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Patho-histological features of fibroblastic sarcoid in horses

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Sarcoid is one of the most common skin tumours, which is diagnosed in various breeds of horses at any age regardless of the animal's sex. Such fibroblastic neoplasm manifests itself in several clinical ways with its specific morphological features. As a rule, it metastasizes rarely but can be locally invasive. The disease's etiology is multifactorial and includes the confirmed role of the bovine papillomavirus and genetic and environmental factors. The article introduces the results of a retrospective study of horses of the Ukrainian riding breed with a fibroblastic sarcoid type, which was localized mainly in the area of the external thoracic vein. Neoplasms were represented by two types. The first one included broad base neoplasms without a pronounced pedicle but with signs of hyperkeratosis. Massive rounded dense formations with an ulcerated surface refer to the second type. To verify the studied types of sarcoid, wide surgical excision of the lesion's visible parts was performed, followed by the selection of fragments for the pathohistological diagnosis. Also, additional staining methods were used to detect the connective and muscle tissue (Van Gieson's staining) and elastic fibers (Weigert's resorcin-fuchsin staining). Microscopically, the neoplasm without a visible pedicle showed signs of hyperkeratosis and acanthosis in the peritumoral epidermal layer. Epidermis desquamation with a significant amount of oxyphilic detritus, diapedesis hemorrhages, and dilated vessels was also detected in some areas. Chaotic placement of fibroblasts and fibrocytes was noted, to a greater extent, subdermally. Anisocytosis and anisokaryosis were expressed moderately. Sarcoids, macroscopically presented in the form of massive dense formations with an ulcerated surface, were histologically characterized by the replacement of the epidermis with cellular elements, subdermal hemorrhages, and the characteristic perpendicular orientation of coarse collagen fibers towards the basement membrane. Closely spaced spindle-shaped cells with fibers forming moiré structures were visualized in the deep dermis layers. Nuclei were hyperchromic and elongated in shape, no atypical mitoses were noted. Pathohistological examination during the identification of a sarcoid type for diagnostic purposes can cause rapid growth and formation of an ulcer. The final diagnosis regarding equine sarcoid can be made based on a combination of clinical signs, histopathological examination, and molecular genetic testing with the isolation of BPV DNA.

Keywords: skin; papillomavirus; hyperkeratosis; necrobiosis; collagen fibers; moiré structures; fibroblasts.

Introduction

Equine skin diseases are usually a serious problem that limits the use of horses in professional sports. Sarcoid is common worldwide, being one of the dominant tumorigenic diseases in horses (Ragland et al., 1970; Cotchin, 1977; Miller & Campbell, 1982; Marti et al., 1993; Knowles et al., 2016). These fibroblastic neoplasms of the skin usually do not metastasize but show a variety of clinical manifestations, ranging from aggressive infiltrative growth to "spontaneous" regression (Ragland et al., 1970; Lane, 1977). Immunotherapy and treatment have not produced the expected results, since neoplasms frequently demonstrated locally aggressive growth, were large in size with multiple lesions and their removal was complicated due to their localization in hard-to-reach places. One of the reasons for disqualification of expensive sport horses at competitions was the aesthetic effect (disfiguring the exterior) and anatomical location of the neoplasm (preventing them from fully performing exercises).

Nowadays, the sarcoid etiology is still debated, but most scientists believe its cause in horses is a virus related or identical to bovine papillomavirus (BPV) of types 1 and 2, and probably type 13 (Otten et al., 1993; Marti et al., 1993; Chambers et al., 2003; Koch et al., 2018). The conducted studies revealed that bovine papillomavirus DNA was present in almost 100% of the examined sarcoid tumours (Bogaert et al., 2005; Bogaert et al., 2007; Taylor & Haldorson, 2013; Epperson & Castleman, 2017). In addition, BPV-1 DNA was detected in flies that were in the vicinity of horses suffering from sarcoid, which may indicate their direct role in the transmission and spread of this virus (Taylor & Haldorson,

2013). However, Derek C. Knottenbelt noted in his studies that the role of papillomaviruses in the development of sarcoid remains unclear. Nevertheless, a large part of these neoplasms contained genetic material identical or very similar to the one found in some papillomaviruses (Knottenbelt & Matthews, 2001; Nasir & Campo, 2008; Finlay et al., 2009).

Sarcoid was first described and characterized as a separate clinical entity in 1936 by C. Jackson. The scientist determined that this type of tumour is widespread throughout the world and could affect other equids at any age and of any sex: donkeys, zebras, mules. The virus is transmitted through insect bites, incorporates itself into the DNA of affected skin cells, causing the transformation of these cells into tumours. Olsen and Cook were the first to suggest a possible role of bovine papillomavirus as an oncogenic equine sarcoid virus in 1951 when they investigated the transmissibility of papillomas. Injections of bovine papilloma extract into the skin of horses caused "sarcoid-like" tumours. A study of equine skin pathologies in which DNA of bovine papillomavirus (BPV) was available, especially in normal skin, suggested that the virus may be in a latent state and is activated after stimulation by an unknown agent. It is the virus latency that may explain why sarcoids recur after complete surgical removal and proves their ability to appear at sites of damage or trauma.

