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Case Report

Equine Sarcoid Associated with Cutaneous Habronemosis

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ABSTRACT

Equine sarcoids are benign fibroblastic skin tumors affecting equids worldwide. Infection with bovine papillomavirus types 1 and 2 has been implicated as a major fact in the disease development; however, the cellular mechanisms underlying fibroblast transformation are still largely unknown. In the present study, a diagnosis of sarcoid was histologically assessed along with eosinophilic dermatitis. The sarcoid lesion expressed the viral oncoproteins E5 and E2, suggesting a causative role of the virus and its replication. Ribosomal DNA of the nematode *Habronema muscae* was also revealed in the lesion. This is the first report to describe and discuss an association of cutaneous habronemosis with equine sarcoid.

1. Introduction

Equine sarcoids are fibroblastic skin tumors affecting horses, mules, and donkeys [1]. They are locally invasive, often occurring at sites of previous injury or scarring, the most common sites being the skin of the head, ventral abdomen, legs, and the paragenital region [2]. Bovine papillomavirus (BPV) is considered as the etiological agent of this tumor. Both BPV types 1 and 2 (BPV-1 and BPV-2) have been detected in sarcoid tumors, with BPV-1 being the predominant type [3-5].

Equine sarcoid is a biologically attractive tumor, as it is a known case of natural cross-species infection by a papillomavirus [6]. Moreover, although BPV infection in cattle produces benign lesions that may regress, the sarcoids are nonpermissive for virus production, locally aggressive, and nonregressing [2].

BPV-1 E5 oncogene encodes for the 44-amino-acid-long major BPV oncoprotein [7,8], which has been shown to be expressed in sarcoids [5,9] and to play an important role in transformation in in vitro models [10]. The E2 protein encoded by all papillomavirus types is approximately 42 kDa in size and contains a C-terminal DNA binding and dimerization domain and an N-terminal transactivation domain [11]. BPV E2 has a role in maintenance of episomal DNA of the virus in dividing host cells, simultaneously associating with chromatin and the viral genome during mitosis [12]. BPV-1 E2 protein interacts with viral helicase E1 and is sufficient for initiation of DNA replication. E2 can form heterodimers with two truncated E2 proteins, and the complex serves as activator of E2-dependent transcription and papillomavirus DNA replication [13].

Habronemosis is a parasitic disease of equids (horses, donkeys, mules, and zebras) caused by *Habronema muscae* and *Habronema microstoma*; a similar infection, namely, draschiosis is caused by a closely related species, that is, *Draschia megastoma*. The adult nematodes belonging to the *Habronema* genus inhabit the glandular portion of stomach of the host, with a special predilection for the margo plicatus without internal migration [14]. Embryonated eggs

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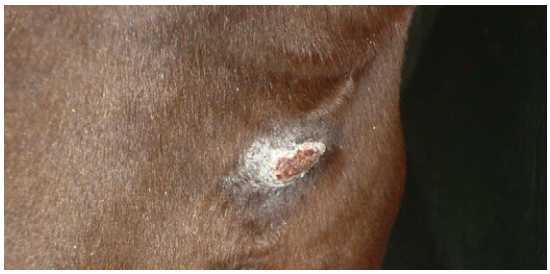

Fig. 1. The subcutaneous mass in the ventral region of the neck had the appearance of a verrucous sarcoid.

are excreted in the feces to the environment, where they are ingested by the larvae of intermediate hosts, represented by flies (e.g., house and stable flies), developing in the manure. Development of the parasite is synchronous with the development of the flies, and third-stage larvae ($L3_S$) of the infectious nematode are deposited on the vertebrate host when the insects feed on the host's skin, mucosae, or wounds. When a fly feeds on the horse's lips, $L3_S$ emerge from the fly and are swallowed by the horse, resulting in the completion of the nematode's life cycle [15]. *Habronema* larvae that are deposited on mucous membranes, on injured skin, or on mucocutaneous transition sites do not complete their life cycle but may induce a local inflammatory reaction causing cutaneous habronemosis (the so-called "summer sores").

