

**Bacteria Associated with Subcutaneous Abscesses of Cattle Caused by
Hypoderma spp Larvae in North of Iran**

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Abstract

This study was performed from February to April 2006; several visits were made to abattoirs in the north of Iran for *Hypoderma* spp infestation. Necropsy inspection of slaughtered and skinned animals were carried out by examination of the inner skin surface and subcutaneous tissues. Warbles were isolated by squeezing nodules from subcutaneous tissues. In the case of abscess presence, aseptic sample were taken from abscesses. The parasitological and bacteriological examinations were performed on the samples. The results indicated that 104 out of 958 of slaughtered animals were infested to *Hypoderma* spp in which 48 (46.15 %), 34 (32.69 %) and 22 (21.15 %) were infested to *Hypoderma bovis*, *Hypoderma lineatum* and both species, respectively. Following bacterial analysis, the following bacteria were isolated: *Escherichia coli*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Klebsiella pneumoniae*.

Key words: Cattle, Subcutaneous abscess, *Hypoderma* spp, Micro-organism

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Introduction

Cattle hypodermosis is a myiasis caused by the larvae of *Hypoderma bovis* and *Hypoderma lineatum* (Diptera, Oestridae) and characterized by the presence of subcutaneous warbles in the dorsal parts of thorax and lumbar of the infested animals.¹ This myiasis is present worldwide in tropical and subtropical areas and causes economic losses by reducing milk and meat production accompanied with hide damage.² These two species of *Hypoderma* infest mainly cattle, horses and occasionally humans.³

For the past 50 years, cattle hypodermosis has represented one of the most significant parasitic diseases in many countries of the northern hemisphere. Hypodermosis greatly impairs livestock production not only by inducing mechanical damage to the internal organs and skin but also by suppressing the host's immune responses.^{2,4,5} As subcutaneous abscesses are mostly infected with different species of zoonotic bacteria, hence they may be ruptured during the skinning process, and their bacterial load can contaminate the surface of the carcass. Therefore, consumption of this infected meat may have public hygienic importance.

At the best of our knowledge, there has not been any study on the bacterial contamination of subcutaneous abscesses related to the *Hypoderma* spp larvae infestation in cattle. The aim of this study was to assess the *Hypoderma* spp in indigenous cattle and the analysis of bacterial contamination associated with the formation of abscesses.

Materials and Methods

This study was performed from February to April 2006 in north of Iran. Study area has a humid and temperate climate, with mean rainfall of about 1200 mm, relative humidity between 40 and 100 % and mean temperature of 17.5 °C.⁶

Necropsy inspection of slaughtered and skinned animals were carried out by examination of the inner skin surface and subcutaneous tissues. Warbles (L₃) were isolated by squeezing nodules from subcutaneous tissues. The larvae isolated from each infested animal were preserved in 70% alcohol for later identification. Animal age group was estimated on the basis of the dentition formula.⁷ On this basis, the animals were divided into two groups namely ≤ 2 and > 2 years old. Subcutaneous abscesses caused by myiasis were dissected with sterile surgery blade and sampled by the sterile swabs. The swabs were placed into sterile tubes containing Peptone Water Medium. Then, the samples were cultured on Brain Heart Infusion Agar (BHIA) and Blood Agar (BA) plates. Finally, the media were incubated at 37 °C for 24h in aerobic and anaerobic conditions. After incubation the appeared colonies were identified by routine bacteriological techniques such as gram staining and biochemical tests (Catalase, Coagulase, Simmon citrate, Urease, Gelatin melting, Nitrate reduction, TSI, OF and Carbohydrate fermentation). *Hypoderma* spp larvae were identified according to Zumpt based on the peritreme structure. In *H. bovis* posterior spiracular plates surrounding the button has a narrow funnel-like channel whereas, in *H. lineatum*, it has a broad channel.⁸

Results

Nine hundred and fifty-eight cattle, 732 males and 226 females, were examined. Out of 958 examined animals, 104 (10.86 %) were parasitized, in which 72 (69.33 %) were males and 32 (30.77 %) were females. The results also indicated that 24 (23.08 %) and 80 (76.92 %) of the infested cattle were belong to ≤ 2 and > 2 years age groups, respectively. The number, sex and age of the examined and infested animals according to the month of collection were shown in Table 1. The infestation to *H. bovis*, *H. lineatum* and both species were

