus. Further radiographic examination of the joint was performed in two projections, anteroposterior and lateral. Significant diffuse destructive-lytic changes in the epiphyseal part of the forearm bones with metaphyseal involvement were established (Fig. 4). Given the anamnestic data, the course of the disease, and the results of the clinical and instrumental examination of the joint, the animal was euthanised, followed by a pathomorphological examination of the neoplasm of the carpal joint tissues. During the autopsy, extensive areas of destruction with growths of a voluminous invasive formation were found. The tumour tissue was light grey in colour, rough, and finely granular in consistency, with areas of brownish-red discolouration and a crumbly, brittle consistency in these zones, which corresponded to foci of necrotic changes with haemorrhagic infiltration. The structure of the joint appeared completely disrupted, with tissue breakdown (Fig. 5).

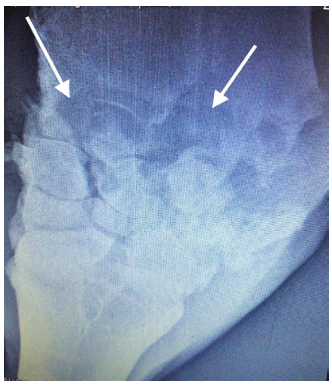

Figure 4. X-ray of the joint tumour
Note: *destructive-lytic changes in the joint*
Source: *authors' photo*

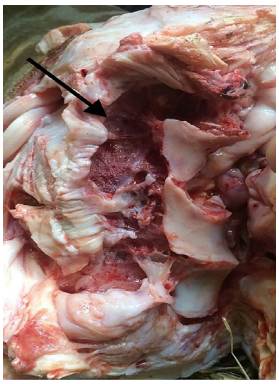

Figure 5. Macroscopic changes in the joint tumour
Note: *visualisation of the joint cavity during necropsy*
Source: *authors' photo*

A morphological study established the presence of growths of mesenchymal tumour with cortical destruction, with an invasive character of growth into the surrounding soft tissues. The tumour tissue was composed of an extracellular fibrous matrix and tumour cells, which demonstrated a variety of structures and had a sarcomatoid, spindle-cell structure with pronounced signs of cytological and nuclear pleomorphism; neoplastic cells were mostly large in size, had a moderate amount of eosinophilic cytoplasm, in places with indistinct intercellular boundaries; the nuclei of the cells had signs of anisonucleosis, were hyperchromatic, and multinucleated cytological forms were focally found, which in their structure were similar to osteoblasts; in the volume of the tumour tissue, signs of tumour incomplete osteogenesis were noted in the form of the formation of a significant number of immature bone trabeculae (Fig. 6-9).

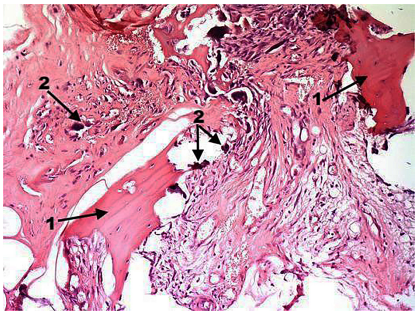

Figure 6. Focal incomplete tumour osteogenesis with immature bone trabeculae and proliferation of atypical cells
Note: *1 – osteoid; 2 – tumour cells of different histotypes. Decalcification, haematoxylin-eosin staining x200*
Source: *authors' photo*

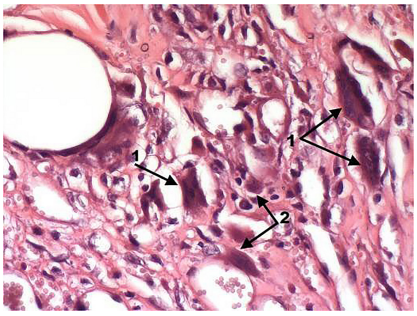

Figure 8. Atypical multinucleated cells of the osteoblastic series
Note: *1 – multinucleated cells; 2 – tumour cells of different histotypes. Decalcification, haematoxylin-eosin staining x400*
Source: *authors' photo*

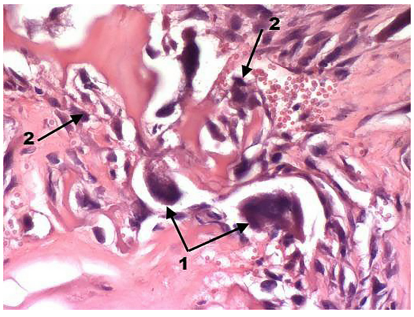

Figure 7. Focal incomplete tumour osteogenesis with immature bone trabeculae and proliferation of atypical cells
Note: *1 – multinucleated cells; 2 – tumour cells of different histotypes. Decalcification, haematoxylin-eosin staining x400*
Source: *authors' photo*