"Summer sores" are severe, proliferative, exuberant, and granulomatous lesions, often showing seasonal recurrence during the warm weather, coinciding with the peak of fly populations and may evolve in tumor-like masses [16]. Although this severe form of habronemosis is often described, especially in temperate regions [17], little is known about the prevalence of the disease, mainly because of clinical diagnostic limitations. More specifically, a clinical diagnosis of summer sores is unreliable, as there is a range of equine skin diseases that should be considered as differential diagnoses and, among these diseases, sarcoids [18].

The aim of the present report is to describe for the first time, a case of cutaneous habronemosis associated with equine sarcoid.

2. Materials and Methods

A case of subcutaneous mass arising in an 8-year-old Maremmano horse from Lazio Region (Fig. 1) was referred by a practitioner in May 2010 in an ongoing epidemiological survey about the prevalence of equine sarcoids in Italy. The horse underwent surgery, and the lesion was fixed in 10% formalin for histological examination.

The origin of the eosinophilic inflammation (see Results) was further investigated by a polymerase chain reaction (PCR)-based assay. In particular, genomic DNA was extracted from formalin-fixed paraffin-embedded tissue samples using the DNEasy Tissue Kit (QIAGen, Milan, Italy) according to the manufacturer's protocol. DNA sample was subjected to a duplex seminested PCR to amplify a species-specific fragment ($\sim$200 bp for *H microstoma* and $\sim$400 bp for *H muscae*) internal to the ribosomal ITS2 of *Habronema* spp, with slight modifications to the previously described protocol [19].

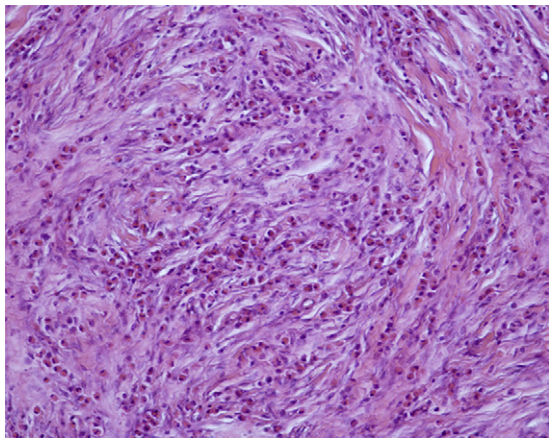

Fig. 2. Equine sarcoid and eosinophilic inflammation. Dermal proliferation of fusiform and spindle-shaped fibroblasts can be observed. A massive eosinophilic infiltration is evident. Hematoxylin and eosin, $\times$240.

For immunohistochemistry, polyclonal rabbit antibody specific for E2 oncoprotein (1:300, gift from Prof. E. Androphy, University of Massachusetts) and polyclonal sheep anti-E5 (1:5000, a gift from Prof. M. S. Campo, Institute of Comparative Medicine, University of Glasgow) were used. Secondary detection was performed with a commercial streptavidin–biotin kit (LSAB kit, DakoCytomation, Glostrup, Denmark), as previously described [5].

3. Results

The cutaneous lesion was histologically diagnosed as sarcoid. The dermal proliferation of fusiform or spindle-shaped fibroblasts typical of this tumor was associated with inflammatory condition and massive eosinophilic infiltration (Fig. 2). To establish whether BPV-1 viral proteins were expressed in this sarcoid sample, the presence of the E5 and E2 proteins was analyzed by immunohistochemistry. Almost all neoplastic cells from the tumor sample displayed cytoplasmic E5 immunoreactivity. E5 was located mostly within the cytoplasm of neoplastic fibroblasts. In particular, a typical juxtanuclear immunoreactivity was also observed. Normal fibroblasts in the area adjacent to the tumor did not stain (data not shown). E2 expression was detected in almost all neoplastic fibroblasts in the tumor sample, and the immunosignal was nuclear (Fig. 3). Normal fibroblasts adjacent to neoplastic area as well as other cell types in the mesenchyme were negative for E2. No parasitic larvae were identified in the histologic cuts.

