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A report of the unusual lesions caused by *Thelazia gulosa* in cattle

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A B S T R A C T

This is a case report of unusual lesions caused by *Thelazia gulosa* in cattle. After several ineffective treatments of suspected infectious keratoconjunctivitis in calves and cows, *T. gulosa* was found associated with retrobulbar granuloma-like masses. The masses with multiple digit-like protrusions on conjunctival surface ejected multiple worms on firm pressure on clinical examination. Samples of tissues that contained worms were surgically removed, fixed in ethanol and submitted to the parasitology and histopathology labs for morphological identification of worms and the nature of the tissue masses, respectively. The infestation was present only in young calves (<3 months) and high-producing cows. Histopathology showed fibrovascular granulation tissue, containing a moderate to marked inflammatory infiltrate. Ivermectin treatment (200 μg/kg, SC, once) with and without surgical excision resolved the infestation. To the best of our knowledge, this is the first report of tissue invasion by *T. gulosa*.

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1. Introduction

*Thelazia* spp. (Spirurida, Thelaziidae), commonly called “eyeworms”, parasitize the eyes and associated tissues of mammals (Naem, 2011; Otranto and Traversa, 2005). Depending on the species, adults *Thelazia* live under eyelids and nictitating membrane, on the surface of the conjunctival sac, in the lachrymal glands and nasolachrymal ducts of ruminants, canids, felids, equids and humans (Anderson, 2000; Skrjabin et al., 1967; Yamaguti, 1961). The female *Thelazia* deposits first-stage larvae into lachrymal secretions of the host. Intermediate hosts, i.e. non-biting muscid flies, ingest the larvae while feeding from lachrymal secretions. Ingested larvae penetrate the wall of midgut and then migrate to ovaries and, in some species, to testes or fat body. In these locations larvae develop into infective third-stage. Finally, when an infected fly is feeding on eye secretions, third-stage larvae emerge from the labelleum and infest the definitive host (Anderson, 2000). The larvae reach adulthood in about 12–30 days depending on the *Thelazia* species (Sousby, 1982).

Mechanical removal (Naem, 2011), local levamisole (1%) or ivermectin (200 μg/kg, SC) (Taylor et al., 2007), doramectin pour-on (Marley et al., 1999) oral levamisol and topical formulation of imidacloprid and moxidectin (Bianciardi and Otranto, 2005) have been recommended for the treatment of the classic infections of the lacrimal sac (without tissue invasion) by *Thelazia* spp. in various animals.

We report an unusual presentation of *T. gulosa* in a dairy cattle farm from central Iran (35°12′N & 51°43′E).

2. Presentation of the case

Five calves (under three months of age) and four high producing cows in a 400 head dairy herd were treated by a local veterinarian for keratoconjunctivitis of presumed infectious origin for four weeks duration using topical disinfectants (including povidone-iodine 10%, Najo Co., Iran) and systemic oxytetracycline (10–15 mg/kg IM 5 days; Erfamycin® 10%, Erfan Daru™ Co., Iran). Due to persistence/deterioration...
of the condition specialist help was sought. The local people reported that
there were human cases of “eye worm” infestation in a nearby village.

The signs were typical of keratoconjunctivitis including hyperemia
of the conjunctiva, abundant opaque creamy white discharge, variable
corneal opacification and mild itchiness. One cow exhibited a conjuncti-
tival mass which protruded when the eye was pushed in for routine ex-
amination of the conjunctiva. Subconjunctival abscesses are common
in the region and based on a tentative diagnosis, local antibiotic treat-
ment was administered. Intraconjonctival injection of $3 \times 10^5$ units
of procaine penicillin (Erfan Darou Pharmacy™ Co., Iran) as 1 ml aqueous
suspension in superior palpebral conjunctiva and systemic flunixin
meglumine (Flunex®, 1.1 mg/kg, Razak Laboratories™, Iran) was
carried out and repeated after 14 days with no success.