It has been determined that sarcoid neoplasms can occur in any part of the body, both individually and in groups, but the head, ventral part of the abdomen, and limbs are affected most often. Skin injuries, surgical wounds, microtraumas after injections, or even insect bites promote the progression or recurrence of neoplasms (Bergvall, 2013; Knottenbelt et al., 2016; Oghuszka et al., 2021). Some scientific sources report that only one

type of sarcoid is usually recorded in one animal, but there have been cases when sick horses were observed to have several sarcoids during their lifetime. Those neoplasms might remain static for months or years and then slowly or suddenly become aggressive and progress for no apparent reason.

As a rule, sarcoid is locally invasive and does not metastasize, except in the malignant form, where the spread occurs by the lymphogenic route with the formation of numerous neoplasms along the lymphatic vessels and in the lymph nodes (Kasperowicz et al., 2006; Knottenbelt et al., 2016). Spontaneous regression is rarely reported, and new sarcoid tumours usually do not develop in such horses. Only one study in a population of Franches-Montagnes horses in Switzerland found a high rate of spontaneous remission (Berruex et al., 2016). Equine sarcoid had a high recurrence rate after treatment, especially after surgical removal. Recurrent neoplasms are more aggressive than the primary lesion, demonstrate a tendency for rapid growth and occupy a larger lesion area. Recurrent sarcoid can emerge weeks to months or years later.

Depending on macroscopic structure, sarcoids are divided into six different types: warty, nodular, fibroblastic, mixed, malignant, and latent.

Warty sarcoid is a neoplasm localized on the muzzle, at the ear base, and in the groin. Those lesions are alopathic and cause neither itching nor pain. They have a typical warty appearance with pronounced hyperkeratosis. Warty sarcoids mostly grow slowly but can progress to a more aggressive form, especially as a consequence of trauma (Scott & Miller, 2003).

Nodular sarcoid is a subcutaneous, easily mobile mass, often but not always spherical, covered by intact, clinically normal skin. Nodules are divided into types A and B. There is no strong connection between the skin and the subcutaneous tissue in case of type A nodules. Type B nodules have a clear skin lesion and the skin surface cannot move independently (Foy et al., 2002; Gebre et al., 2018).

Fibroblastic sarcoid is the most aggressive form that can develop from any other type of neoplasm after accidental or iatrogenic manipulation, including biopsy (Foy et al., 2002). The neoplasm has a fleshy appearance, sometimes with ulcers, which are covered with a scab under which the granulation tissue is formed. Ulcerous surfaces attract flies, which contribute to irritation, local infection with bacteria, and the development of miasms. Groin, abdomen, wounds, and distal parts of the limbs are the typical locations of fibroblastic sarcoids. Cutaneous wounds, especially on the distal extremities, are also at risk for fibroblastic sarcoid. Fibroblastic sarcoid is divided into two types. Type 1 is characterized by a pedicle without thickening at the neoplasm's base, mainly with a fleshy head from normally pigmented skin or the formation of ulcers due to constant trauma. Type 2 has a broad local base with the skin thickening in the form of a large callus (Scott & Miller, 2003; Yuan et al., 2011; Wilson et al., 2013).

Mixed sarcoid (occult, warty, nodular, and fibroblastic) is a combination of two or more of these types and may represent a progressive or transitional state between warty or occult, fibroblastic or nodular (Hartl et al., 2011). Most sarcoids can be classified as mixed because some types (verrucous, nodular, and fibroblastic) are often present in different variations within the same lesion. If it is difficult to determine which type of sarcoid predominates, and the classification of mixed sarcoid is used. This can happen in the phase of transition or progression from one clinical type to another. The muzzle, eyelids, groin and medial part of the thigh are the places where the mixed type of sarcoid is detected most often.

Malignant sarcoid is a particularly invasive type of tumor which can invade the lymphatic vessels, causing the formation of multiple tumours along the vessels and local lymph nodes. The malignant type is most often localized on the skin of the jaw and muzzle, elbow, and medial part of the thigh (Knottenbelt, 2005).

Occult sarcoid is a small area of skin without hair or with thin hair and altered pigmentation. Typically, it is a rounded alopecia-like formation with nodules about 2–5 mm in diameter localizing on the skin around the mouth, eyes, and neck, especially in those places where the number of hair follicles is smaller. However, occult sarcoid can affect large surfaces, and some horses can have even several such lesions (Giraldo et al., 2020). Itching and pain are absent. This type of sarcoid grows slowly and can turn into a verrucous one. Occult sarcoid rarely affects the extremities.

