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The investigation of lipid peroxidation, anti-oxidant levels and some hematological parameters in sheep naturally infested with *Wohlfahrtia magnifica* larvae

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ABSTRACT

Wohlfahrtia magnifica is the main agent of traumatic myiasis in many central and eastern European countries as well as in the Mediterranean basin and Asian countries. The present study was aimed to investigate the effect of myiasis infestation on lipid peroxidation, antioxidant levels and several hematological parameters in the sheep naturally infested with *W. magnifica* larvae. A total of 38 sheep, including 19 awassi sheep naturally infested with *W. magnifica* larvae and 19 clinically healthy awassi sheep, were studied. The infected animals were divided into three groups depending on the number of larvae (Group 1: <50 larvae; Group 2: 50–100 larvae; Group 3: >100 larvae). In blood samples, red blood cell counts, hemoglobin, hematocrit concentration and white blood cell, neutrophil, lymphocyte, eosinophil, basophil counts, plasma malondialdehyde, erythrocyte glutathione levels and erythrocyte glutathione peroxidase activity were studied. The results revealed a marked decrease in red blood cell counts and hemoglobin concentrations along with a significant increase in white blood cell and neutrophil counts. The elevation in plasma malondialdehyde levels, a function of lipid peroxidation, established a significant difference between the control group and groups 2, 3. Decreased activity of erythrocyte glutathione peroxidase was found significant in the control group and all parasitemia.

We conclude that in natural infestations with *W. magnifica*, as in infestation with *Lucilia cuprina*, there is a notable increase in inflammatory activities resulting from the movements, secretions, and toxins of the larvae and form the toxins secreted by the bacteria – which leads to an impression of anemia – and that the tissue injury results in an increase in level of free radicals in the organism.

1. Introduction

Wohlfahrtia magnifica (Schiner) is regarded as the major causative agent of traumatic myiasis in the majority of central and eastern European countries as well as in the Mediterranean basin and Asia countries (Hall, 1991; Hall and Farkas, 2000; Farkas et al., 2009; Zumpt, 1965). Infestations with the larvae of *W. magnifica* (wohlfahrtiosis) are demonstrated in many domestic animal species (Hall and Farkas, 2000; Farkas et al., 2001, 2009). Its larvae are obligatory parasites and their most vulnerable hosts are reported to be sheep (Hadani et al., 1971; Martinez et al., 1987). Previous studies show that *W. magnifica* is the primary species that causes traumatic myiasis in sheep in Turkey (Özdal, 2004; Sayın İpek and Şaki, 2010; Şaki and Özer, 1999a,b).

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Table 1
Date of infections, age, body location, species and number of larvae with referans farms of sheep suffering from traumatic myiasis.

Sheep (no.)	Date	Age	Body location	Species	Number of larvae 2, 3	Referans farms
1	May 2008	0–1	Leg	*W. magnifica*	89	C
2	May 2008	1–2	Leg	*W. magnifica*	79	E
3	May 2008	0–1	Tail	*W. magnifica*	120	E
4	May 2008	2–3	Udder	*W. magnifica*	48	E
5	June 2008	0–1	Ear	*W. magnifica*	93	A
6	June 2008	0–1	Tail	*W. magnifica*	113	A
7	July 2008	0–1	Tail	*W. magnifica*	110	D
8	July 2008	1–2	Leg	*W. magnifica*	75	D
9	July 2008	2–3	Leg	*W. magnifica*	77	A
10	July 2008	2–3	Udder	*W. magnifica*	47	B
11	August 2008	2–3	Leg	*W. magnifica*	70	B
12	May 2009	1–2	Head	*W. magnifica*	74	C
13	May 2009	1–2	Leg	*W. magnifica*	94	E
14	June 2009	2–3	Udder	*W. magnifica*	43	B
15	June 2009	2–3	Udder	*W. magnifica*	44	A
16	June 2009	2–3	Udder	*W. magnifica*	41	A
17	July 2009	0–1	Tail	*L. sericata*	70	C
18	July 2009	1–2	Leg	*W. magnifica*	89	D
19	July 2009	0–1	Udder	*W. magnifica*	40	E
20	July 2009	2–3	Tail	*W. magnifica*	141	B