The seminested PCR yielded an amplicon of the expected size for *H muscae*. The sequencing of the amplicon demonstrated that the sequences represented appropriate regions internal to the ITS2 of *H muscae*.

4. Discussion and Conclusion

To the best of authors' knowledge, the present study shows the association of habronemosis with equine sarcoid for the first time. Cutaneous habronemosis is a skin

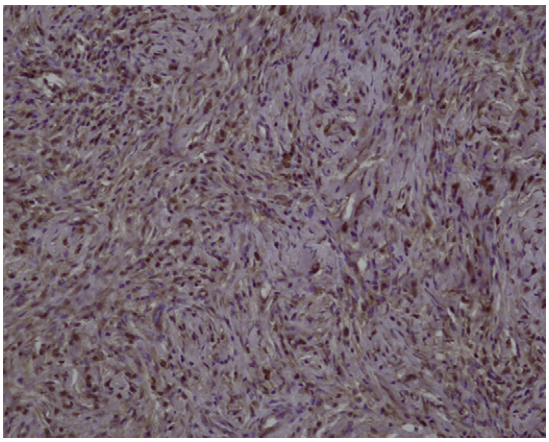

Fig. 3. Equine sarcoid stained with specific antibody for BPV-1 E2 protein. Almost all nuclei of neoplastic fibroblasts show E2 nuclear immunosignal. Streptavidin–biotin–peroxidase method was used. Mayer's hematoxylin nuclei counterstain.

disorder affecting horses and other equids, which is caused by the atypical deposition of the larvae of *Habronema* on skin wounds, mucocutaneous junctional areas around the eyes, and on other abnormal skin areas that have moisture or discharges to attract infected flies. The larvae migrate internally, irritate the tissue, and cause inflammation and induce an allergic immune response characterized by eosinophilic infiltration [20,21]. In the present case, a dermal proliferation of fibroblasts, an inflammatory condition, and a dense eosinophilic infiltration were observed microscopically, and the skin lesion was molecularly revealed to be positive for *H muscae* DNA. Given that no larvae were found histologically, it is likely that the nematode could have been already disintegrated, but the dermatological lesion remained.

Viral proteins E5 and E2 were also detected in the lesion, and specifically, to the best of authors' knowledge, this is the first time that E2 protein expression has been found in a sarcoid, suggesting that the BPV-1 DNA had undergone replication and transcription and hence the infection was active.

BPV-associated cancer is a multifactorial disease, and several steps are required before full neoplastic transformation is achieved. Like many viruses, BPV can establish a latent infection. Normal epithelia are the accepted site of viral latent infection [22], and the damage of the epithelium, possibly through production of inflammatory cytokines, induces expression of viral genes leading to tumor formation [23]. There is also evidence that normal skin in horses may also function as reservoirs of BPV [22]. We speculate that normal skin contained latent BPV-1 whose gene expression was triggered by the inflammatory condition due to *H muscae*, and this in turn induced sarcoid.

Additionally, from a comparative point of view, it is noteworthy that the strong association of schistosomiasis and bladder cancer harboring human papillomavirus DNA is well known, suggesting a role of parasitosis as a cofactor in PV-associated tumors [24]. Further studies are required to determine whether *Habronema* infection may act as a cofactor in BPV-induced carcinogenesis in equine hosts.

Finally, as habronemosis and sarcoid may coexist in the same patient, and the diagnosis of habronemosis is difficult owing to the existence of different equine skin conditions with overlapping clinical appearance [18], it is suggested that histological biopsy is a useful tool for a reliable differential diagnosis.

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