In practice, veterinarians have tried several protocols for sarcoid treatment, which generally showed positive dynamics, but the response to the therapy was not always consistent and effective in various breeds of horses. Modern sarcoid treatment primarily involves antitumour therapy, but the development of preventive measures in the future may be directed against the causative papillomavirus agent (Hallamaa et al., 2005; Scagliarini et al., 2012; Knottenbelt, 2019). In Ukraine, like in many European countries, sarcoid remains an important clinical problem, especially for thoroughbred horses. However, almost no cases of this disease have been found in Ukraine until recently.

Our work aimed at verifying sarcoid, determining its type, and finding out some pathohistological features of organization of this neoplasm.

Materials and methods

During the studies, we followed the general rules of the appropriate GLP laboratory practice (1981), positions of the General Ethical Principles of the Experiments on Animals, approved by I National Congress of Bioethics (Kyiv, 2001). The whole experimental part of the study was conducted according to the requirements of the International Principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986), Rules of Studies using Experimental Animals, approved by the Decree of the Ministry of Healthcare No. 281 as of November, 1, 2000, "On Measures for Further Improvement of Organization Forms of Studies using Experimental Animals" and the corresponding Law of Ukraine "On Protection of Animals against Abuse" (No. 3447-IV as of 21.02.2006, Kyiv).

This epizootological study is based on a retrospective analysis of 152 horses during 2019–2022, mainly of the Ukrainian riding breed in the country's western region, of which 52 animals were clinically diagnosed with sarcoid. Neoplasms were localized on the abdomen (19), chest area (17), limbs (9), and neck (7). Tumours localized in the abdominal area were surgically removed along with the removal of neoplasms under general and local anesthesia in a standing position. During the clinical examination, all horses were healthy, except for existing neoplasms. Surgically, using a scalpel, a wide excision of the lesion's visible parts was performed. The preventive measures to avoid autoinoculation with tumour cells were taken, following all rules of antiseptics. A punch biopsy was performed when the neoplasm was localized in hard-to-reach places (chest area, neck) near large vessels and occupied minor, mostly limited foci of up to 0.5 cm. All the manipulations were performed with the use of analgesic and sedative drugs, depending on the weight of the animal and operative removal method in compliance with the fixation techniques and the rules of the European Convention on animal treatment. The article describes the cases of neoplasms localized in the abdomen.

Fragments of neoplasms had been selected for histopathological examination and were fixed in a 10% neutral formalin solution. After fixation, the neoplasm was excised, taking into account its tissue localization, followed by washing in tap water during 24 hours. Histological cassettes with fragments were dehydrated in an ascending series of alcohols, starting with 70°, and increasing the concentration by 10% with exposure for 12 hours each. After bringing it to absolute ethanol, the exposure was reduced to six hours. The next stage was transferring the fragments to a 1:1 mixture of xylene and absolute ethanol for 1 hour, then xylene I for 1 hour and xylene II for 1 hour. Finally, the fragments were kept in a 1:1 mixture of xylene and paraffin. Paraffin was soaked in two portions in a thermostat at the temperature of 56 °C with exposure for 1 hour. The material was poured into blocks and cooled. Histological 7 µm-thick sections were prepared from the obtained blocks using a sledge microtome MC-2. The sections were mounted on glass slides by the method of extraction from distilled water. The mounted sections were dried in a thermostat for 12 hours at the temperature of 37 °C. Deparaffinization was carried out using xylene and followed by rehydration through a descending series of alcohols to distilled water (Merkulov, 1969). Staining was performed using Ehrlich's hematoxylin and eosin. After dehydration in an ascending series of alcohols through xylene, the sections were enclosed in a synthetic medium, and covered with a cover glass. To verify the sarcoid type, additional staining according to the Van Gieson method was used for collagen fibers, and resorcin-fuchsin according to Weigart for elastic fi-

bers. Histological examination and photofixation were performed using a Leica DM-2500 light microscope with a Leica DFC 450C camera and Leica Application Suite 4.4 software.

Results

Macroscopically, sarcoid in some horses was localized in the area of the external thoracic vein in the form of a neoplasm with a broad base without a pronounced pedicle, round and elongated in shape, of a dense consistency with signs of hyperkeratosis, 3 to 8 cm in diameter (Fig. 1a).

In other horses, it occurred in the form of massive rounded tumours with a narrower base (pedicle) and an ulcerated surface (Fig. 1b), which attracted insects. After its surgical removal, the sarcoid sections were examined. Neoplasms were represented by dense fibrous tissue with the expressed massive and perpendicularly located strands at the periphery (Fig. 1c, d).

Histological examination of skin fragments around the sarcoid revealed slight hyperkeratosis. Sebaceous glands of a typical morphological structure were located in the reticular layer of the dermis, which opened into the hair follicle channel (Fig. 2a). The preserved hair shafts in the hair follicles were visible on the cross-sectional micro-preparations (Fig. 2b).



