# Seroprevalence of Hypodermosis in Cattle in Nigde Province of Turkey by Comparison of Commercial and Indirect-ELISA Methods

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## ABSTRACT

The aim of this study was to determine the seroprevalence of hypodermosis in cattle of Nigde province, located in the centre of Turkey. A total of 183 serum samples were collected between December 2009 and February 2010 for this goal. The serum samples were analyzed with a commercial ELISA kit (Pourquier ELISA Hypodermosis bovine serum screening) and an indirect-ELISA (using antigen Hypodermin C) methods. The results of the tests with the commercial ELISA kit showed that 53 (28.9%) out of the 183 cattle were seropositive for hypodermosis. Eleven (18.3%) out of the 60 female and 42 (34.1%) out of the 123 male, were found to be seropositive. The seropositivity rate was calculated as 21.8% (40/183) by indirect-ELISA, and the seroprevalence was 18.3% (11/60) and 23.5% (29/123) in female and males, respectively. A statistically significant correlation was observed when the two test methods for detecting seropositivity of *Hypoderma* were compared ($p<0.001$). This study is to the best of our knowledge the first serological survey on hypodermosis in cattle from the Nigde province of Turkey.

**Key Words:** *Hypoderma* spp., hypodermosis, Enzyme-Linked Immunosorbent Assay (ELISA), seroprevalence, cattle, Nigde, Turkey.

## INTRODUCTION

Amongst ectoparastites, warble fly infestation is a common disease in cattle. *Hypoderma bovis* and *H. lineatum* are the most important species of *Hypoderma* as their larvae are their specific host and they are parasitic to cattle. Both species display palearctic distribution properties (1). In many cases there are no detectable clinical signs due to infestation however *Hypoderma* spp. can cause economic losses by reducing milk production, increasing the meat trim and damaging the hide. Cattle often become very agitated when flies attempt to lay eggs. They will often try to escape from female flies and will seek shade or water to avoid the flies, and in some cases breaking through fences in attempt to escape resulting in physical damage. Loss of weight due to their attempts by running from the adult flies may be considerable. Interruptions of normal grazing behavior may also lead to decreases in weight gain.

Anti-hypoderma antibodies appear within 4-8 weeks post infection during the migration of first instar larvae and persist at diagnostic levels for 3 or 4 months after the emergence of the third instar larvae (2). The antibody levels peak between November and March in Europe. This represents the optimal period for the collection of sera in order to obtain an early diagnosis (3).

The migration and the development of the *Hypoderma* larvae in the cattle take about 10-11 months leading to the condition of hypodermosis (1, 4). A visible swelling in the skin of the dorsum may indicate the presence of hypodermosis, however a small number of subcutaneous *Hypoderma* larvae may not result in any symptoms. Warble fly infestation with a large number of larvae can lead to weight loss, loss of appetite, and reduction in meat and milk production. After the larvae leave the body, the dermal lesions heal with granulation tissue. Hides from cattle with warble fly infestation have a reduced commercial value (5).

Various studies carried out in Turkey have reported that hypodermosis in cattle caused by *H. bovis* and *H. lineatum* is common and that it leads to significant economic losses (6-8). Those studies determined the *Hypoderma* spp. infestation rate in Turkey to be between 0.3% and 68%, and that the occurrence of *H. bovis* is more wide spread than *H. lineatum* (7, 9-16). The study carried out by Sayin *et al*. (14) to investigate the epidemiology of warble fly infestation in cattle encompassed all the regions of Turkey. The study was carried out from grazing cattle and detected *Hypoderma* infestation rates of 28.3% in the Black Sea region, 28% in the Marmara, 41.6% in the Aegean, 33% in the Mediterranean region, 38.9% in the Central Anatolia, 41.9% in the East Anatolia, and 47.8% in the Southeastern Anatolia regions.

In recent years diagnosis of warble fly infestation in Turkey was performed by using serological methods. Some of these studies used the ELISA, and reported the seroprevalence rate was between 23.3% and 38.6% (17-19). The aim of this study is to investigate the seroprevalence of hypodermosis in cattle slaughtered in Nigde abattoir using the commercial ELISA kit and the indirect ELISA test methods and also to compare the performances of the two test methods for the serological diagnosis of hypodermosis.

## MATERIALS AND METHODS

The animals used in this study consisted of cattle of at least one year of age slaughtered at a local abattoir in Nigde province of Turkey. Blood samples were obtained from a total of 183 cattle between December 2009 and February 2010. The sera were separated and stored at -20°C until assayed.