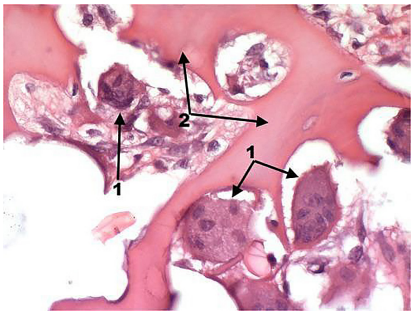

Figure 9. Atypical multinucleated cells of the osteoblastic series among foci of incomplete tumour osteogenesis in the form of the formation of immature bone trabeculae
Note: *1 – multinucleated cells; 2 – tumour osteoid. Decalcification, haematoxylin-eosin staining x400*
Source: *authors' photo*

Therefore, the morphological changes identified are consistent with osteoblastic osteosarcoma. Accordingly, the histopathological diagnosis of osteoblastic osteosarcoma was established. The conducted studies confirm the data on osteosarcoma in various animal species. A challenging issue remains the detection and early diagnosis of oncological diseases in wild animals kept in artificially created conditions. Most of them are not domesticated, and any diagnostic procedure (blood sampling, X-ray examination, ultrasound, etc.) requires the use of general anaesthesia. More often than not, tranquillisation is insufficient, and it is necessary to anaesthetize the animal to a surgical level of anaesthesia. Accordingly, it is difficult to study large animals such as giraffes, elephants, rhinoceroses, antelopes, and others.

The presented study involved a complex set of preparatory measures, which included: equipping an enclosure where the clinical and instrumental examination of the giraffe was carried out, pre-anaesthetic preparation of the animal, training specialists to perform diagnostic procedures, and checking the equipment used (X-ray machine, electrocardiograph, necessary set of surgical instruments). Induction into anaesthesia and its course proceeded bypassing the excitation stage, slowly, gradually the animal reached the surgical level of anaesthesia necessary for the safe performance of all diagnostic studies.

J. Nguyen *et al.* (2022) and D. O'Neill *et al.* (2023) established that the radiographic appearance of osteogenic sarcoma depends on the morphological variant of the tumour (osteoblastic, osteolytic, mixed), location, and growth rate. The area of involvement in radiographs, in most clinical cases, does not have clear boundaries. The most characteristic features are manifested by the destruction of the cortical layer and the appearance of extraosseous components of the tumour. One of the most pathognomonic criteria for sarcoma in these cases is the detachment of the periosteum in the form of a formation at the edge of the cortical layer defect.

In the presented radiographic study, the tumour node had indistinct contours with signs of a massive soft tissue component of the epiphyseal part of the bone of the carpal joint with involvement of the growth plate and cartilage, more likely of intramedullary location. This clinical manifestation of the disease in the giraffe was manifested by a progressive increase in the volume of the affected joint, and lameness and limited movement indicated pain for several months. At the same time, when flexing the joint, which was determined by palpation, a slight crunch was manifested, indicating destructive changes in the bones of the joint.

S. Myagkov *et al.* (2019), M. Harkusha & R. Mihraliev (2021) and M. Kudlya (2024) reported the spread (metastasis) of tumour cells through lymphatic vessels to lymph nodes. In general, they should be examined during an examination and surgically removed, especially those located near the tumour. The examination of lymph nodes is important for two reasons: the first is the collected information that is used to determine the stage of the disease; the second is the increased risk of spread to other organs of the body. In human medicine, this criterion is used to provide additional therapy for the patient (chemotherapy, radiation therapy or immunotherapy).

The use of various individual drug therapy methods for appendicular osteosarcoma in dogs was investigated by A. Poon *et al.* (2020). The authors focused on those clinical trials that may have a high degree of efficacy in terms of prolonging the life of the animal. Y. Nakano *et al.* (2021) evaluated and described the frequency of metastasis in cats. In 67 patients with histologically confirmed osteosarcoma of the appendicular skeleton, surgery was performed

to amputate the limb. The frequency of distant metastases was 46.3% (31/67). The average survival time after surgery was 527 days. It is clear that these aspects in this study of the giraffe can only be informative in terms of the spread of the pathological process. Macroscopically, the surrounding lymph nodes in the giraffe did not change.

