Pathology of natural *Przhevalskiana silenus* infestation in goats

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Abstract. Among the arthropods causing diseases to animals, myiasis causes a broad range of infestations depending on the location of larvae and its developmental stages on the body of the host. These infestations reduce host physiological functions, destroy host tissues and cause significant economic losses to livestock worldwide. This study was conducted to find out the pathological changes of goats tissue infested with *Przhevalskiana silenus*. Goat warble fly infestation (GWFI), improperly named goat hypodermosis, is a myiasis caused by larvae of *P. silenus*. Out of 16,250 goats examined in the slaughter house in the studied area, 433 (2.67%) were infested with warble fly. The minimum and maximum rate of infectivity was 7 and 84 with an average of 32.4 warbles per animal. Histopathological examinations were carried out on the infested subcutaneous tissues. Infiltration of the mononuclear cell types, tissue necrosis, pyogranulomatous reaction, hyalinization, mineralization, muscle fragmentation, oedema, and hyperemia of arterioles and capillaries were the most important microscopic findings associated with different developmental stages of *P. silenus* instars in the goats. The results of this survey indicated that GWF is a widespread infestation in Shiraz, Fars Province, southern part of Iran.

INTRODUCTION

Ectoparasite infestation is a major veterinary problem affecting livestock in many parts of the world (Hourrigan, 1979). Although myiasis has been known since very ancient times, at the beginning of the third millennium, they still remain an unresolved problem for animal production. Myiasis are responsible for severe economic losses to the livestock industry through abortion, reduced milk production, losses in weight and fertility, and poor hide quality in developing and developed countries (Otranto & Stevens, 2002; Oryan et al., 2008). Amongst these, warble fly infestation is a notorious and common disease of herbivores in Iran. Goat warble fly infestation (GWFI) is a myiasis caused by the larvae of Przhevalskiana silenus (Brauer, 1858) an insect belonging to

order Diptera, suborder Brachycera, family Oestridae, subfamily Hypoderminae and is characterised by warbling the larval form of this fly under the skin of back and flanks regions and rarely other subcutaneous areas of goats (Otranto & Puccini, 2000). Goats in Fars Province, southern Iran are mostly freely grazing in underdeveloped and mountainous areas. In such areas, this fly is endemic and is accounted as an important veterinary problem (Oryan *et al.*, 2009).

It is not easy to calculate the economic impacts due to the loss of body weight, growth retardation, and decrease in milk/meat production, and hide deterioration due to this infestation. Its presence also necessitates carcass trimming and downgrading (Rahbari & Ghasemi, 1997). The tanning industry encounters with severe losses due to the holes in animals' hide (Abul-Hab & Al-S'adi, 1974; Abo-Shehada *et al.*, 2006). It is evident that infestation with *P. silenus* is associated significantly with poor carcass condition because goats are restless and do not feed enough and this results in production losses. The larval fly also damages the host immune system and predisposes them to an array of biological agents such as bacteria, fungi, viruses (Boudler, 1989; Lo'pez *et al.*, 2005), and it is extremely difficult to evaluate these losses economically. In addition, *P. silenus* larvae can parasitise humans, resulting in irritation and hypersensitivity responses (Abul-Hab & Al-S'adi, 1974).

The adult *P. silenus* is active from April to June in different areas of Iran. The adult fly lacks mouthparts and survives on resources accumulated during the larval period. During the periods that the fly is active, the first instar larvae emerge from eggs laid directly on the hairs of the hind legs (mainly tarsal and femoral regions) of the goat. The larvae then penetrate the epidermis and dermis to enter into the subcutaneous tissue to migrate for a short distance to reach the flanks and sacrum. The migration pattern inside the body of animals seems to be exclusively subcutaneous (Cheema, 1977; Otranto & Puccini, 2000; Abo-Shehada et al., 2006). Five months later, the first instar larvae (L_1) reaches the back of the animal and remains almost inactive and initiate a minimum tissue reaction at this stage. Then the L_1 develop into second (L_2) and third stage larva (L_3) in the subcutaneous tissue of the back without performing further internal migration (Soni, 1942; Grunin, 1962; Otranto & Puccini, 2000). The third stage larvae makes a perforation outward in the skin of the back and drops on the ground and develops to pupae by enveloping in a tough barrel structure in which the adult fly grows up.

