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Review

Molecular diagnosis of certain nematode infections can save life and beauty, and preserve breeds of socially relevant and sporting animals

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Abstract

The recognition that the health and welfare of some humans are improved through contact and relationships with animals is now established. Two commonly recognized assistance animals are dogs and horses. Both provide therapeutic benefits to humans with some physical and mental illnesses and both assist people with disabilities. Moreover, the public and scientific attention to the health and conservation of many animal breeds is also increasing worldwide.

In the past few years, two potentially life-threatening nematode infections that can induce tumours or tumour-like masses in canids and equids, spirocercosis and draschiosis/habronemosis, respectively, are emerging in several areas of the world. This article reviews and comments how recent insights into the molecular early diagnosis of these diseases can save and preserve life, beauty and breeds of socially relevant and sporting animals.

Keywords: Spirocercosis; Draschiosis/Habronemosis; Dog; Horse; Molecular diagnosis

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1. Introduction

Chronic infections by bacteria, viruses and parasites lead up to the 18% of world's tumours (Parkin, 2006).

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Among parasites, helminths play an important role in the aetiology of several cancers, even though the mechanisms by which they induce malignant transformation of host cells is still a moot issue (IARC, 1994). The best investigated parasite-induced tumours are those caused by platyhelminthes (e.g., *Schistosoma* and *Opistorchis*), in which it has been demonstrated that inflammatory and molecular events may cause malignant transformation of normal tissues (IARC, 1994; Mostafa et al., 1999).

Nonetheless in the past few years two potentially life-threatening nematode infections causing tumours or tumour-like masses in dogs and equids, spirocercosis and draschiosis/habronemosis, respectively, are emerging in several areas of the world. The aim of the present article is to report and comment how recent insights into the molecular early diagnosis of these diseases can save and preserve life, beauty and, in some instances, breeds of socially relevant and sporting animals.

2. The diseases

Canine spirocercosis by *Spirocerca lupi* (Nematoda, Spirurida) is characterized by the induction and promotion of malignant neoplasms in infected animals, such as metastasizing oesophageal tumours, fibro- and osteosarcomas (Mazaki-Tovi et al., 2002; Klainbart et al., 2007).

Although such disease has been underestimated and neglected for several years, the interest of the Scientific Community has recently increased, due to the increase in the number of reports in the Mediterranean Basin, the Middle East, South America, USA and Africa (rev. in Traversa et al., 2007). *S. lupi* causes the carcinogenetic transformation of normal host cells, possibly by excretory/secretory products (ESPs), by nitric oxide or reactive oxygen intermediates and/or osteogenic metabolites acting as a bone-growth factors. In particular it is believed that living *S. lupi* adults in oesophageal nodules release genotoxic factors in their metabolites, thus inducing genetic instability in normal surrounding fibroblastic cells, which can lead to malignant transformation (Melendez and Suarez-Pellin, 2001; Mazaki-Tovi et al., 2002; Klainbart et al., 2007). In such situation the reactive oxygen and nitrogen components can damage DNA, for example by breaks, point and frameshift mutations (Feig et al., 1994). The well-known instability of the host genome is central to the triggering of the malignant transformation, and this accounts for the high incidence of this disease in certain breeds, such as Labrador and Golden Retrievers, that are subjected to high selection pressure by breeders and are sometimes overexposed to such life-threatening parasitosis.

Draschia megastoma, *Habronema microstoma* and *Habronema muscae* (Nematoda, Spirurida) are widespread fly-borne nematodes that cause gastric, cutaneous and/or muco-cutaneous tumour-like masses in equids. Adults living in the stomach induce local eosinophilic inflammation and tumorous growths on the luminal surface of the organ. Larvae in cutaneous sites or muco-cutaneous transitions induce local chronic granuloma which are related to exuberant keloids and tumour-like masses, i.e. ‘‘summer sores’’ (Soulsby, 1982; Pusterla et al., 2003). The pathogenesis of these lesions is unknown, even though the local eosinophilic inflammation and the increased proliferative response of host cells repairing affected tissue damage, together with some epigenetic events, might impair the cell proliferation control and enables the cellular clonal expansion, thus enhancing the development of tumour formation. Also the high level of eosinophilia present in the lesions might induce genes important for tumour transformation (e.g., NF-κB and nuclear protooncogenes), as suggested also for dog spirocercosis (Melendez and Suarez-Pellin, 2001).

3. The diagnosis

The early diagnosis of these two serious and potentially fatal diseases is crucial for a prompt treatment to preserve animal health and welfare and possibly to save the life of the infected animals.