K. Makielski *et al.* (2019) and K. Silver *et al.* (2023) described classic osteosarcoma as an intramedullary malignant tumour whose cells produce osteoid. It is termed primary when it develops in unaltered bone and is the most common primary high-grade sarcoma. It can appear after trauma to an area of bone, implantation of metal osteosynthesis, etc. It is believed that osteosarcoma develops from mesenchymal stem cells with minimal osteoblastic differentiation, however, the "cell of origin" remains unknown. It is more commonly observed in various genetically determined syndromes. In the conducted study, the tumours of the relatively young giraffe do not exclude a genetic factor in the aetiology of the disease. After all, it is known that specific changes in genes can increase the risk of developing osteosarcoma and other types of cancer.

T. Fossum (2019) and K. Dittmer & S. Pemberton (2021) proved that during the macroscopic examination of osteosarcoma tumours, an affected zone is revealed involving the metaphyseal part of the bone. The tumour can be located intramedullary, grey-white in colour and heterogeneous. Upon direct examination of the tumour, intensely mineralised areas of bone are revealed, and mucous foci and myxoid matrix, haemorrhages, areas of necrosis, and cystic formations can also be found. At the same time, the periosteum, in the form of a soft tissue component, is without clear contours and of various sizes.

In this study, during the autopsy of the giraffe's joint, longitudinal destruction of the articular cartilage of irregular shape was established, its fragmentation with the spread of the tumour intramedullary and with signs of lytic structures in the direction of the metaphysis. On the section, a brittle consistency, haemorrhages, and necrosis were noted. D. Meuten (2017) indicated that during the histological examination of osteosarcoma, a fairly wide spectrum of morphological changes was determined. The tumour grows from the bone marrow area and changes and destroys bone trabeculae. Neoplastic cells are characterised by moderately expressed cellular atypia and pleomorphism: they can be epithelioid, plasmacytoid, spindle-shaped or rounded. The cytoplasm is more often eosinophilic or light. The presence of neoplastic bone tissue is pathological osteoid. The amount of the latter does not matter, it is produced by neoplastic cells. According to the author, osteosarcoma is divided into several histological variants: osteoblastic, chondroblastic, fibroblastic, giant cell, osteoblastic-like, epithelioid, clear cell, and chondroblastic-like.

Based on the results of the histological study of the tumour of the carpal joint of the giraffe presented in the article, the presence of incomplete tumour osteogenesis in the form of the formation of immature bone trabeculae was established, atypical multinucleated cells of the osteoblastic series and atypical pleomorphic cells of other histotypes were found, which confirmed the diagnosis of osteoblastic osteosarcoma.

Chondrosarcoma has been described in the research of A. Zając *et al.* (2021), A. Gazendam *et al.* (2023) and G. Kask *et al.* (2023). The distinguishing feature of chondrosarcoma from osteosarcoma lies in the spread of the primary malignant bone sarcoma. As with osteosarcoma, this tumour affects long bones, especially those of the pelvic limbs, and can also occur in the bones of the pelvis and ribs. Chondrosarcoma

can be of varying degrees of malignancy: aggressive low-grade, non-metastasising tumour, and high-grade malignancy. Most conventional chondrosarcomas, both low-grade and high-grade, are primary and begin to develop in the medullary part of the bone. At the same time, some proportion of tumours can occur secondarily within an enchondroma or on the surface of the bone from a pre-existing osteochondroma. The final diagnosis is based on a morphological study. J. Vetter *et al.* (2023) provided information on guinea pig (*Cavia porcellus*) disease with fibroblastic osteosarcoma. The diagnosis was confirmed at the Faculty of Veterinary Sciences of the National University of Asunción (Paraguay).

Thus, based on the clinical, instrumental, and morphological studies presented in the study, the relevance of studying oncopathology in wild animals has been confirmed, in particular, the identified and histologically proven features of the course of osteosarcoma in giraffes. It has been established that in the diagnosis of joint bone tumours, all components of a clinical examination play an important role: collecting anamnestic data, conducting radiological and histological studies, and interpreting the obtained results.

Conclusions

Osteosarcoma is a malignant bone tumour that arises from multipotent mesenchymal stem cells capable of differentiating into cartilage and bone. It is characterised by the production of atypical osteoid and bone structures by malignant proliferating tumour cells. According to the results obtained from the study, the development of a malignant neoplasm in a giraffe (*Giraffa camelopardalis reticulata*) was accompanied by a characteristic clinical course observed in animals with bone oncopathology. Conducting a clinical and diagnostic examination was complicated by many factors, one of which was the natural stress response during direct contact with the animal, which hindered the conduct of even simple, at first glance, diagnostic procedures. When an increase in the volume of the carpal joint was detected, clinical observation of the animal and the course of the disease was carried out. Over several months, marked lameness was noted with increased limitation of movement when walking, the joint increased, which indicated a pain syndrome. Therefore, to achieve the goal of diagnostic measures, general anaesthesia was used.

