Prevalence and economic significance of Hypoderma bovis in Ardahan

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Abstract
The aim of this study was to determine the prevalence of cattle infected by Hypoderma, a parasite that causes great economic losses in the world, especially in the Ardahan region. A total of 31,095 cattle skins were randomly selected between 15 November 2020 and 15 March 2021 and examined. The prevalence of warbles was 47.3%. The proportions of cattle with warbles on the skin on their backs were 76.6% in one-year-old and younger animals, 60.9% in two- and three-year-old animals, and 16.5% in cattle that were four years old or more. It was determined that 53.6% of the cattle with warbles on the back skin were indigenous breeds, 47.6% were Simmental and Montofon crosses, and 36.2% were domestic breeds. The average number of larvae was 8.2. Interviews with the owners of the infected animals revealed that hide loss was on average 10% in liveweight, with a 5% to 8% in the milk production of dairy cows. In the animals under investigation the total economic loss from warbles caused by damage to the hide was calculated as US$114 188. Thus, it is important to carry out preventive practices regularly to limit the spread of the parasite and prevent economic loss in Ardahan.

Keywords: cattle, economic loss, Turkey, warble
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Introduction
Ardahan has an ideal environment for cattle breeding in Turkey and contains approximately 330 151 animals (Ayvazoğlu & Demir, 2020). Thus, cattle breeding is one of the most important sources of income for the people in the region, with some 60% of the population employed in this field.

Infection by hypoderma is characterized by warbles, which are seen seasonally in the dorsal and lumbar regions of animals, especially in autumn, winter and, in some instances, spring (di Regalbono et al., 2003; Oğuz, 2013; Atalge et al., 2021). The period of parasitism by hypoderma larvae in cattle is 9 - 10 months. If pupa and adult fly periods are included, the life cycle is approximately 1 year. Adult flies lay their eggs in the hairs of the lower body of cattle. First instar larvae (L1) migrate in the connective tissues of cattle from summer to autumn. L1 then develops into second (L2) and third (L3) larval stages. The L3 stage occurs on the back and creates swellings under the skin called warbles. Then L3 falls to the ground and becomes a pupa. After about five weeks, adult flies emerge to complete their biological cycle. Adult flies live only 1 - 2 weeks (Taşcı et al., 2018). The presence of hypoderma larvae can be detected by palpating L2 and L3 instar larvae on the backs of the infested animals in autumn and winter or by examining the internal organs and subcutaneous tissues of the carcass (Kettle, 1990). Morphological differentiation of L3 stage larvae is not usually difficult, although sometimes it can be because hypoderma species have similar ecological, biological, and morphological characteristics (Otranto et al., 2003). The spread of Hypoderma bovis and the infestation rate vary according to geographical location and climatic conditions of the regions (Özkutlu & Seygili, 2005). According to studies in various countries, the infestation rate can reach up to 100% (Otranto et al., 2005; Ahmed et al., 2012; Saidani et al., 2016; Patra et al., 2018). The larvae of Hypoderma lineatum and Hypoderma bovis cause significant economic losses owing to decreased meat-milk yield and growth, and perforation of the hide (Hall & Wall, 1995, Boulard, 2002; Ahmed et al., 2016; Taylor et al., 2016; Patra et al., 2018; Merhan et al., 2020). Therefore, the aim of this study was to determine the prevalence of
Hypoderma infection of cattle in Ardahan by palpating the skin in autumn and winter and to attract attention to the economic importance of the disease.

Materials and Methods

Ethical approval for this study was received from Ardahan University Scientific Publication and Ethics Committee; decision number E-67796128-000-2100006245.

Animal barns in the Merkez, Çıldır, Posof, Hanak, Damal, Göle districts of Ardahan were visited between November 2020 and 15 March 2021. These dates corresponded to the period between the return date of the cattle from the pasture and the date of going to the pasture. The backs of cattle were palpated to detect the boils or warble sacs caused by Hypoderma infection. A total of 31 095 randomly chosen animals were examined by hand. Age, breed, and gender of affected cattle were also recorded. The number of warbles was divided into three groups according to their frequency. Cattle with fewer than five warbles on the back skin were in Group 1, those with six to ten were in Group 2, and those with 11 or more were in Group 3.