Fig. 1. Sarcoid: *a* – a neoplasm with a broad base without a pronounced pedicle, rounded and elongated in shape with signs of hyperkeratosis; *b* – a massive neoplasm with a surface covered with ulcers; *c, d* – a cross-sectional view of sarcoid

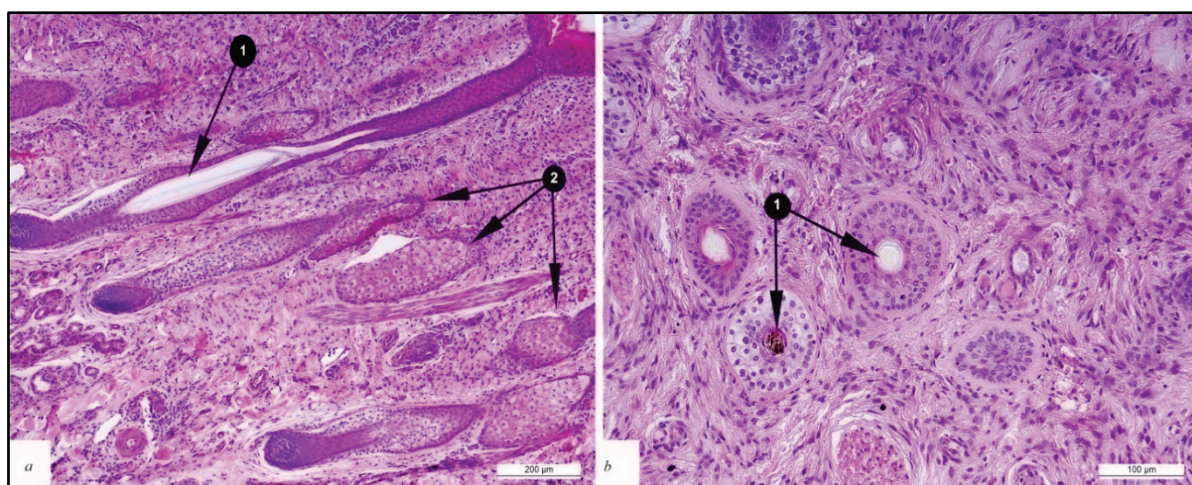


Fig. 2. Horse skin around sarcoid: *a* – reticular layer of dermis with a hair bulb and hair (1), sweat glands (2); *b* – cross section of the dermal layer with hair follicles (1); hematoxylin and eosin

Dilated capillaries, diapedesis hemorrhages, and signs of hyperkeratosis and acanthosis (Fig. 3a, b) with the epidermis desquamation and the formation of a significant amount of oxyphilic detritus were noted in the

peritumorous epidermal layer (Fig. 4a, b). The demarcation line was weakly expressed. A significant number of lymphoepithelial elements, segmented neutrophilic granulocytes, and activated macrophages were

visualized in places of necrobiosis (Fig. 4b). Chaotic placement of fibroblasts and fibrocytes with hyperchromatic nuclei was noted subdermally. Coarse collagen fibers were localized, and to a greater extent, in the deep layers of the dermis, vessels were dilated, vascularization was insignificant, anisokaryosis and anisocytosis of tumorous elements were moderately pronounced, and mitoses were rare (Fig. 4c, d). Neoplasm growth was infiltrative and invasive.

The replacement of the normal dermis components by fibroblasts and the proliferation of coarse collagen fibers is a characteristic morphological feature of all sarcoids. Staining with picrofuchsin according to the Van Gieson method revealed that the neoplasm stroma was mainly represented by stellate fibroblasts and coarse spindle-shaped fibers, which were stained bright red (Fig. 5a, b, d).