The typical lesion caused by *S. lupi* are oesophageal nodules and granulomas, that potentially can progress to neoplastic transformation. The importance of an early diagnosis is pivotal as it has been demonstrated that an early anthelmintic treatment of infected dogs can lead to the resolution of the oesophageal nodules—thus preventing their transformation into tumours (Berry, 2000; Lavy et al., 2003). Routine faecal diagnosis is impaired by low (i.e., down to 3%) sensitivity and by the prepatent period, and requires a well-trained operator and microscopist (Evans, 1983; Fox et al., 1988). Repeated faecal floatations on samples collected at least in three consecutive days as well as well-skilled technicians are required to achieve a true positive result. Other methods are endoscopy and radiography. However, both of these modalities are characterized by inherent limits. Radiography lacks sensitivity and specificity and its findings are often variable. Furthermore, it is difficult to radiographically discriminate *S. lupi* nodules and neoplasms from other opaque soft-tissue masses. Oesophagoscopy has been shown to be

specific and sensitive and can differentiate between a typical nodule and a neoplasm; however, endoscopic biopsies of oesophageal masses might not allow differentiation between a tumour and a granuloma (Berry, 2000; Mazaki-Tovi et al., 2002; Ranen et al., 2004). Radiography often requires restraint or sedation, while endoscopy always requires general anaesthesia of the patient, all of which may be expensive, time consuming and in some cases can be risky, and life threatening for the infected animal (Joubert et al., 2005). Thus, these approaches do not permit reliable aetiological (i.e., parasitological) diagnosis, and, therefore, does not enable an effective anthelmintic treatment.

The sole means to diagnose gastric draschiosis/habronemosis is *via* necropsy, because symptoms associated with gastric infection may overlap with other types of colic. Also, classical faecal examination lacks sensitivity (0–3%) (Traversa et al., 2004).

Additionally, diagnosis of the summer sores is problematic since the granulomatous lesions are similar to those of a broad range of other dermopathies of equids, such as botryomycosis, pythiosis, onchocercosis, equine sarcoid and squamous cell carcinoma (Inzana and Carter, 1990; Pascoe, 1990; Chaffin et al., 1995). In many instances, microscopic examination of stained histological sections of dermal lesions can be unreliable, since larval parasites are scarce in the skin masses and, since they live only for less than 1 month in the skin, the lesions often do not contain nematodes but only exuberant, necrotic or calcified tissue. Therefore, skin biopsies, squeezes or smears often result negative at the microscopical examination (Dunn, 1969).

Given these limitations, the extraordinary specificity and sensitivity provided by DNA technology and the significant merit in developing and applying improved diagnostic tools recently led to studies aimed at molecular diagnostic techniques for canine spirocercosis and equine draschiosis/habronemosis.

4. Molecular insights

With respect to canine spirocercosis, a recent study provided important information on an informative region within the mitochondrial (mtDNA) gene encoding for the cytochrome *c* oxidase subunit 1 (*cox1*) of *S. lupi* (Traversa et al., 2007). This showed to be a promising objective for innovative diagnostic methods using easily collectable biological samples, such as mucus or faeces. When the classical diagnostic approach is not reliable for the clinician and/or parasitologist, the efficacy of these methods are of paramount importance in cases of suspected spirocercosis. A PCR-based assay targeting the *S. lupi cox1* gene may facilitate the diagnosis at an earlier stage, when tumorous lesions have yet to be induced. This is particularly true in the cases of oesophageal carcinoma, when the parasite is not present in the mass and the faecal samples from the diseased animal may become negative for the parasite. Hence, all classic diagnostic approaches are prone to failure, and thus may delay an effective and prompt treatment, necessary to save the infected animal or may become positive too late in the disease course. In particular, it has been demonstrated that early anthelmintic treatment of an infected dog can lead to the resolution of the oesophageal nodules—thus preventing their transformation into tumours (Berry, 2000; Lavy et al., 2002, 2003): hence, it is easy to appreciate the importance of an early and specific diagnosis that, at the moment, is not possible with the classical techniques.

A simple and fast PCR-based assay, which was recently developed, permits the identification and delineation of causative agents of equid draschiosis/habronemosis irrespective of their life cycle stages, thus having important implications for both epidemiologists and, mainly, clinicians. In particular, this tool is the only method available for the diagnosis *in vivo* of gastric draschiosis/habronemosis with exquisite sensitivity (97%, down to 0.02 fg of parasite DNA) and specificity (100%). Clearly, this method overcomes the limitations of the traditional diagnosis, and it provides a powerful approach under clinical conditions (Traversa et al., 2004). It allows the diagnosis of the gastric disease *in vivo* and a prompt and effective anthelmintic treatment that is particularly important in those animals suffering from colic. This technique also is applicable to the cutaneous and mucocutaneous forms of these parasitoses; these require an unequivocal diagnosis because of the necessity for applying specific and particular therapies. Also, it is essential that these infestations are diagnosed and treated promptly because these induced lesions usually ulcerate and predispose the affected equid to opportunistic infection by bacterial and mycotic pathogens that can spread and become life threatening (Pusterla et al., 2003). Also, it is proposed that this molecular tool may be employed in experimental trials to evaluate the efficacy of compounds against *Draschia/Habronema via* the monitoring of the decline or absence of parasite-specific DNA in faeces after treatment in live equids, thus circumventing the need to sacrifice them (Traversa et al., 2004).