Goat warble fly infestation is cosmopolitan and is reported from many countries of the world including, Pakistan, Afghanistan, Cypress, Iraq, Iran, Siberia, Cyprus, India, Saudi Arabia, Syria, Isreal, Turkey, Pamir, former Yugoslavia, Albania and Greece (Pugliese & Zanchi, 1984; Alani *et al.*, 1991; Giangaspero & Lia, 1997; Otranto & Puccini, 2000; Azizi *et al.*, 2007). Goat warble fly is a very common infestation in Jordan where the prevalence rate of 10% for *P. silenus* infestation is recorded in slaughtered goats (Abo-Shehada *et al.*, 2006). In another investigation during monthly visits to North Sinai, 2.11% of goats were found infested with *P. silenus* (Morsy *et al.*, 1998). Prevalence ranges from 30 to 90% in Italy (Puccini *et al.*, 1986; Otranto & Puccini, 2000), from 53 to 94% in Turkey (Sayin *et al.*, 1973), from 22 to 25% in Iraq (Abul-Hab & Al-S'adi, 1974), 24% in Albania (Tagari & Manehasa, 1973), 26.42% in Jammu, India (Yadav *et al.*, 2006) and 5.3% in Masjed-Soleyman, South-western Iran has previously been reported (Azizi *et al.*, 2007).

Goats are the main source of income for nomads and other farming communities of Iran and improving the health of these animals will result in better return to the farmers leading to socioeconomic upliftment of the rural population. Despite many investigations that concentrated on P. silenus aetiology, taxonomy, diffusion, therapy and immunology (Tassi et al., 1986, 1987; Khan et al., 1994; Giangaspero et al., 1996; Otranto & Puccini, 2000; Tajik et al., 2011) there are few data on the parasite internal life cycle and tissue damages encountered in the host by this myiasis. The pathological changes associated with this infestation have not been adequately documented too. Therefore, the present study was undertaken to provide supplementary information about important tissue changes occurring due to larval stage of P. silenus in naturally infested goats.

MATERIALS AND METHODS

This study was carried out in goats of local breed slaughtered at the Shiraz slaughterhouse, Shiraz, Fars Province, southern part of Iran. Carcasses of 16,250 goats were examined for the presence of *P. silenus* larvae from October 2008 to December 2010. The carcasses were examined either at weekly intervals or twice a week. Animal hairs and skin were carefully examined for *P. silenus* eggs before and after skinning. The animals were palpated at the back, flanks and chest to detect presence of the subcutaneous

warbles. Oesophagus and spinal canals were examined for larvae and/or lesions caused by them. Necropsy of the slaughtered and skinned animals was carried out by examination of the inner skin surface and subcutaneous tissue. The warbles were isolated from the skin and subcutaneous areas of each carcass, counted, measured, photographed and stored in separate containers containing 0.9% normal buffered saline at 4°C, after having registered their location on animal body diagrams. Due to the small size of the first stage larvae pieces of tissues from the appropriate locations of the carcasses were dissected under a stereomicroscope. The larvae were measured, identified and classified at different larval stages according to the keys (Zumpt, 1965; Otranto & Puccini, 2000). The first instar larvae were detected from early August to the end of the September. A mixture of L_1 and L_2 were present from early to mid October and the second instar larvae were the only larval stage from mid October to the end of November. From the early December to 20^{th} December a mixture of L_2 and L_3 were present in the carcasses while all the larvae recovered from the last trimester of December to mid-March were only L_3 and had the largest size. The larvae were photographed and stored in 70% ethanol.

Appropriate tissue sections from the infested subcutaneous tissues were fixed in 10% neutral buffered formalin, dehydrated in graded ethanol, embedded in paraffin, sectioned at 5 µm thickness, stained with hematoxylin and eosin, and studied with a routine light microscope. The larvae were studied and photographed using an ordinary stereomicroscope.

