Serological diagnosis for *Hypoderma* infestation in cattle of Wasit province by using of an indirect-ELISA

Abbas Hassan Khlaty Al-Sray¹  Ghassan Jabar Khalaf ¹  Khawala H. Sabbar²

1. Department of Microbiology, College of Veterinary Medicine, Wasit University, Iraq.  
2. Department of Animal Production, College of Agriculture, University of Al-Qadisiyah, Iraq.

Corresponding Author Email: gabbashassan@uowasit.edu.iq  
Co-Authors Emails: Dr rn_1988@yahoo.com  Khawalasabbar8@gmail.com

(Received 13/3/2017, Accepted 24/4/2017)

Abstract

The aim of present study was to detect the specific *Hypoderma* IgG-antibodies in blood samples of cattle by using of indirect-ELISA. For this purpose, an overall of 476 cattle from different regions in Wasit province / Iraq, were submitted for blood samples collection and then for serological assay that revealed on 249 (52.31%) as a total result for serologically positive cattle. Also, the study was discussed the seropositive prevalence of hypodermosis with a number of epidemiological risk factors (age, sex, breed, and herd management), which showed that the highest seropositive rates have been reported in ≥3 years group (59.39%) more than ≤3 years group (43.72%) for age factor, in females group (56.72%) more than males group (36.54%) for sex factor, and in bad group (59.48%) more than good group (32.81%) for herd management factor. Statistically, at a level of (P≤0.05) the significant differences were reported within the age, sex, and herd management factors; but it was not detected between both groups of breed factor (local and cross-breed).

Keywords: Hypoderma, cattle, serological diagnosis, indirect-ELISA, Wasit province

Introduction:

*Hypoderma* is an obligating parasitic pathogen belongs to Osteridae family of Diptera order, which infesting some domesticated animals, mainly, cattle to resulting in a hypodermosis, disease (1). Worldwide, bovine hypodermosis is caused by two species of *Hypoderma* genus (*H. bovis* and *H. lineatum*) that diagnosed morphologically, serologically and molecularly (2). In China, a third species of bovine hypodermosis (*H. sinense*) was detected, in last decade, through depending on molecular characterization of the 3rd stage of *Hypoderma* larvae (3). However, hypodermosis is endemic in most northern hemisphere countries in North America, Europe, Africa and Asia involving Iraq (4). It has been considered to be responsible on substantial high economic losses to livestock industry worldwide related to hide damage, lowered productivity and high emaciation of infested cattle (5). In addition, bovine *Hypoderma* species may, accidently, infest humans causing a seldom sever condition (6). *Hypoderma* is characterized by development of larval stages of *Hypoderma* in animal’s tissue and presence of warbles under the skin of infested animals. The first endoparasitic stage migrates in deep connective tissues of their hosts during summer to reach to the dorsal subcutaneous tissues in the following spring, to molting into the second and third larval stage, producing swellings on the skin along the back. The mature third larvae fall on the ground and pupate at the end of spring (7, 8). Although, the clinical condition can, easily, diagnosed through detection of maggots that being visible, the migratory, cavitory and pseudo-hypodermosis have many diagnostic challenges, particularly, for those veterinarians unacquainted with practical and actual skin infections (9). During the recent years, immunodiagnostic techniques for cattle hypodermosis have been improved so that their increased diagnostic sensitivity allows investigators to avoid...
Materials and Methods:
Study area and sample collection
A totally of 476 cattle was selected, randomly, from different herds and fields belonging to some regions in Wasit province / Iraq, during the period of (March-July / 2016). All study’s cattle were submitted for receiving data related to epidemiological risk factors (age, sex, breed, and herd management), and for blood samples collection. In accordance to these factors, cattle were divided into two aged groups (≤3 years, and >3 years), two sex groups (male and female), two breed groups (locals and cross -breeds), and two herd’s management groups (bad and good) that determined in depending on the feeding and medication systems. In addition, about 10 ml of blood samples were collected from jugular vein of each animal by using disposable syringes. Blood samples inserted into free numbered anticoagulant tubes, centrifuged at 4000 rpm for 15 minutes, and the sera kept into 1ml micro-tubes at -20°C until used (13).

