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

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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 ($P \leq 0.05$) 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, cavitary 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 visual examinations, palpation of warbles or post-mortem examination (10). In infested cattle, the main antigens that can be detected by serological tests are three enzymes secreted by L1 larvae, called hypodermin (A, B, and C), which play an important role during the life cycle of *Hypoderma* in body of a host. Whilst, the hypodermines A and B play a major role in an inhibition of inflammatory and immune response, the

hypodermin C is actively collagenolytic enzyme secreted by the first instars (L1) during its migration through a host's connective tissues (11,12). The present study was aimed for serological detection of specific *Hypoderma* IgG-antibodies against L1 hypodermin C in cattle blood serum samples of some regions in Wasit province / Iraq; through an application of indirect-ELISA for first time in Iraq; and for estimation of the relationship between seropositive results with a number of selected epidemiological risk factors included the age, sex, breeds, and herd management.

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	< 0.350
	Positive Control	> 0.350
	Minimal ratio of positive and Negative Controls	3.5
Calculation	S/P $\dfrac{\text{Value of Sample} - \text{Value of Negative Control}}{\text{Mean Value of Positive Control} - \text{Value of Negative}}$	
Interpretation	Negative Results	S/P < 50 %
	Positive Results	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 %)

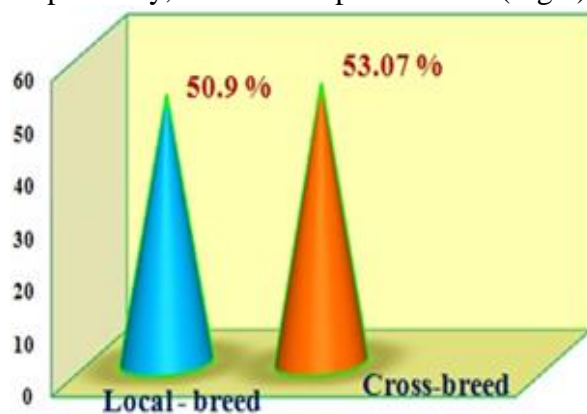**Table (3): Relationship of seropositive cattle with the epidemiological risk factors**

	Epidemiological Risk Factors	Groups	Total No.	Seropositive	Seronegative
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≤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).
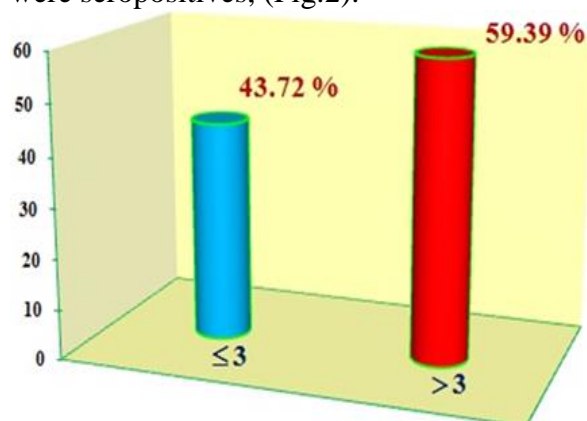
Fig. (1): Seropositive results of age factor
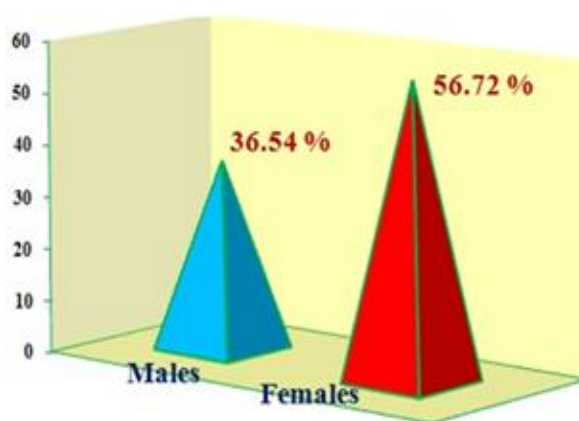
Fig. (2): Seropositive results of sex factor

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 cross-breed, 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).

Fig. (3): Seropositive results of breed factor

Fig. (4): Seropositive results of herd management factor

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.

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