The SPSS 20 program (IBM Corp., Armonk, New York, USA) was used for statistical analysis. Chi-square test was used to evaluate the effects of age, sex and location on the incidence of Hypoderma bovis. The general linear model procedure was used to assess these effects on the intensity of infestation. When significant ($P < 0.05$) effects were detected, the means were compared with Tukey’s test.

Results and Discussion

Warbles caused by hypoderma larvae were detected on the backs of 47.3% of the 31 095 cattle that were examined for hypodermosis (Table 1). The frequency of warbles from hypoderma infection varied ($P < 0.01$) between the Ardahan Merkez, Çıldır, Posof, Hanak, Damal, and Göle districts. A total of 37.7% (550) of the cattle had fewer than five warbles, 46.4% (6 821) had five to ten, and 15.9% (2 345) had more than ten. Infection was detected at higher rates in areas with the largest numbers of cattle (Çıldır and Göle) and lower in areas with the smallest number (Hanak and Posof). The incidence of warbles in places at an altitude of 1800 or more metres above sea level, such as Damal, Göle and Çıldır, was 48.7% and in Posof at a lower altitude (1500 m), warbles were found in 29.8% of the cattle ($P < 0.01$).

Table 1 Distribution of warbles from hypoderma infection in regions of Ardahan, Turkey

<table>
<thead>
<tr>
<th>District</th>
<th>Incidence of hypoderma infection</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Warbles</td>
<td>No warbles</td>
</tr>
<tr>
<td>Centre</td>
<td>3 184 (46.2%)</td>
<td>3 706 (53.8%)</td>
</tr>
<tr>
<td>Çıldır</td>
<td>4 410 (51.1%)</td>
<td>4 216 (48.9%)</td>
</tr>
<tr>
<td>Posof</td>
<td>404 (29.8%)</td>
<td>952 (70.2%)</td>
</tr>
<tr>
<td>Hanak</td>
<td>842 (46.8%)</td>
<td>956 (53.2%)</td>
</tr>
<tr>
<td>Damal</td>
<td>1 786 (36.8%)</td>
<td>3 070 (63.2%)</td>
</tr>
<tr>
<td>Göle</td>
<td>4 090 (54.0%)</td>
<td>3 479 (46.0%)</td>
</tr>
<tr>
<td>Total</td>
<td>14 716 (47.3%)</td>
<td>6 379 (52.7%)</td>
</tr>
</tbody>
</table>

Of these animals, 61% were Simmental and Montofon crossbreds, 23.7% were imported breeds, and 15.4% were native breeds, 38.7% were male, 61.3% were female, and 32.0% of them were under one year old, 26.1% were 2-3 years old and 41.9% were 4 years old and over. Table 2 shows the incidence rates of the disease by breed, age, and gender. The incidence of warbles caused by hypoderma larvae was twice as high in males as in females ($P < 0.01$). The age of the cattle likewise affected whether they had been infected by hypoderma, with the cattle that were one year old and less and those four years old and older being more likely to be infected than those that were two or three years old ($P < 0.01$). Finally, the imported breeds had the highest percentage of cattle infected with hypoderma, Simmental and Montofon crosses were intermediate, and the local breeds experienced the lowest rate of affliction ($P < 0.01$).
Table 2 The influence of breed, age and gender on the incidence of hypoderma infection in cattle from Ardahan, Turkey