Serological diagnosis (Indirect-Enzyme-Linked Immunosorbent Assay)
Animal sera were examined to detect the presence of specific Hypoderma IgG-antibodies by an indirect-ELISA kit (Institut Pourquier, France). This serological kit is a screening test for detection of specific antibodies against L1 antigens of Hypoderma genus in bovine serum samples, and it’s prepared in this way to ensure their characteristics for 9 months with a high sensitivity and specificity that could be near 100 % (14, 15). According to manufacturer’s instructions, all sera samples were subjected to the steps of the test and, finally, the results were read by using a microplate photometer reader (BioTek®, USA), at 450 nm of optical density (OD). Then, the validation for ensure reliability, calculation and interpretation of obtained results have been done as shown in (Table 1).

Statistical Analysis
All received data were arranged, tabled and analysed by using of two computerized programs, Microsoft Office Excel (2013) and IBM/SPSS (v.23). Chi-square test was applied to detect the significant differences at P≤0.05 level (16).

Table (1): Validation, calculation and interpretation of obtained results

| Validation Criteria                  | Negative Control | Positive Control | Minimal ratio of positive and Negative Controls |
|-------------------------------------|------------------|------------------|-----------------------------------------------|
|                                     | < 0.350          | > 0.350          | 3.5                                           |

| Calculation                         |                   |                   |
|-------------------------------------|-------------------|-------------------|
| Still # P                           | Value of Sample – Value of Negative Control | Mean Value of Positive Control – Value of Negative |

| Interpretation                      | Negative Results   | Positive Results  |
|-------------------------------------|-------------------|-------------------|
|                                     | S/P < 50 %        | S/P ≥ 55 %        |
Results:
A totally of 476 cattle’s blood samples were tested by an indirect-ELISA, and the result reported that 249 (52.31 %) samples of cattle were seropositive with *Hypoderma*, while 227 (47.69 %) samples of cattle were seronegative, as shown in (Table 2). The results of (Table 3) were showed the association of seropositives between the groups related to each epidemiological risk factor, separately. The studied factors involved age, sex, breed, and herd management; as shown in (Figures 1, 2, 3, and 4).

Table (2): Serological prevalence of *H. bovis* by an indirect-ELISA

| Total No. | Seropositive Results | Seronegative Results |
|-----------|----------------------|----------------------|
| 476       | 249 (52.31 %)        | 227 (47.69 %)        |

Table (3): Relationship of seropositive cattle with the epidemiological risk factors

| Epidemiological Risk Factors | Groups       | Total No. | Seropositive | Seronegative |
|-----------------------------|--------------|-----------|--------------|--------------|
|                             |              |           | Seropositive |              |
| 1 | Age | ≤ 3 | 215 | 94 (43.72 %) b | 121 (56.28 %) |
|   |     | > 3 | 261 | 155 (59.39 %) a | 106 (40.61 %) |
| 2 | Sex | Males | 104 | 38 (36.54 %) b | 66 (63.46 %) |
|   |     | Females | 372 | 211 (56.72 %) a | 161 (43.28 %) |
| 3 | Breed | Local | 167 | 85 (50.9 %) b | 82 (49.1 %) |
|   |     | Cross-breed | 309 | 164 (53.07 %) b | 145 (46.93 %) |
| 4 | Herd Management | Bad | 348 | 207 (59.48 %) a | 141 (40.52 %) |
|   |     | Good | 128 | 42 (32.81 %) b | 86 (67.19 %) |

In each factor groups, variation in small letters, vertically, referred to a significant difference at level of $P \leq 0.05$

The results of age factor showed that 94 (43.72 %) and 155 (59.39 %) of cattle samples were seropositives in both ≤ 3 and >3 years age’s groups, respectively, (Fig.1). The results of sex factor appeared that 38 (36.54 %) of males samples and 211 (56.72%) of females samples were seropositives, (Fig.2).