RESULTS

Out of 16,250 goats carcasses examined at Shiraz Slaughterhouse, 433 (2.66%) were found infested with *P. silenus*. The minimum and maximum rate of infectivity was 7 and 84 with an average of 32.4 warbles per animal (Fig. 1a). The first instar larvae (L_1) were found on infected animals from early August to end of September, the second larval stage

 (L_2) from early October to end of November and third-stage larvae (L_3) from early December to mid-March. No larvae were found on skin or subcutaneous tissues from end of March to late July. While there was no significant difference in the prevalence of infection between males and females, the goats of 7-24 months age were more infected than those of the elder and younger ones. Goats younger than 6 months were not apparently infected.

The tissue reaction to the live first instar larva was minimal and the live L_1 larvae did not initiate host cell degeneration and tissue necrosis and the skin remained intact during the months that the first stage larval infestation was dominant. The infested tissue response to the live first instar larva was mostly of mild mononuclear cell type infiltration and lymphocytes, plasma cells and macrophages were the main cell populations present in the infested tissue. In some instances a capsule of fibrous connective tissue surrounded the live L_1 (Fig. 1b). However, few eosinophils were also infiltrated in the infected areas of most cases of this type of L₁ larval infestation. In the cases of larval death and degradation, that was a common finding in case of infestation with L₁ larvae, tissue necrosis with neutrophil infiltration and pus formation in the center surrounded by lymphocytes, plasma cells, macrophages, eosinophils, giant cells and a capsule of fibrous connective tissue of various thickness, forming a pyogranulomatous reaction, was an ordinary finding in this type of larval stage infestation. In this situation langhans and foreign body giant cells were present in the vicinity of the degraded larva (Figs. 1c and 1d). The remnants of the fragmented cuticle were present in the center of some of these pyogranulomatous reactions and the center of the lesions in others was mineralized.

When the first instar molted into the second instar larva and fragments of its separated outer wall were added to the surrounding debris, a severe inflammatory reaction and massive tissue necrosis started that continued until the end of infestation phase. The muscle over the larva became pale, necrotic, fragmented and many



Figure 1. a) Numerous second stage larvae of *Przhevalskiana silenus* are seen in the subcutaneous area of a goat

b) Live first instar larva is localized in the skeletal muscle of the subcutaneous tissue. Inflammatory cell infiltration around the larva is minimal and the muscle fibers close to the larval localization (left bottom corner) is almost normal (H and E, scale bar=410 µm)

c) The dead first instar larval stage is degraded and a granulomatous reaction with the degenerated larva, epitheloid macrophages (thick arrow) and giant cells (thin arrows) in the center that is surrounded heavily by lymphocytes is seen (arrow head) (H and E, scale bar=105 μ m)

d) A pyogranuloma is formed surrounding the dead 1st instar larva with degraded neutrophils, forming pus-like materials (thick arrows) surrounded by epitheloid macrophages and giant cells (thin arrow). Lymphocytes heavily infiltrated this structure (*). Part of the thick fibrous connective capsule (F) is seen on the right top corner (H and E, scale bar=250 µm)

e) The live second instar larva heavily destructed the subcutaneous muscle fibers and the tissue in close vicinity to it is necrotized (thick arrow). The injured tissue section is severely infiltrated with lymphocytes, macrophages and eosinophils. Normal cell borders and the cell nuclei of the larva are seen in the center of the lesion (thin arrow) (H and E, scale bar= $275 \,\mu\text{m}$)

f) Parts of the cuticle of the larva (L) are seen in subcutaneous tissue. It initiated tissue necrosis (N) and mild inflammatory cell infiltration in the surrounding tissues (H and E, scale bar= 275μ m)

g) Heavy tissue necrosis (arrow) due to 3^{rd} instar larva is evident. The tissue is severely infiltrated with inflammatory cells (*) (H and E, scale bar=105 μ m)