<table>
<thead>
<tr>
<th>Classification</th>
<th>Incidence of hypoderma infection</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Warbles</td>
<td>No warbles</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8 308 (68.9%)</td>
<td>3 751 (31.1%)</td>
<td>12 059</td>
</tr>
<tr>
<td>Female</td>
<td>6 425 (33.7%)</td>
<td>12 628 (66.3%)</td>
<td>19 053</td>
</tr>
<tr>
<td>≤1</td>
<td>7 656 (76.7%)</td>
<td>2 327 (23.3%)</td>
<td>9 983</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 - 3</td>
<td>4 929 (60.9%)</td>
<td>3 168 (39.1%)</td>
<td>8 097</td>
</tr>
<tr>
<td>≥ 4</td>
<td>2 148 (16.5%)</td>
<td>10 884 (83.5%)</td>
<td>13 032</td>
</tr>
<tr>
<td>Crossbred</td>
<td>9 018 (47.6%)</td>
<td>9 917 (52.4%)</td>
<td>18 935</td>
</tr>
<tr>
<td>Imported</td>
<td>3 984 (53.6%)</td>
<td>3 442 (46.4%)</td>
<td>7 426</td>
</tr>
<tr>
<td>Native</td>
<td>1 731 (36.4%)</td>
<td>3 020 (63.6%)</td>
<td>4 751</td>
</tr>
</tbody>
</table>

Males were more severely infected than females (P <0.01). The greatest severity of infection was observed consistently in younger animals, and it decreased as the animals became older (P <0.01). The densities of warbles by gender and age are given in Table 3.

Table 3 Relationships of gender and age with the severity of hypoderma infection as measured by the number of warbles on the back of an infected animal

<table>
<thead>
<tr>
<th>Classification</th>
<th>Severity of infection groups, warbles</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;5</td>
<td>5 - 10</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2 869 (51.7%)</td>
<td>3 998 (58.5%)</td>
</tr>
<tr>
<td>Female</td>
<td>2 681 (48.3%)</td>
<td>2 840 (41.5%)</td>
</tr>
<tr>
<td>≤1</td>
<td>3 297 (59.4%)</td>
<td>3 083 (45.1%)</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–3</td>
<td>1 768 (31.9%)</td>
<td>2 504 (36.6%)</td>
</tr>
<tr>
<td>≥ 4</td>
<td>485 (8.7%)</td>
<td>1 251 (18.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>5 550</td>
<td>6 838</td>
</tr>
</tbody>
</table>

Hypoderma infection causes cutaneous myiasis in domestic and wild ruminants and great economic losses in the world (Boulard, 2002; Çiçek et al., 2011, Yadav et al., 2017; Atalge et al., 2021). Although Hypoderma spp. has a cosmopolitan distribution, this insect is commonly found in tropical and subtropical countries (Patra et al., 2018). The prevalence of infection varied from approximately 3% to 100% (Abul-hab & S'adi, 1973; Preston, 1984; Otify & Mansour, 1994; Puccini et al., 1997; Yin et al., 2003; di Regalbono et al., 2003; Papadopoulos, 2004; Otranto et al., 2005; Balkaya et al., 2010; Çiçek et al., 2011; Ahmed et al., 2012; Ilie et al., 2012; Dehghani et al., 2012; Yadav et al., 2013; Yagoob et al., 2014; Saidani et al., 2016; Taşcı et al., 2018). In this study, the prevalence of infection by hypoderma in Ardahan was 47.3%. This province is important to Turkish animal agriculture and this rate was higher than in most other regions (Martinez-Moreno et al., 1996; Boldbaatar et al., 2001; di Regalbono et al., 2003; Otranto et al., 2005). The reasons might include intensive use of pasture, absence of formal control programmes, and the less than satisfactory implementation of control programmes by individual breeders.