Figure (1): Seropositive results of age factor

The seroprevalence of hypodermosis according to breed factor has been reported that 85 (50.9 %) and 164 (53.07 %) of cattle samples in local and crossbreed, respectively, were positives (Fig.3). According to herd management factor, hypodermosis prevalence was showed that 207 (59.48 %) and 42 (32.81 %) of cattle samples in bad and good groups, respectively, has been seropositive, (Fig.4).
Discussion:

Hypodermosis is a cosmopolitan disease of large importance in about fifty five tropical and subtropical countries, with great regional variation in prevalence that reported in many countries to be up (100%) depending on studied herds or regions, and diagnostic methods (17,18). In Iraq, this study was the first one that directed toward diagnosis of bovine hypodermosis through detection of specific *H. bovis* IgG-antibodies by indirect-ELISA, and the result was higher than those studies reported by (7) (28.6%), (19) (28.81%); and lower than those reported by (13) (74.8%) and (20) (80%). Although, all efforts for *Hypoderma* control, it is still widespread especially in some countries with low socioeconomic status, lack of basic sanitation, inadequate garbage disposal, and leaving or incorrect application of insecticides that can lead to increasing spread of problem (8). The early reliable diagnosis represented great challenges in eradication because of the detection of L1 at beginning of migratory phase will allow for treatment and avoiding of damage in host tissues (20). In the last three decades, several serological tests have been developed as alternative and confirmatory diagnostic techniques for hypodermosis. Indirect-ELISA has the ability to detect infection from the early first stage, L1, whereas a direct diagnosis cannot be made until the appearance of larvae on the animal’s back. Thus, indirect-ELISA enabled the detection of *Hypoderma* larvae in a herd long before their visual appearance, and in this way, allows greater flexibility of insecticide treatment to break the cycle of re-infestation (21,22). As well as, several studies reported the high degree of sensitivity and specificity of indirect-ELISA in detecting of hypodermines C in sera of infected animals that could be reaching about to (95.6-98.1%) and (92.2-96.4 %), respectively (23, 24). Recently, indirect-ELISA has been used, widely, for monitoring the occurrence of hypodermosis in Britain and other European countries, and has been recommended for surveillance when clinical diagnosis becomes impractical due to the low level of infestations (25). Many other factors affecting the extent of *Hypoderma* invasion have been described, in this study, including age, sex, breed, and herd management. The prevalence of bovine hypodermosis was varies significantly among different age stages of cattle. It was evident from the study’s results that the older cattle have a higher rate of hypodermosis than younger one. Although, the older cattle have hard and thick skin as compared to the younger cattle and the larvae of *Hypoderma* confronted difficulties in penetrating the old cattle thick skin (26). However, the immunity is developed, progressively, as a result of previous frequent exposures to parasite, which conferred a specific protection after subjection to an initial infection to persist for several weeks. Hence, the specific IgG-
antibodies have been increased in specificity and intensity after exposure for frequent infections to persist for more duration than first infection (27,28). The high rate of hypodermosis in females than males could be attributed to the physiological variations between both genders or to the management practices that applied in study’s area and allowed for females to be grazed in pastures or field to be more prone to infested with hypodermosis, and keeping of the males at special attention in houses (29). In relating to breed factors, no significant differences were showed between the results of local and cross-breeds groups. This result could mean that the susceptibility for infestation with hypodermosis was not influenced by genetic variations of both breeds, and all cattle might be at the same risk for Hypoderma parasite. The bad group of herd management was detected to be having a high prevalence in seropositive cattle than good group, and this could because of unhealthy sources of feeding and/or drinking water that contaminated with parasite, abominable stocking rate, unhealthy breeding system, and lack or absence of control and prevention schedules. Although, the previous insecticide treatment had an important influence in prevalence of causative parasite, the grazing pattern had, mostly, an effective role in maintaining the infection especially in extensive management system (30, 31, 32). In fact, the inappropriate industrial management indicated a favorable conditions for surviving and development of different Hypoderma stages (pupal and adults) (33). In conclusion, the indirect-ELISA used in present study, was provided a baseline data for the high efficacy of technique in diagnosis of bovine hypodermosis, and great prevalence of specific Hypoderma IgG-antibodies in cattle of Wasit province/Iraq. Also, the study detected the role of age, sex, and herd management (as epidemiological risk factors) in prevalence of the seropositive Hypoderma infestation.