Subsequently, all cases were re-examined in more detail and com-
plete ophthalmoscopy and swabbing for culture of the conjunctival sac
was carried out. During the examination one cow and one calf showed
two free worms in the conjunctival sac. Fleshy masses (2–4 cm) with
multiple digit-like processes (up to 1.5 cm in length and 2–4 mm in
diameter) were observed extruding when mild pressure was applied
on the bulb in three out of four cows and one out of five calves. Palpation
of the masses showed their connection through a firm tubular structure
with other masses that could be partially palpated at the side/behind the
eye globe. Firm pressure led to release of abundant entangled masses of
worms through a fistula-like orifice in the conjunctival fold next to the
mass.

Surgical removal via an incision in the nasal (medial) canthus under
local anesthesia was carried out on two cows with the largest masses
and then all animals were treated using ivermectin (Erfamectin®
Co., Iran), and ketoprofen (Vetoßen®, 3 mg/kg, IM, bid, for the first 3 days and afterwards, every-
other-day for as long as discomfort was present, up to 7 days; Aburaihan
Pharmaceuticals™ Co., Iran). Pieces of fleshy tissue and entangled worm
masses were removed from the medial and caudomedial to retrobulbar
aspects of the eye. Worms were residing mainly in 2–3 connected small
pouches, but worms also were visible individually burrowing in the tis-
sue. During surgical removal, a few connected masses were noticed
behind the globe and they were removed by blunt dissection as far as
possible (Fig. 1).

The surgical site was closed using fine absorbable material (4–0
polyglycolic acid) leaving a fine drainage. The treatment was followed
up and considered successful by the farmer and local veterinarian.

3. Histopathological and parasitological examinations

The excised tissues and the extracted masses of worms were placed
in 70% ethanol and then transferred into 10% neutral buffered formalin.
Paraffin-embedded sections of the tissues (5 μm) were stained with
hematoxylin-eosin (H&E) for histological examination.

A total of 50 nematodes were cleared in lactophenol. Identification
was made using light microscopy and based on morphometrical charac-
teristics, including the length of spicules in the male and the distance of
the vulva from anterior end in female nematodes by two parasitologists;
and using the characteristics of worm sections within the tissue by two
pathologist in different institutes, all unaware of the source of the
samples. These identification criteria have been presented by Soulsby
(1965) and Arbuckle and Khalil (1978).

Over 100 flies were captured and sent to a single laboratory for spe-
ciation, to identify possible vectors.

4. Results

Parasitological studies based on the size of the buccal capsule and
the shape/size of the spicules identified the worms as T. gulosa
(Fig. 2). Sections of conjunctiva showed extensive erosion or ulceration,
with partial to full-thickness epithelial loss overlain by a thick
haemorrhagic or serocellular crust. There were foci of degenerate and
non-degenerate leukocytes, particularly granulocytes, surrounding
moderate numbers of nematode profiles, in both transverse and longi-
tudinal sections (Fig. 3). The helminths were approximately 500 μm
in diameter and had a smooth cuticle, coelomary musculature with
well-developed lateral chords and small, uninucleate intestinal cells
with prominent microvilli, consistent with Thelazia sp. Fibrovascular

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**Fig. 1.** Pieces of fleshy tissue and entangled worm masses (fixed in 70% ethanol) excised from the medial and caudomedial aspect of the left globe of a cow. Masses of entangled worms (black arrows) within few interconnected pouches (pieces of the biggest pouch walls marked with *) and digit like protrusions at the conjunctival side of the lesion (white arrows) were the most notable aspects of the lesion.
granulation tissue within the submucosa contained a moderate to marked inflammatory infiltrate, mainly lymphohistiocytic, with fewer eosinophils and plasma cells, merging with the deeper, hyaline episcleral collagen (Fig. 3).

Ivermectin treatment with or without surgical excision resolved the infestation to the assessment of the farmer and local vet. Both surgical removal and anthelmintic therapy resulted in mild to moderate tissue swelling, soreness, epiphora and pruritus in the eyes. This reaction to surgery or dead worms was responsive to non-steroidal anti-inflammatories (ketoprofen) and resolved in one to two weeks - according to the farmer.