5. Open avenues for socially helpful and sportive animals and breed conservation

The molecular approach to the diagnosis of parasitic diseases is, in general, central to an effective and prompt treatment and, in the case of spirocercosis and draschiosis/habronemosis is undoubtedly the most specific and effective approach. In fact, for these emerging diseases, the molecular tools can actually establish the certainty of diagnosis that is lacking in conventional methods and, thus, open new and important modalities to save the life of dogs and horses that are more often employed as socially helpful animals.

Dogs give strength and mobility for the physically disabled, provide comfort for people suffering emotional difficulties, and they are part of circumstances that unite dogs and people. In particular, after an appropriate early socialization, some breeds such as Labrador Retrievers and Golden Retrievers are employed for their ability to provide companionship, and are used especially for elderly people or for children with autism. These breeds are the ones most often used as ''service dogs'', animals highly trained to assist people with strength and movement disabilities (e.g., people with muscular dystrophy, disseminated sclerosis or cerebral palsy). Dogs provide aid for the hearing-impaired, and Retriever breeds are used worldwide as guides for blind/visually impaired individuals to ''see'' in their everyday lives. Also, miniature horses have been proposed as a alternative aid for mobility functions for the visually impaired or blind people, especially for those who are allergic to dogs, and for those who want a guide animal with a longer lifespan than dogs, and for those who prefer a tiny horse. In addition, horseback riding of ponies and standard-size horses is a recognized cure for some illnesses including neurological disorders or depression, and for children with autism, mental retardation, learning disabilities, epilepsy, and cerebral palsy. In all of these circumstances, a deep, meaningful and close relation is established between the person and their personal animal assistant. Animals living outdoors (e.g., riding and sport horses) and genetically predisposed dog breeds are more likely to be parasitized and, thus, are in danger themselves and for the assisted people to whom they are assigned. Therefore, each situation which endangers the health and the welfare of the dog or the horse inevitably threatens the health and the welfare of the person who is assisted by the therapeutic animal. The typical example would be a Labrador Retriever ''close friend'' of a child with autism that becomes infected with *S. lupi* and develops a tumour because of a lack of an early diagnosis. The likely death of the dog (the prognosis for dogs with *S. lupi*-inducted sarcomas is very poor and surgery and chemotherapy are usually only palliative treatments) could be a tremendous trauma for the child's psyche. The same could happen with a horse that becomes unable to walk and ride because of a skin mass caused by *Draschia/Habronema*. These lesions are often moist and purulent, which hamper normal gait, and, thus, could deprive the assisted individuals of their therapeutic companionship. Importantly, summer sores are also deleterious in horses employed for sport competitions and exhibitions, since they impair the affected animals' performances throughout their life. Moreover, once these lesions appear, they can be permanent and disfiguring; these scars could be avoided with an early specific diagnosis.

Summer sores can be an important threat for the natural reproduction of affected animals. In fact it has been verified that affected males are not able to mate with females, since animals with genital lesions exhibit difficulty in copulating and frequently bleed during or following copulation; moreover, the lesions cause pruritis and the normal healing process is very prolonged and can result in penile scars which definitively impair the extension and retraction of the penis. In addition, leg lesions can prevent the normal and physiological ambulation of affected limbs during mating. In some cases the amputation of the urethral process is necessary (Stick, 1979). For example, this is particularly true for a popular donkey breed in Italy, the ''Martina Franca'' donkey. Presently, the natural reproduction of these donkeys is endangered, and they are vulnerable and face a high risk of extinction in the near future because they are very susceptible to the occurrence of lesions on the glans penis and on their hocks. Even though this example is referred to as a particular situation in which this nematode infection is actually threatening the conservation of a breed, the reproductive problems caused by draschiosis/habronemosis are fully applicable to equid breeds in general.

It is therefore noteworthy that Thoroughbred, Quarter horses and Arabian horses are among the most susceptible horses to (muco)- cutaneous summer sores, as reported in several areas of the World (Stick, 1979; Rebhun et al., 1981; Pusterla et al., 2003).

In this respect, a prompt diagnosis of gastric draschiosis/habronemosis is not only necessary in equids suffering from often fatal gastric colics, but, more importantly, it is central in the prophylaxis of summer sores. Also, because the gastric lesions are caused by adult nematodes living in the stomach, they

represent a source for further infections by parasitic flies. Thus, an early diagnosis of this gastric infection in an animal kept on a farm or in a stable would allow prompt treatment, which would prevent the spread of the skin lesions in the animals living nearby.

6. Conclusions

In conclusion, the intention of this article is to stimulate thought regarding two important parasitic diseases of domestic canines and equids, and how new avenues have been opened by molecular diagnostic techniques. By employing new diagnostic methods, lives can be saved, and the health and physical beauty of breeds of socially relevant companion animals can be preserved. The advent of DNA technology has revolutionized several areas in the medical sciences; with respect to parasitology, these approaches have been incredibly effective for the specific diagnosis of many infections. Today, DNA-based techniques are often relatively simple, fast, targeted, and more reliable than other methods. The laboratory equipment and consumable reagents are readily available to most diagnostic laboratories. It is suggested that these tests become the routine choice for important situations with animals as they presently are routine for many human infections.

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