References:
1-Hassan MU, Khan MN, Abubakar M, Waheed HM, Iqbal Z, Hussain M. Bovine hypodermosis-a global aspect. Tropical Animal Health and Production, (2010); 42(8), 1615-1625.
2-Jaiswal AK, Sudan V, Kumar P, Srivastava A, Shanker D. Bovine hypodermosis in indigenous cattle herd and its successful therapeutic management. Journal of Parasitic Diseases, (2016);40(1), 166-168.
3-Otranto D, Traversa D, Colwell DD, Guan G, Giangaspero A, Boulard C, Yin H. A third species of Hypoderma (Diptera: Oestridae) affecting cattle and yaks in China: molecular and morphological evidence. Journal of Parasitology, (2004); 90(5), 958-965.
4-A’aiz NN, Kashash KH, Al-Kaabii NA. Prevalence of bovine hypodermosis in Babylon province in Iraq. Al-Anbar J. Vet. Sci., (2011); 4 (2), 99-102.
5-Khan MN, Iqbal Z, Sajid MS, Anwar M, Needham GR, Hassan M. Bovine hypodermosis: Prevalence and economic significance in southern Punjab, Pakistan. Veterinary Parasitology, (2006);141(3), 386-390.
6-Otranto D. The immunology of myiasis: parasite survival and host defense strategies. Trends in Parasitology, (2001); 17(4), 176-182.
7-Balkaya I, Simsek S, Saki CE. A serological and molecular survey of cattle hypodermosis in east-Turkey. Veterinary Parasitology, (2010); 173(3), 287-291.
8-Francesconi F, Lupi O. Myiasis. Clinical Microbiology Reviews, (2012); 25(1), 79-105.
9-Dehghani R, Sedaghat MM, Esmaeli N, Ghasemi A. Myiasis among slaughtered animals in Kashan, Iran: descriptive a veterinary entomological problem in the tropics. The Iranian Journal of Veterinary Science and Technology, (2014); 4(1), 19-28.
10-Bouard C, Alvinerie M, Argenté G, Languille J, Paget L, Petit E. A successful, sustainable and low cost control-programme for bovine hypodermosis in France. Veterinary Parasitology, (2008); 158(1), 1-10.
11-Cencek T, Karamon J, Sroka J, Zdbyel J. Role of hypodermines in the adaptation of *Hypoderma bovis* larvae to the parasitic mode of life. Medycyna Weternaryjna, (2009); 65(9), 593-596.

12-Jan S, Lateef M, Abbas F, Maqbool A, Jabbar MA, Kakar H, Kakar E. Serological and epidemiological studies on goat hypodermosis in Northern Upland Balochistan, Pakistan. Pakistan Journal of Zoology, (2014); 46(1), 153-160.

13-Panadero R, López C, Dacal V, Vázquez L, Sánchez-Andrade R, Díaz P, Diez Baños P. Seroprevalence by Sandwich and Indirect-ELISA of bovine hypodermosis in Galicia (NW of Spain). Revista Ibérica de Parasitologia, (2006); 66(1-4), 95-98.

14-Cencek T, Ziömko I, Karamon, J. Development of the ELISA kit for the detection of *Hypoderma bovis* antibodies in cattle. III. Stability and usefulness of ELISA components preserved under different conditions. Wiadomosci Parazytologiczne, (2000); 47(3), 511-520.

15-Karatpe M, Simsek S, Karatepe B, Cayvaz M, Seyvili M, Balkaya I. Seroprevalence of hypodermosis in cattle in Nigde province of Turkey by comparison of commercial and indirect-ELISA methods. IJVM, (2013); 68, 38-42.

16-McDonald JH. Handbook of biological statistics. Baltimore, Maryland (USA): Sparky House Publishing, Vol. 2, (2009); Pp: 39-45.