Table 1. The frequency of *Hypoderma* larvae on the infested cattle, in North of Iran

| Months | Number of animals | Infested animals | Age and sex of infested animals | | | |
|--------------|-------------------|------------------|---------------------------------|-----------|-----------|-----------|
| | | | Male | | Female | |
| | | | ≤ 2 years | > 2 years | ≤ 2 years | > 2 years |
| February | 294 | 27 | 3 | 16 | 2 | 6 |
| March | 511 | 61 | 9 | 33 | 4 | 15 |
| April | 153 | 16 | 3 | 8 | 3 | 2 |
| Total | 958 | 104 | 15 | 57 | 9 | 23 |

Table 2. The results of bacteriological study of subcutaneous abscesses, divided according to the *Hypoderma* spp, sex and age groups

| Number | Sex | Age | <i>Hypoderma</i> spp | <i>E. coli</i> | <i>S. pyogenes</i> | <i>S. aureus</i> | <i>S. epidermidis</i> | <i>K. pneumonia</i> |
|--------|-----|--------------|----------------------|----------------|--------------------|------------------|-----------------------|---------------------|
| 1 | F | ≤2 years old | <i>H. bovis</i> | + | - | + | - | - |
| 2 | F | ≤2 years old | <i>H. bovis</i> | + | + | - | - | - |
| 3 | F | ≤2 years old | <i>H. lineatum</i> | + | + | - | + | - |
| 4 | M | >2 years old | Both spp. | + | - | + | + | + |
| 5 | F | >2 years old | <i>H. bovis</i> | + | + | - | - | - |
| 6 | M | ≤2 years old | <i>H. lineatum</i> | + | + | - | + | + |
| 7 | F | ≤2 years old | <i>H. bovis</i> | - | + | + | - | - |
| 8 | F | ≤2 years old | <i>H. bovis</i> | + | + | - | + | - |
| 9 | F | ≤2 years old | Both spp. | + | + | - | - | - |

seen in 48 (46.15 %), 34 (32.69 %) and 22 (21.15 %) samples, respectively. The bacteriological results indicated that, from 23 sampled abscesses 9 of them had bacterial contamination. The following bacteria were isolated: *Escherichia coli*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermicus* and *Klebsiella pneumonia* (Table 2).

Discussion

In the past years, no detailed research has been conducted on the bovine hypodermosis in Iran. Besides, the information related to basic epidemiology of the disease in Iran is scanty. Up to now, the only published work on cattle hypodermosis was showed the annual

prevalence of 3 % infestation was reported in Southern regions of Iran.¹⁰ In this study, animals' infestation to *H. lineatum* was higher than *H. bovis* whereas our findings revealed that *H. bovis* was predominant species in the study regions. It could be explained by the fact that our study regions, just as described above, are of relatively moderate and humid climate while the climatic conditions of Southern regions of Iran are tropical and dry. Therefore, our findings are more similar to those of Berkenkamp *et al.* that *H. bovis* is commonly observed in moderate and cool climate.⁹ Our results indicated that most infested animals were over 2 years old; the same results were obtained by some authors.^{10,11} This study demonstrates that aerobic and facultative bacteria could

isolate from subcutaneous abscesses. However, based on to previous reports, anaerobic bacteria were isolated more frequently from liver abscesses and their isolation was associated with colonic and biliary origins.¹² The isolation of *E. coli*, *S. pyogenes*, *S. aureus* and *S. epidermidis* from subcutaneous abscesses of cattle are in agreement with the results of Tadayon *et al*, who showed the most frequented bacteria that isolated from subcutaneous abscesses of sheep and goat belong to the following genera: *Corynebacterium*, *Staphylococcus*, *Streptococcus*, *Pasteurella*, *E.coli*.¹³

In this work male animals showed higher mixed bacterial contamination than females. We did not find the plausible explanation for the less sensitivity of female animals than males to mixed bacterial contamination.

The third stage larva (L₃) of both *Hypoderma* species after migration reaches to the dorsal region of thorax and lumbar, finally it penetrates into the skin for releasing from animal. It should be pointed out that this migration is occurred in the last of winter and at this time of the year animals are kept in closed stalls with high density. On the other hand, the high prevalence of *E. coli* in these conditions is a good explanation for more isolation than the other bacteria from subcutaneous abscesses in cattle. Finally, based on our knowledge and the review of literatures, there are not enough studies about subcutaneous abscesses in cattle. On the other hand, because of economic lost and contamination of the abscesses by microorganisms, more studies should be done to learn about incidence and antibiotic resistance profile in the other parts of the world.

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