In this condition, it was possible to conduct a radiographic examination of the joint and establish a tumour lesion of the bones and periarticular tissues. Subsequently, the selected and fixed in 10% buffered formalin material was subjected to histological examination. As a result of the histopathological studies, a sufficient spectrum of morphological changes characteristic of osteoblastic osteosarcoma was obtained, namely: the presence of atypical cells with invasive growth and multinucleated cells of the osteoblastic series, incomplete tumour osteogenesis, extracellular matrix with the growth of pleomorphic cells. Thus, it should be noted that for the diagnosis of bone osteosarcoma, it is important to compare the radiographic findings of the affected area with the subsequent analysis of histological preparations.

Further directions for conducting scientific research on bone oncopathology in animals kept in zoos and wild animals living in nature have great prospects for continuation, as this expands the understanding and information about the morphological and biological characteristics of tumours that form bone.

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Conflict of Interest

The authors declare no conflict of interest.

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Остеосаркома у самця жирафа (*Giraffa camelopardalis reticulata*)

Андрій Марунчин
Кандидат ветеринарних наук, головний лікар
Ветеринарна клініка «УніВет»
02002, вул. Микільсько-Слобідська, 6Е, м. Київ, Україна
https://orcid.org/0009-0001-1035-457X

Беата Квятковська-Паліс
Ветеринар
Приватна практика, ветеринарний кабінет «AGAMA»
32-046, вул. Міноґа, 181, м. Міноґа, Польща
https://orcid.org/0009-0000-4836-7627

Марсін Паліс
Ветеринар
Приватна практика, ветеринарний кабінет «AGAMA»
32-046, вул. Міноґа, 181, м. Міноґа, Польща
https://orcid.org/0009-0002-8359-6470

Анастасія Ковальова
Завідувач патологоанатомічного відділення
Медичний центр «Універсальна клініка «Оберіг»
03057, вул. Зоологічна, 3, м. Київ, Україна
https://orcid.org/0000-0002-9886-3685

Катерина Терещенко
Лікар ветеринарної медицини, магістр
Ветеринарна клініка «УніВет»
02002, вул. Микільсько-Слобідська, 6Е, м. Київ, Україна
https://orcid.org/0009-0009-8786-8163

Анотація. Актуальність дослідження полягала у відсутності достатньої інформації щодо описаних клінічних випадків захворювання парнокопитних тварин, що відносяться до родини Жирафових (*Giraffidae*), на онкопатологію, а саме остеогенну саркому. При цьому, для оцінки ступеня ураження кістки та віддаленого метастазування у дрібних свійських тварин застосовують комп'ютерну томографію, магнітно-резонансну томографію та рентген. Однак, для диких тварин такі методи візуальної діагностики провести вкрай складно. Тому мета дослідження полягала у верифікації клінічного діагнозу – остеогенна саркома в жирафа. Для вирішення цього питання тварині застосовано загальний неінгаляційний наркоз, проведено діагностичне клінічне та рентгенологічне дослідження суглобу, а також патоморфологічну верифікацію тканин із пухлинним ростом. Відібраний від тварини матеріал досліджували гістологічно. В результаті клінічного огляду жирафа відмічалося збільшення в об'ємі та потовщення зап'ясткового суглобу лівої грудної кінцівки. Згідно прояву клінічних симптомів, патологічний процес поширився через кортикальний шар і розтягнув окістя зап'ясткового суглобу. Окрім цього, відмічали характерні прояви хронічного болю та виснаження тварини. Відповідно до результатів рентгенівського знімку з'ясували,

що у жирафа присутні ознаки ураження кісток та хряща суглобу з картиною агресивного і деструктивного характеру, а також остеолітичні зміни з незначною кальцифікацією. При цьому, кортикальний шар кістки залишився неушкодженим, лише епіфізарно-метафізарна ділянка кісток передпліччя. Завдяки проведенню патологогістологічних досліджень встановили остеобластичну остеосаркому. Таким чином, за результатами комплексних досліджень отримали нові данні щодо особливостей перебігу остеосаркоми у жирафа, патоморфологічно – характеристику злоякісної пухлини, що формує кістку. Відповідно, отримані результати розширили та доповнили інформацію щодо патогенезу онкопатології кісток у диких тварин, що є важливим для ветеринарної практики і науки. Тому практичне значення цього дослідження полягає у поглибленому розумінні патоморфології онкологічних захворювань апендикулярного скелета

Ключові слова: пухлинний остеогенез; нуклеарний плеоморфізм; атипові клітини; остеоїд; клініко-діагностичне дослідження; патологогістологічне дослідження