17-Colebrook E, Wall, R. Ectoparasites of livestock in Europe and the Mediterranean region. Veterinary Parasitology, (2004); 120(4). 251-274.

18-Wall R. Ectoparasites: future challenges in a changing world. Veterinary Parasitology, (2007); 148(1), 62-74.

19-Ahmed H, Panadero-Fontan R, Lopez-Sanez C, Khan MR, Asif S, Mustafa I, Qayyum M. Development of Indirect ELISA for the Diagnosis of Bovine Hypodermosis (*Hypoderma lineatum*) in the Cattle of Subtropical Region of Pakistan. Kafkas Universitesi Veteriner Fakultesi Dergisi, (2013); 19(6), 215-219.

20-Yin H, Ma M, Yuan G, Huang S, Liu Z, Luo J, Guan G. Hypodermosis in China. J Anim Vet Adv, (2003); 2, 179-83.

21-Gorcea FC, Cálescu N, Gherman CM, Mihalca AD, CozmaV. Diagnostic values of clinical, pathological and serologic findings in cattle hypodermosis in Pestișani, Gorj County Romania. Sci Parasitol, (2011); 12, 173-6.

22-Blank D, Yang W. Behavioral Responses of Goitered Gazelle (*Gazella subgutturosa*) to Parasitic Activity of Botflies. Journal of Parasitology, (2014); 100(1), 66-72.

23-Panadero R, Vázquez L, Colwell DD, López C, Dacal V, Morrondo P, Diez-Baños P. Evaluation of antigen captures ELISA for the early diagnosis of *Hypoderma lineatum* in cattle under field conditions. Veterinary Parasitology, (2007); 147(3), 297-302.

24-Ahmed H, Afzal MS, Moeen M, Simsek S. An overview on different aspects of hypodermosis: Current status and future prospects. Acta Tropica, (2016); 162, 35-45.

25-Saidani K, Lopez-Sánchez C, Diaz-Fernandez P, Cabanelas-Dopazo E, Perez-Creo A, Morrondo-Pelayo P, Benakhla A. Bovine Hypodermosis in the Maghreb: Sero-epidemiological Study in Algeria by Indirect ELISA. Kafkas Universitesi Veteriner Fakultesi Dergisi, (2016); 22(5). 757-763.

26-Khan MN, Iqbal Z, Khan IA, Chaudhry SA, Sajid MS. Surveillance of cattle hypodermosis in district Chakwal, Pakistan. International Journal of Agriculture and Biology, (2008);10(3), 337-339.

27-Sadd BM, Schmid-Hempel P. Insect immunity shows specificity in protection upon secondary pathogen exposure. Current Biology, (2006);16(12), 1206-1210.

28-Cabanelas E, Panadero R, Fuertes M, Fernández M, Benavides J, López C, Pérez V. Histological and immunohistochemical characterization of *Hypoderma lineatum* (Diptera: oestridae) warbles. Veterinary Parasitology, (2015); 212(3), 361-367.

29-Negm-Eldin MM, Elmadawy RS, Hanan GM. *Oestrus ovis* larval infestation among sheep and goats of Green Mountain areas in Libya. Journal of Advanced Veterinary and Animal Research, (2015);2(4), 382-387.

30-Boulard C. Durably controlling bovine hypodermosis. Veterinary Research, (2002); 33(5), 455-464.

31-Di Regalbono AF, Capelli G, Otranto D, Pietrobelli, M. Assessment of cattle grub (*Hypoderma spp.*) prevalence in northeastern Italy: an immunoepidemiological survey on bulk milk samples using ELISA. Veterinary Parasitology, (2003); 111(4), 343-350.
Guan G, Luo J, Ma M, Yang D, Wang Y, Gao J, Boulard C. Sero-epidemiological surveillance of hypodermosis in yaks and cattle in north China by ELISA. Veterinary Parasitology, (2005); 129(1), 133-137.

Saidani K, Benakhla A, Díez-Baños P, Panadero R. Chronobiology of Hypoderma spp. in north-central Algeria as a basis to establish a control program. Rev IberoLatinoam Parasitol, (2011); 70(2), 157-162.