As a result of their feeding activity, *W. magnifica* larvae may cause severe damage to tissues within only a few days. The continuous abrasion caused by the larvae further enlarges the inflamed wound in the skin, such that the wound may reach a diameter of 0.5–20 cm and a depth of 5–8 cm. Besides, the infested skin appears tense, warm and sometimes edematous. Epidermal necrosis develops along the margins of advanced wounds. The areas which do not harbor any larvae are malodorous due to suppuration. The general condition of the animals deteriorates with time. Clinical symptoms of *W. magnifica* infestation include restlessness, anxiety, inappetence, fever, and increased respiratory rates (Farkas et al., 1997). Apart from their toxic and irritating effects, myiasis larvae may lead to reproductive disorders, lameness, blindness, and reduced yields in infested animals as a result of the secondary infection of wounds. If not treated, animals suffering from traumatic myiasis may die from intoxication, septicemia, shock, histolysis, and secondary infections (Farkas et al., 1997; Martinez et al., 1987; Zumpt, 1965).

As long as they do not exceed certain levels, free radicals play a significant role in the defense of the organism against foreign substances and infectious agents. However, in the cases of infection and tissue breakdown or inflammation, lipid peroxidation occurs and the level of free radicals increases (Erenel et al., 1992; Wolff et al., 1986).

Unsaturated fatty acids found in the cell membrane react with oxygen metabolites and break down into a variety of end-products, including peroxide, alcohol and aldehyde. This process is referred to as lipid peroxidation. Once initiated, lipid peroxidation continues by means of auto-catalysis and eventually results in the generation of a toxic end-product, malondialdehyde (MDA) (Köse and Doğan, 1992). The enzyme glutathione peroxidase (GSHPx), which is found in the cytoplasm of cells, reduces the adverse effects of detrimental hydroxy acids (Aras et al., 1976). Normally, reduced glutathione (GSH) is found at very high levels in tissues. GSH protects the tissues against free radicals by limiting lipid peroxidation (Akkuş, 1995).

Several studies have been devoted to the sheep infested experimentally with the larvae of *Lucilia cuprina*, in an attempt to understand the infestation pathology of the parasite. The infestations carried out with concentrated larval applications indicate that severe inflammatory response develop against bacteria and the toxins found in the wound, and that cruel toxic effects build up in the animals affected with myiasis (Broadmeadow et al., 1984). The larvae are reported to stimulate massive cellular infiltration on the wound surface and dermis (Colditz et al., 1996). Moreover, a previous study reports that ammonium bicarbonate found in the larvae of *L. cuprina* can cause the death of the animal by increasing the ammonium concentration in the blood of the host (Guerrini, 1997). However, no studies that examine the effect of infestation on blood and biochemical parameters in the sheep suffering from natural traumatic myiasis and *W. magnifica* infestation concerning the number of larvae and lesion size and deepness are available up to now.

With the present study, we aimed to determine the effect of natural infestation of sheep with *W. magnifica* – the primary factor for traumatic myiasis – on several hematological parameters, ammonium, MDA, GSH, and GSHPx.

2. Materials and methods

2.1. Study period and study area

The present study was conducted between January 2008 and December 2009 in Diyarbakir province, located in south-eastern Anatolia. Five areas were chosen for the present study: (A) the city center (37°55′N, 40°14′E) the counties which were (B) 40 km (Çınar, 38°96′N, 35°24′E), (C) 50 km (Ergani, 38°26′N, 39°76′E), (D) 70 km (Silvan, 41°01′N, 38°14′E) and (E) 90 km (Lice, 40°65′N, 38°47′E) far from the center.