The prevalence of hypoderma varies according to the geographical characteristics of the region (altitude, climate, etc.) and the methods used in fattening cattle (pasture or confined feeding) (Özkutlu & Sevgili, 2005; Taşçı et al., 2018). In the present study, animals were more frequently affected at high altitude than at lower elevation. For example, Ahmed et al. (2012) observed the prevalence of affected cattle was 8.9% in plains and 20.6% in hilly areas. The risk of infestation was also higher in pastured animals than in those fed in confinement (di Regalbono et al., 2003; Özkutlu & Sevgili, 2005). These results might provide...
insight into the high prevalence of hypoderma infestation in Ardahan where pasture use is intense. Previous studies reported that the average number of larvae – and hence the severity of infestation – varied between 5.1 and 13.8 per animal (Sayın et al., 2000; Kara et al., 2005; Karatepe & Karatepe, 2008; Taşcı et al., 2018). The number of larvae per animal in the present study (8.2) fell within this range.

Although the prevalence of hypoderma infestation varied with gender in the present study, previous results were conflicting. Higher rates of infestation were found in females (Şimşek et al., 2008; Balkaya et al., 2010; Ciçek et al., 2011) and in males (Kara et al., 2005; Taşcı et al., 2018). These results might be attributable to differences in management. In Ardahan, males are commonly pastured, and females are kept indoors for milk production.

Animals less than one year old were reportedly infested more frequently (Saidani et al., 2016; Kara et al., 2005; Khan et al., 2006) and with greater severity (Kara et al., 2005, Taşcı et al., 2018) than older animals. The observation that infestation decreased with age might be explained by a degree of immunity developing with age and resistance to the disease increasing (Panadero et al., 2009). Thus, whereas seroprevalence tended to increase with age, the true prevalence and number of larvae under the skin of the back decreased with age.

The prevalence of hypoderma infestation varied according to breed (Saidani et al., 2016). Higher seropositivity was found in Brown Swiss and Brown Swiss crosses than in Simmental and Simmental crosses on the Kars Plateau of Turkey (Taşcı et al., 2018). The present finding that imported breeds had a higher incidence of warbles than the native breeds might be attributable to the local adaptation of the latter animals.

Interviews with the owners of the animals revealed that warbles affected the value of the hide. Hassan et al. (2010) reported losses in liveweight and milk production by dairy cows as additional sources of loss that could be attributed to hypoderma. In addition, the activities of flies and larvae cause discomfort and injuries resulting in lower animal welfare. The mean price of a hide infested with hypoderma larvae was reduced by US$ 0.59/kg. With the average weights of hides being 10 kg, 15 kg and 20 kg for one-year-old animals, two-year-old animals, and animals three years old and over (Taşcı, 2018), the economic value of losses because of hide defects was estimated to be US$114 188 [(10 × 7 656 x 0.59) + (15 × 4.929 x 0.59) + (20 × 2 148 x 0.59)] (US$ 1 = 13.56 Turkish lira in February 2022). In 2011, skin-related loss in the value of cattle in Afyon was calculated as US$12 192 per year (Ciçek et al., 2011) and in a study in Kars in 2018, the annual loss attributable to hide damage was US$113.000 (Taşcı et al., 2018). In other countries, the annual economic loss due to hypoderma infestation has been estimated at US$22.8 million in Pakistan (Khan et al. 2006), US$ 119 million in Greece and US$11.5 million in Italy (Macchioni, 1984).

Conclusions
Considering the attitude of cattle farmers in Ardahan preventive medicine practices seemed to be avoided, insecticides were not applied regionally but on an enterprise basis, and that at the time of study there was no regular protection control programme. Although the prevalence of hypoderma infestation should be lower, it was higher than in the western region of Turkey and many European countries. Therefore, timely vaccination and the use of effective control strategies were imperative to limit economic losses. Training programmes for education producers about the effects of hypoderma infestation are of vital importance in protecting animal welfare.

Authors’ Contributions
CA (ORCID: 0000-0003-2064-0657) conceptualization, methodology, writing and original draft preparation; ŞK (ORCID: 0000-0003-3727-8893) data collection, methodology and review; PAD (ORCID: 0000-0002-7010-0475) analysis, writing, review and editing.

Conflict of Interest Declaration
The authors declare that they have no conflicts of interest relative to the content of this paper.

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