h) Numerous newly regenerated blood vessels (thin arrows) could be seen in the fibrous connective tissue capsule. The capsule possess numerous fibroblasts and connective tissue bundles infiltrated with many lymphocytes and macrophages (H and E, scale bar=105 μ m)

neutrophils and eosinophils infiltrated into this necrotic area and in the vicinity of the live larva (Fig. 1e). However, at the later phases of infection with the same larval stage, neutrophils were substituted with lymphocytes, macrophages, plasma cells and giant cells (Figs. 1f and 1g). Infiltration of eosinophils was also seen at the later stage of L_2 infestation too. The subcutaneous area, dermis, and finally epidermis close to L_2 became necrotic and similarly were infiltrated by inflammatory cells. The L_2 moulted at this stage and the third stage larvae (L_3) started their life cycle. Head of the larva then penetrated through the necrotic area of muscle and skin and the third instar larva started its aerobic life cycle. A wall of fibrous connective tissue that in most cases

was usually infiltrated by lymphocytes, macrophages and eosinophils surrounded different parts of the larva except the head region. In some instances, an abscess was formed around the live L₃ and the larva was floating in pus consisting of neutrophils together with tissue debris. Generally the head was either covered by a scab consisting of dried tissue debris, dead neutrophils and necrotic fibrino-purullent exudates or it was uncovered and directly in contact with the outside environment. Many cavities containing tissue debris and inflammatory cells indicating recent separation of the third instar larva from the skin as well as multifocal pinpoint scar tissues showing that the instars left the skin long time ago were present in the dermis and epidermis. The scar tissue was unorganized, highly vascularized and numerous mononuclear cells were still present among the connective tissue fibers. In some instances the capillaries and arterioles were hyperaemic and in some places the dermis was heavily oedematous. Rupture of the delicate blood vessels which resulted in haemorrhages in this granulation tissue was another ordinary finding. The skin adenexae such as sebaceous glands, hair follicles and other adenexae in the vicinity of larval localization disappeared and was replaced with granulation tissue (Fig. 1h). Even 40 days after the last L_3 left their hosts still some pustules and cutaneous abscessations were seen in the previously infested animals.

DISCUSSION

Although goats are important sources of milk and meat for food purposes, wool and hides for clothing and other textile articles, they have not received adequate attention so far as their parasitic diseases are concerned, particularly those caused by arthropods. This is probably due to the underdeveloped environments in which the goats are normally raised. Consequently, improving the health of goats will reflect positively on financial returns of farmers. Systematic studies of the disease conditions, particularly those of GWFI are few. In this study prevalence, internal life cycle and pathology of GWF were investigated. The results showed that this fly is an important veterinary problem and is responsible for serious animal-health problems and considerable economic losses. It is evident that infestation with *P. silenus* is significantly associated with poor carcass condition.

When the eggs are laid directly on the back and flank of an animal, the first instar larvae (L_1) emerged from the eggs in 5-6 days and penetrated into the subcutaneous tissue by the collagenolytic enzymes of its salivary and intestinal glands (Madel & Nahif, 1971; Tassi *et al.*, 1989). L_1 have a club-shaped pseudocephalon ending with a pair of spiracles, and is subdivided into 11 segments with spines at the conjunction of segments (Cheema, 1977).

Observation of carcasses pointed out that P. silenus larvae penetrated directly from the place where the eggs were laid and entered into the subcutaneous connective tissue and muscle fibers of the same area. The present observation confirmed the findings of Otranto and Puccini (Otranto & Puccini, 2000) that the eggs are only laid on animals' back and flanks and the first instar larvae emerged from eggs in the same place and penetrated into subcutaneous tissue and muscle fibers without migration because larvae at different developmental stages were only found in the dorsal region of the animals. This study also confirmed the findings of some of the previous studies that the adult fly has adapted to local climatic condition and hence has only one cycle of infestation each year (Puccini et al., 1986; Khan et al., 1994; Oryan et al., 2009).