The presence of both *Musca domestica* and *Musca autumnalis* was confirmed in the farm based on the morphology of the flies and their eggs (Taylor et al., 2007).

5. Discussion

_Thelazia rhodesi_, _Thelazia gulosa_ and _Thelazia skrjabini_ are the principal species adapted to cattle (Anderson, 2000). These species have been reported in slaughtered cattle from the region in a case study at a nearby abattoir (52 km apart from the under discussion farm (Naem, 2011)), and they are of cosmopolitan distribution (Anderson, 2000). Reported-ly, the prevalence of the infestation in slaughtered animals is 2% (Naem, 2011), however, there are no data on clinical prevalence of the nematodes in the region. In the past, infestation rates of 4.5, 25.7% and 41.9% with different species of _Thelazia_ have been reported for cattle from Australia, Canada and United Kingdom (Arbuckle and Khalil, 1978; Kennedy et al., 1990; Overend, 1983). The differences of the prevalences in different regions may relate to climatic conditions, species of the vector flies (Overend, 1983) and possibly the genetic differences of the parasites themselves.

Infestation with _Thelazia_ can be subclinical or with clinical signs. According to Soulsby (1982) in many cases, _Thelazia_ spp. have no pathogenic effect on hosts. In cases with clinical manifestations, lachrymation, conjunctivitis, keratitis, corneal ulceration, eyelid edema and photophobia are seen (Otranto and Traversa, 2005; Soulsby, 1982).

Miller and Campbell (1992) removed a large subconjunctival cyst from the left eye of a seven month-old calf and found two females _T. gulosa_ in the cyst wall. They attributed the cyst formation to the penetration of normal tissue barriers by worms or entrance via an earlier conjunctival wound and stimulation of an inflammatory response.

In the current case report, a new form of lesions due to _T. gulosa_, i.e. tissue invasion in large numbers and formation of fibrovascular granulation tissue was recorded for the first time. Both host immunity elements and/or parasite virulence could be the cause of the invasive pathogenesis of the nematodes. To explore these possibilities, we further investigated some factors we suspected to play a role in the unusual pathogenesis of the infestation.

Interestingly, out of several hundred animals, only calves less than three months of age and high producing cows were clinically affected on this farm. It was hypothesized that immune depression associated with production stress of cows (Goff and Horst, 1997; Hoeben et al., 1997) and a developing immune system in calves play a role in the unusual tissue invasion presentation of the disease reported here. To our knowledge the calves were not directly related to the infested cows as dams, but the common sires were not investigated. Therefore, genetic susceptibility or hereditary immune deficiencies cannot be excluded. Similar to this finding, a study from Canada reports younger calves

Fig. 2. Photomicrographs of the buccal capsule (left panel) and posterior end of a male (right panel) _Thelazia gulosa_ (10× objective). To reveal maximal details, different settings were used to image the two photomicrographs (the reason for color difference). Fig. 3. Histological sections showing longitudinal and transverse nematode profiles with characteristic features of _Thelazia_ spp. within a marked lymphoplasmacytic, histiocytic and eosinophilic inflammatory response. Haematoxylin and Eosin).
and those over 2 years (the age which cows approach peak production) had highest rate of infestation (Kennedy et al., 1990).

The fly species we detected within the farm (Musca domestica and Musca autumnalis) are compatible with those that were involved in cattle thelaziosis in Italy, Northern and Eastern Europe and United States (Giangaspero et al., 2004).

Regarding the severe keratoconjunctivitis and deep periorbital tissue inflammation, the reported type of lesions could affect the productivity and welfare of the animals to some extent. Although it is known that different species cause human thelaziosis (Naem, 2011), because of the unusual pathogenesis we investigated the anecdotal reports of human infestations in a nearby village, which was unsuccessful as “infested people had left the area”. The unusual and invasive nature of the worms and anecdotal human infestations in the region warrant further investigation.

References