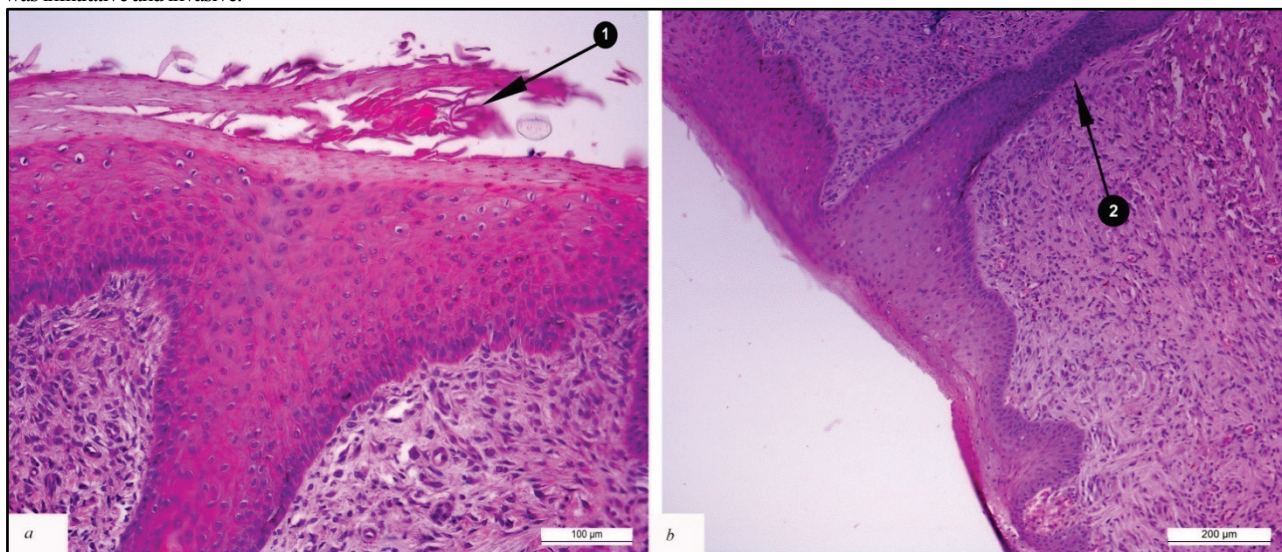


Fig. 3. Horse skin around sarcoid: *a* – hyperkeratosis (1); *b* – acanthosis (2); hematoxylin and eosin

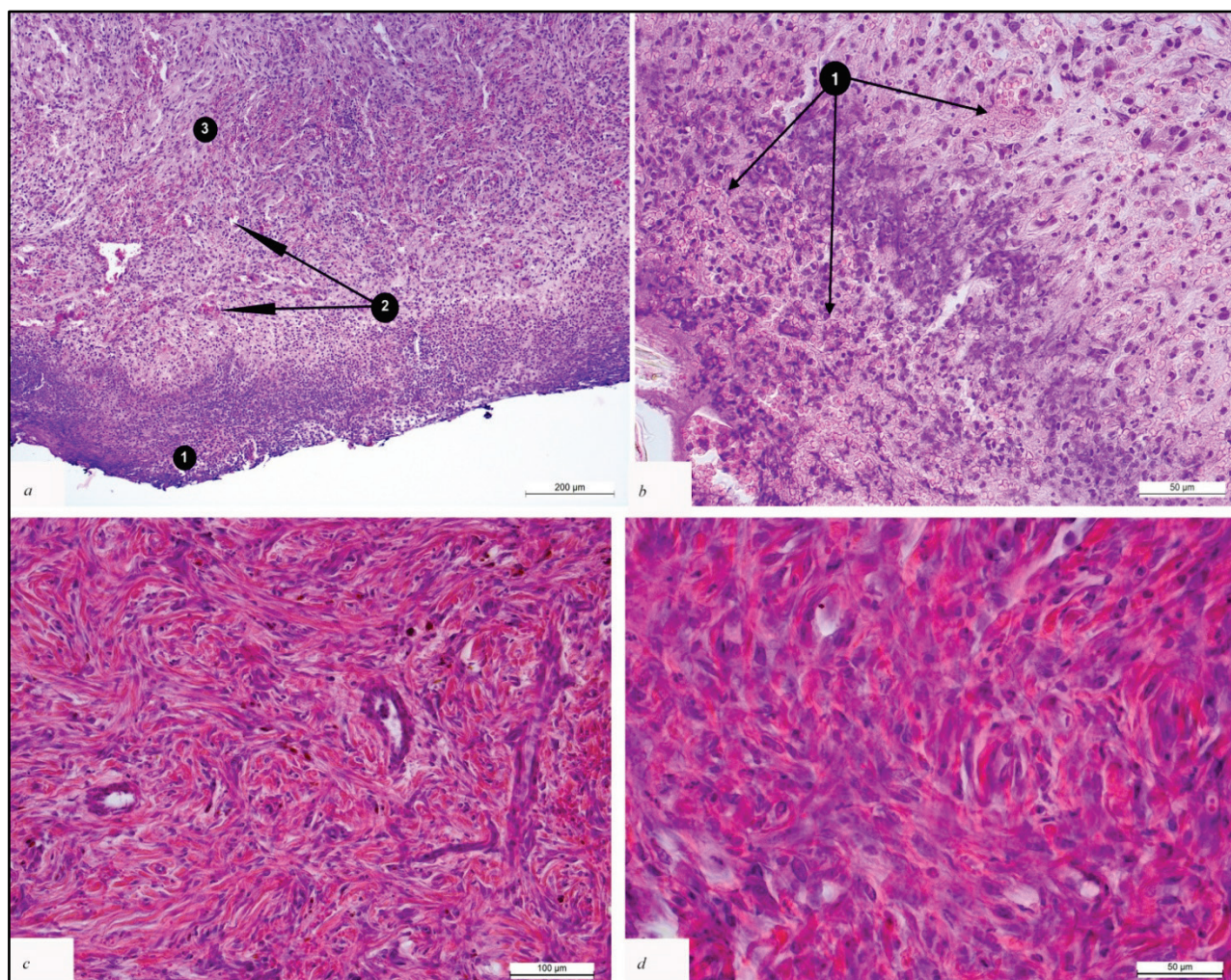


Fig. 4. Fibroblastic sarcoid: *a* – the epidermal layer desquamation (1), expansion and blood filling of the capillaries (2), foci of fibroblastic cellular elements' with atypical growth (3); *b* – cellular infiltration with hemorrhages in the peritumorous epidermal layer; dilated and blood-filled capillaries (1); *c, d* – spindle-shaped neoplastic cells in the deep layers of the dermis with hyperchromatic nuclei that form moiré shapes; hematoxylin and eosin