The sera samples were separated by sex. In order to investigate *Hypoderma* antibodies, the study utilized ELISA Test Kit (ELISA Pourquier Hypodermosis bovine serum screening). The ELISA test was carried out according to the manufacturer's instructions. The values obtained through reading the microtiter plates at a wavelength of 450nm using the ELISA microplate reader (MR-96A) were then calculated by the equation specified in the kit's procedures:

$$\text{Value \%} = \frac{\text{Samples' Optical Density} - \text{Negative Control's Optical Density}}{\text{Positive Control's Optical Density} - \text{Negative Control's Optical Density}} \times 100$$

Test sample's percentage value of ≥ 55%, were considered positive and results of ≤ 45% were considered to be negative. The same samples were then tested using Hypodermin C as the antigen in the analysis of the samples with an indirect laboratory devised ELISA. The technique was performed according to Simsek *et al.* (18). The plates (Dynatech Laboratories, IA, USA) were coated with l00 µl of 2.5 µg/ml of antigens in 0.1 M carbonate/bicarbonate buffer (pH 9.6) per well. Sera were diluted 1:200 in PBST and the conjugate (anti-bovine IgG conjugated to rabbit peroxidise, Sigma A5295) were added at a 1:7500 dilution. Each plate was read out 450 nm using an ELISA reader (Bio-Tek Instruments, USA). The results were expressed as the mean of the optical density (OD). All samples were tested in duplicate and were repeated when there was a difference up to 10% between the duplicates. The cut-off value was calculated as the mean of the negative control sera absorbance values plus three standard deviations (18).

To analyze and compare the results obtained from the commercial and the indirect ELISA tests with respect to gender, the Yates's-corrected chi-square test and Cramer's V coefficient was applied. The data was analyzed with the SPSS 17.0 for Windows. $P<0.05$ was regarded as statistically significant.

## RESULTS

The results of the tests showed that out of the 183 cattle examined, 53 (28.9%) samples were determined to be seropositive for hypodermosis with the commercial ELISA, while 40 (21.8%) were seropositive with the indirect-ELISA (Table 1).

When the both tests were compared, out of the 183 cattle, the results for the 117 (63.9%) samples were negative for hypodermosis in both of them, while 27 samples (14.7%) were positive in both tests. Out of the 39 samples that were

**Table 1:** Comparison of the commercial and the indirect-ELISA tests in the diagnosis of hypodermosis.

| | Commercial test kit | Indirect ELISA |
|---|---|---|
| Overall total cattle | 183 | 183 |
| Tested Positive | 53 | 40 |
| Tested Negative | 130 | 143 |

**Table 2:** Comparison of the commercial and the indirect-ELISA tests in the diagnosis of hypodermosis in terms of gender

| | Commercial test kit | Indirect ELISA |
|---|---|---|
| Overall total males positive | 42 | 29 |
| Overall total females positive | 11 | 11 |

dissimilar in their test outcomes, 13 (7.1%) samples were negative with the commercial ELISA and positive with the indirect-ELISA, whereas the other 26 (14.2%) samples tested positive with the commercial ELISA and negative with the indirect-ELISA. Therefore, seropositivity rates between the two tests have been determined to be moderately statistically correlated using the Cramer's V coefficient as 0.449 ($p < 0.001$) and the compatibility of the two tests were calculated as 78.6%.

The seropositivity of both diagnostic tests was compared according to gender. Out of the 11 females that were determined as positive with the commercial ELISA, 7 of them were also positive with the indirect-ELISA. Out of 42 positive male with the commercial ELISA, 20 were also positive with the indirect-ELISA. Likewise, out of the 11 female that were determined as positive with indirect-ELISA, 7 were also positive with the commercial-ELISA. Furthermore, out of 29 positive males with the indirect ELISA, 20 were also positive with the commercial-ELISA. The correlation in terms of sexes was determined to be 86.66% for females and 74.79% for males (Table 2).

Sensitivity and specificity values of indirect-ELISA were calculated using the commercial-ELISA as a reference (Table 3). According to this calculation, sensitivity and specificity values were 50.9 and 90% respectively. False negative ratio of indirect-ELISA was 49.1% while false positive ratio 10%. Besides, diagnostic value of the test was 78.7%.

Similarly, sensitivity and specificity rates of commercial-ELISA were assessed using the indirect-ELISA as a reference (Table 4). As a result, sensitivity and specificity values were 67.5 and 81.8%, respectively. False negative ratio of commercial-ELISA was 32.5% while false positive ratio 18.2%. However, diagnostic value of the test was 78.7%.