During its mutation from L_2 to L_3 the body of larvae (8x15-18 mm) becomes dark because of chitin accumulation. The spiracles function as breath organs since the larvae are aerobic at this stage and breathe by means of skin hides produced by collagenolytic enzymes accumulated during migration. In spring the mature larva wriggles out of its cyst and falls on the ground into which it penetrates to pupate. The pupal case is black and the fly emerges from it (Tajik *et al.*, 2011).

Pathological studies showed that the first instar live larvae results in milder lesions

than those caused by the L_2 and L_3 in the subcutaneous tissue. However, abscessation and pyogranulomatous reactions were usually present in the vicinity of the dead and degraded L_1 . The results of Tafti *et al.* (2012) study also showed two main pathological lesions around live or dead larva including non-purulent and granulomatous or pyogranulomatous dermatitis associated with variable infiltration of eosinophils, and also different degrees of peripheral fibrosis. Purulent inflammation at this stage could be due to the enzymatic lyses of the subcutaneous tissue. Degradation of the dead L_1 could also result in the release of different suppurative bacteria from the gut of the larvae that are not only responsible for further enzymatic changes of the affected area but they also initiate pus formation and purulent inflammation. However, presence of abscesses in vicinity of some of the live L3 or even long time after the L_3 left their hosts is possibly due to the contamination of the infested area through the orifice of the perforated skin. It has been stated that if the pyogenic bacteria burst into the grubs, a suppurative cavity may form in which the L_3 is immersed in a pus-like liquid (Cheema, 1977). Tajik et al. (2011) also isolated Escherichia coli, **Staphylococcus** epidermidis, Staphylococcus aureus, Streptococcus pyogenes and Klebsiella *pneumonia* from the abscesses containing *P. silenus* in goats. However, they did not culture the abscesses contents for anaerobic microorganisms and *Mycoplasma* species but in a previous study by Tadayon et al. (1980), anaerobic bacteria and different species of Corynebacterium and Pasteurella have also been isolated from the cutaneous abscesses of the goats in the same area. Therefore *P. silenus* not only has severe economic impact on tanning industries and also is responsible for impaired milk and meat production, growth retardation, immune suppression and carcass depreciation, but also make the infected animals more susceptible to further microbial diseases. In future, the relation of this infestation with diseases such as black leg, malignant oedema and tetanus should be followed up.

In addition tuberculosis and paratuberculosis diseases caused by *Mycobacterium* species such as *Mycobacterium* tubeculosis, *Mycobacterium* bovis and *Mycobacterium* paratuberculosis that are prevalent diseases among large and small ruminants of this area could easily be transmitted via the skin holes produced by this myiasis.

In addition, the architecture of the infected skin was severely affected by this myiasis and even 40 days after the last L_3 left the skin, no adenexa such as hair follicles, sweat glands and sebaceous glands were present in the dermis. At this stage, dermis and subcutaneous area consisted of unorganized connective tissue which was infiltrated by mononuclear cells and eosinophils. The blood vessels of the infected area were still hyperaemic and many areas of focal or diffused haemorrhages were still present even long time after clearing from infestation.

In view of the economic losses encountered by GWFI in goats, P. silenus infestation can be considered among the main parasitic diseases and major steps needs to be taken to control the flies or to form mass treatment of goats particularly when the L_1 are present in the host. The lesions are not developed at this stage and particularly in case of live L_1 , the skin has not been secondary infected with pathogenic bacterial or fungal species yet. Knowledge of internal life cycle and the lesions produced by this parasite throughout the year can help in the designing of prophylaxis and eradication programs against GWFI which is still underestimated by veterinarians and goats farmers in spite of its broad distribution and economic impacts. Therefore, it seems myiasis due to *P. silenus* is a real problem which affects the goat population of this area. The internal life cycle and type of tissue reaction to different stages of this myiasis are no doubt issues for considerable discussion among scientists and this investigation aims to offer them further food for thought. Although some aspects of myiasis has been described since ancient times, the host tissue reaction and pathology of this infestation are still unknown and their

understanding could be helpful in designing the best time for eradication of the parasite and treatment of the infected animals.

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