Cell nuclei were dark brown and oval; cellular atypism was expressed weakly. In the subdermal layer, fibroblasts were located mainly perpendicular to the basement membrane in the form of strands (Fig. 5c), while intradermally, a higher density of collagen fibers was noted, which were intertwined in the form of moiré (Fig. 5a, b, d). The amount of collagen matrix in fibroblastic sarcoid varies from minimal to significant, which determines a macroscopic structure of neoplasm. Collagen fibers occupied the bulk of the neoplasm in the examined sarcoids, intradermally forming dense massive conglomerates.

Weigert's resorcin-fuchsin staining method was used to detect elastic fibers in the studied neoplasms of the horses' skin, revealing no elastic fibers in the structure of the examined sarcoids (Fig. 6a, b).

Analyzing the microscopic changes in the studied cases of equine sarcoid, it is possible to note the regularity of the proliferation of dermal fibroblasts. The observed macroscopic and microscopic signs of the fibroblastic-type sarcoid were characterized by the following features: hyperkeratosis, complete epidermis ulceration with massive infiltration of polymorphonuclear cell elements, vessel expansion, moderate vascularization, and subdermal and intradermal proliferation of coarse collagen fibers that formed long twisted moiré structures. The epidermal ulcer was the feature of Type 1 fibroblastic sarcoid, which was observed to have the growth of a massive neoplasm on the pedicle, occupying both the superficial and deep skin layers. The demarcation line is not expressed.

Discussion

Sarcoid tumours are usually characterized by focal thickening of the skin, changes in both skin pigmentation and structure. However, a diagnosis is impossible to make based on macroscopic signs alone, so pathohistological examinations are a key element in verifying this pathology. It is important to note that due to the variable microscopic features of equine sarcoid, small biopsies may not provide sufficient tissue to differentiate sarcoid from other skin lesions, such as granulation tissue, fibroma, or fibrosarcoma. Therefore, preference should be given to excisional biopsy material, which will provide the largest amount of diagnostic material (Knottenbelt, 2005; Wobeser et al., 2010).

According to the scientists, the pathohistological pattern depends on the clinical type of sarcoid (Martens et al., 2000; Wobeser, 2017). Thus, for example, in case of warty sarcoid, the epithelial component is affected more than the dermal one, and is represented mainly in the form of a small band of active fibroblasts in the epidermis. In the case of fibroblastic sarcoid, there is always partial or complete ulceration of the epidermis with infiltration of polymorphonuclear cells (Martens et al., 2000). When it comes to nodular sarcoid, the epidermis is often thinned (Martens et al., 2000). If the dermal proliferation does not contact the epidermis, the latter is normal. In case of occult sarcoid, the epidermis is usually normal or has only minor changes.