**Table 3:** Sensitivity and specificity of indirect-ELISA using commercial-ELISA as a reference.

| | Commercial-ELISA | | | |
|---|---|---|---|---|
| | | + | - | Total |
| Indirect-ELISA | + | 27 | 13 | 40 |
| | - | 26 | 117 | 143 |
| | Total | 53 | 130 | 183 |

Sensitivity = 27/53 = 50.9%
Specificity =117/130 = 90%
False negative ratio = 26/53 = 49.1% or (1-sensitivity) = 49.1%
False positive ratio = 13/130 = 10% or (1-specificity) = 10%
Diagnostic Value = (27+117)/183 = 78.7%

**Table 4:** Sensitivity and specificity of commercial-ELISA using indirect-ELISA as a reference.

| | Indirect-ELISA | | | |
|---|---|---|---|---|
| | | + | - | Total |
| Commercial-ELISA | + | 27 | 26 | 53 |
| | - | 13 | 117 | 130 |
| | Total | 40 | 143 | 183 |

Sensitivity = 27/40 = 67.5%
Specificity = 117/143 = 81.8%
False negative ratio = 13/40 = 32.5% or (1-sensitivity) = 32.5%
False positive ratio = 26/143 = 18.2% or (1-specificity) = 18.2%
Diagnostic Value = (27+117)/183 = 78.7%

## DISCUSSION

Hypodermosis in cattle has been observed in all the countries geographically located between the latitudes 30° and 60° in the northern hemisphere, and has been reported to cause major losses (5). Despite the efforts in the recent years to control hypodermosis using medication, sterile insect production, and vaccination trials, the disease is still relatively widespread in America, Canada, Africa, and Europe (20, 21).

This study determined the seroprevalence of hypodermosis in the cattle slaughtered in the abattoir of Nigde province with the commercial and the indirect-ELISA tests as 21.8% and 28.9%, respectively. These results are similar to the records of Simsek *et al.* (18) and Balkaya *et al.* (19).

In contrast, the results of the present study are lower than those obtained by Ozkutlu and Sevgili (17). The differences in the results could be attributed to the differences in the geographical locations and climates of the study sites as well as the differences in the care of the animals and feeding conditions.

Hypodermosis has been investigated in various countries using serological, immunohistochemical and molecular methods (20-27). The ELISA method is used in many countries in the diagnosis of hypodermosis, because, it has the advantage that it can be applied to many animals rapidly and easily. The test is also relatively cheap and it can use either milk or serum samples. The studies using ELISA reported seroprevalence rate of 42.3% in Spain (28), 96% in Mongolia and 0% in Japan (29), 43.3% in Italy (30), 48.7% in Belgium (31), between 6% and 51.7% in China (32), and 38.6% - 41.2% in Albania (33). In the present study seropositivity rates for the commercial ELISA kit and indirect-ELISA showed moderate statistical correlation. Therefore, it is considered that both commercial and indirect ELISA tests could be used in the serological diagnosis of hypodermosis.

In the recent years, the diagnosis of hypodermosis in Turkey has been carried out using serological and molecular diagnostic methods. Ozkutlu and Sevgili (17) studied the hypodermosis seroprevalence in the cattle of Sanliurfa province with a commercial ELISA and determined the seroprevalence rate as 38.6% (116/300). They also reported that while the seropositivity rates with respect to breed, age and study districts were statistically significant, there was no statistical significance with respect to gender. Simsek *et al*. (18) examined hypodermosis seroprevalence in three provinces in the Eastern and the Southeastern Anatolia regions using indirect-ELISA and reported an average seroprevalence rate of 23.3% (148/634), and also observed higher seropositivity in females than the males. Balkaya *et al.* observed the seroprevalence rate of hypodermosis in females as 28.6% with the indirect-ELISA test in Erzurum province (19).

In conclusion, this study demonstrated that hypodermosis is present in the cattle of Nigde province located in the Central Anatolia region of Turkey. For the randomly selected 183 cattle in Nigde, commercial ELISA and indirect ELISA seropositivity rates were calculated as 28.9% and 21.8%, respectively. The results of the study show a statistically moderate correlation ($p<0.001$) between the two tests, therefore, both these tests can be used in the serodiagnosis of hypodermosis.

Sensitivity and specificity values of indirect-ELISA were 50.9% and 90%, respectively. This shown that indirect-ELISA was more successful than commercial-ELISA for detection of negative cases. Besides, diagnostic value of the test was 78.7%. However, sensitivity rate was 67.5% for commercial-ELISA indicating that it can detect the positive cases more succesfully than indirect-ELISA. We believe that commercial-ELISA is a good test due to the higher sensitivity but indirect-ELISA can also be used for wide seroepidemiological surveys.

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