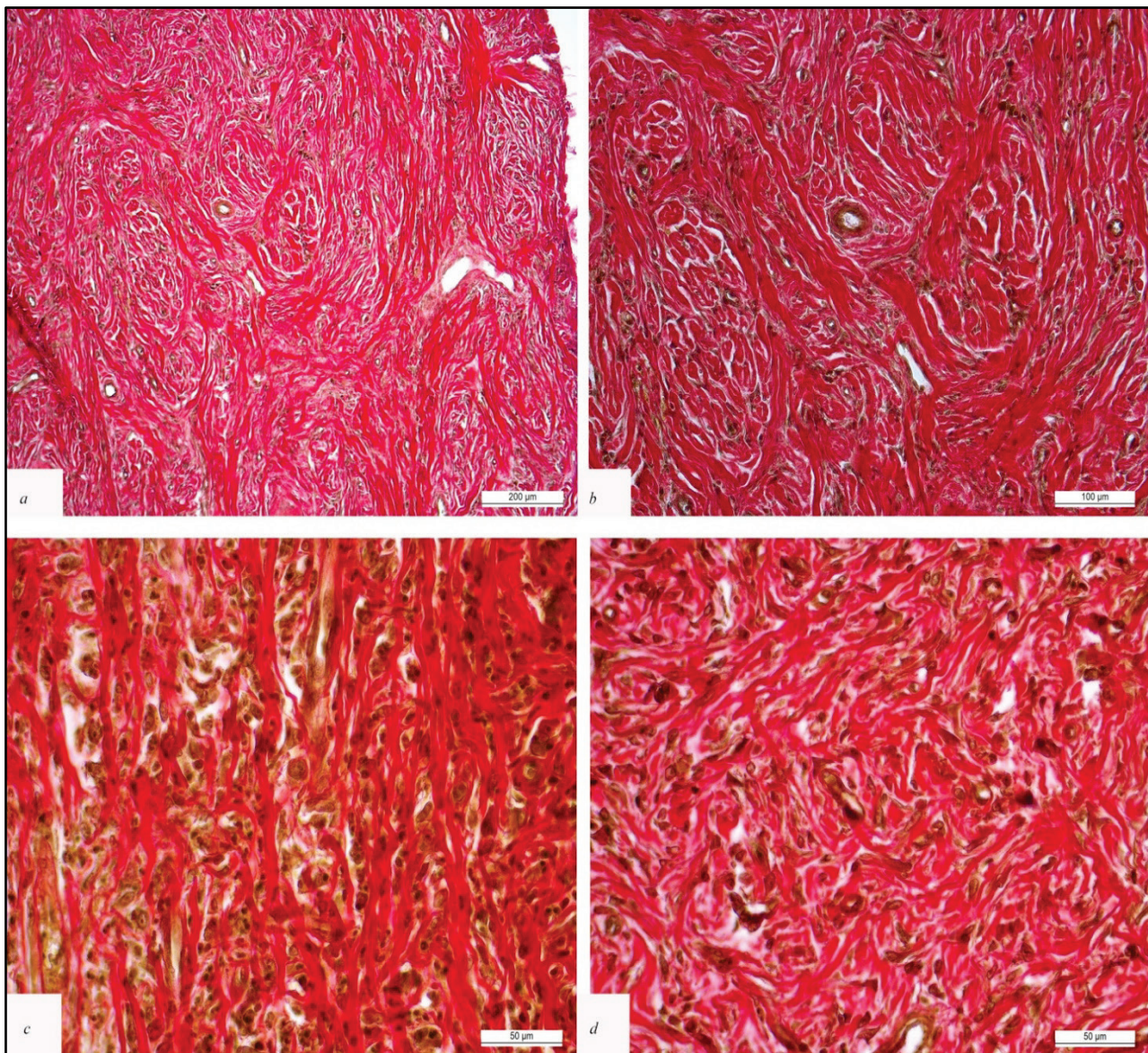


Fig. 5. Fibroblastic sarcoid: *a, b, d* – intradermal placement of coarse collagen fibers in the form of bundles intertwining with each other in a moiré pattern; *c* – fibroblasts are located perpendicular to the basement membrane in the form of strands. Van Gieson

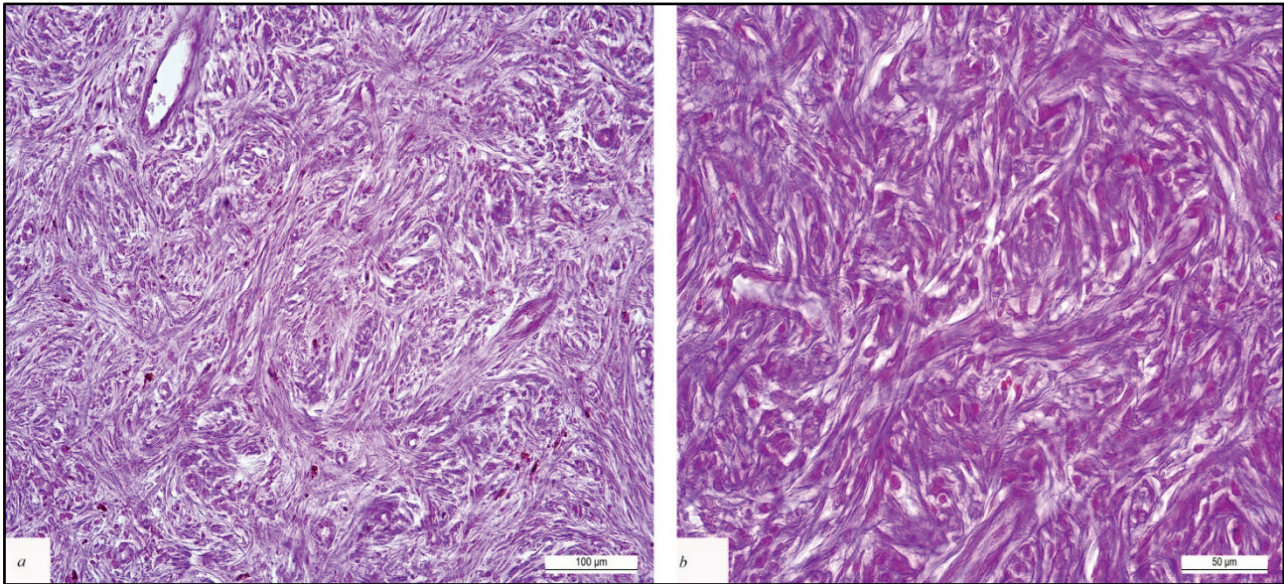


Fig. 6. Fibroblastic sarcoid: *a, b* – the absence of elastic fibers between rough collagen fibers, which are chaotically intertwined; resorcin-fuchsin staining according to Weigert

The only typical aspect of this sarcoid type is the increased density of subepidermal fibroblasts, interspersed between the reduced number of hair follicles and sweat glands (Martens et al., 2000). They do not demonstrate a typical morphology or a specific moiré distribution. The number of dermal fibroblasts is also lower compared with other sarcoid types. The only feature common to all sarcoid types is that dermal fibroblasts are denser than such of the normal skin (Martens et al., 2000). Nodular sarcoid can mimic a schwannoma, although its clinical appearance and the BPV availability indicate the sarcoid origin of tumor (Bogaert et al., 2011).

The results of our pathomorphological examinations of equine sarcoids of the Ukrainian riding breed are coherent with the results of other studies, which reported that the histological feature of all equine sarcoids is an increased density of dermal fibroblasts, as compared with the normal skin. The typical histological changes found in the fibroblastic type of sarcoid included the proliferation of coarse, densely interwoven bundles and coils of spindle-shaped fibroblasts in the dermis. In most cases, the epidermis was partially or completely ulcerated with massive infiltration of polymorphonuclear cellular elements without a visible demarcation line, dilated vessels, and petechial hemorrhages.

The formation of cells and fibers that united into bundles, forming so-called “palisade structures” was noted subdermally and intradermally. Histopathological examinations conducted by Terwid et al. on 100 equine sarcoids also showed a high frequency of epidermal hyperplasia, the formation of “palisade structures”, which were visualized in 67%, 88% and 91% of cases, respectively. Our research is also consistent with the studies reporting fibroblastic proliferation during the sarcoid histological examination, as well as the presence of spindle-like curls that formed chaotic structures in the dermis. The number of mitoses is small, but the appearance of fibroblastic orientation at a perpendicular angle to the epidermal basement membrane is also different but available in a high percentage of tumours. The epidermis, if available, was usually hyperplastic with a characteristic elongated spinous layer, although in many cases, it was normal or even atrophic with marked hyperkeratosis.

Therefore, the diagnosis of sarcoid should include three components: clinical, pathohistological, and molecular genetic studies with the detection of BPV DNA. A thorough clinical examination combined with a detailed history (duration of problems, localization of lesions, age, breed, number of lesions, etc.) should be sufficient in making a preliminary diagnosis. The lack of clinical experience or atypical characteristics of tumour can confuse veterinarians, and therefore additional laboratory diagnostics are needed for a correct diagnosis. Histopathological examination is often decisive, but it should be noted that biopsy, particularly of small and stable lesions, can cause their rapid growth and ulceration. If a non-excisional biopsy is required, sites within the tumour should be carefully selected to include intact epidermis and minimize the factors that would promote sur-

rounding inflammation and granulation (Goodrich et al., 1998; Haspelagh et al., 2018). The possible deterioration of sarcoid after biopsy is the reason why biopsy of warty, occult, and small nodules is contraindicated, even if a definitive diagnosis is impossible in such cases. Another option is to perform complete surgical excision of a sarcoid, including excision of wide margins of the normal skin, and a noncontact approach followed by histological confirmation. Thus, the pathologist would be able to verify the morphological features of tumour and make the correct diagnosis (Goodrich et al., 1998). A more modern approach to the diagnosis of equine sarcoid is the detection of BPV DNA in lesions using polymerase chain reaction (PCR). This can be done on histopathological specimens of the tissue with suspected equine sarcoid but without typical histological features (Angelos et al., 1991). Detection of BPV DNA using PCR has many advantages, namely, it is non-invasive, causing minimal trauma to the tumour (Trewby et al., 2014). Disadvantages include its inability to diagnose occult sarcoid, lower sensitivity compared with histopathologic diagnosis, and low specificity due to the high prevalence of BPV DNA in the normal equine skin (Martens et al., 2001; Bogaert et al., 2011; Gysens et al., 2022).

Conclusion

Diagnostics of skin diseases of horses remains an urgent problem and requires the attention of many Ukrainian and foreign researchers. Unfortunately, the etiology of many diseases is still unknown, and the available treatment methods are not always effective. Sarcoid is the most common type of tumour that horse owners and veterinarians have to deal with. It is a locally invasive skin neoplasm with various clinical manifestations. Therefore early diagnostics with prompt treatment can improve the prognosis. Spontaneous regression is rare, relapses are common, and exacerbations are a possible complication, especially when a wrong therapy is attempted. Animals affected by sarcoid can never be considered disease-free, and horse owners must be properly informed about the characteristics and behavior of this tumour.

The etiology of equine sarcoid is multifactorial, including bovine papillomavirus, genetic, and environmental issues. Usually, the treatment of this pathology depends on the lesion type, its localization, and degree of changes. The fibroblastic type of sarcoid is the most aggressive neoplasm form, characterized by hyperkeratosis and acanthosis with epidermis desquamation, dilation of capillaries, subdermal hemorrhages, lymphoepithelial infiltration in places of necrobiosis, undefined demarcation line, massive proliferation of coarse collagen fibers (especially in the deep dermis layers), and absence of elastic fibers in the formation structures.

The authors declare that they have no potential conflict of interest concerning the authorship or publication of this article.

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