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Detection of *Hypoderma* spp. antibodies in bovine milk in some regions of Nineveh governorate, Iraq

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Abstract

The study aimed to determine hypodermosis using clinical examination and indirect ELISA. Between October 2022 and March 2023, the study was conducted in several Nineveh governorate regions. One hundred ninety cows were clinically examined, collected milk samples, and used the commercial indirect ELISA kit. Seven (3.68%) of the 190 cows examined in the field had hypodermosis conversely, *Hypoderma* spp. Antibodies were detected in twenty-nine (15.26%) of the 190 cow's milk samples. The highest percentage 36.36% was reported in Bazwaia, whereas the lowest 6.66% was reported in Veterinary Teaching Hospital, Ashuwa'yrat, and Qayyarah. The percentage was 20.32% for cows five years or less and 5.97% for those over five. The results also showed that the native breed had a higher percentage 16.07% than the imported breed 9.09%. Cows fed outside have a higher rate of antibodies 28.72% than cows fed indoors 2.08%. Furthermore, October 2022 had more infested cows 23.33% than December 2022 9.37%. The study concluded hypodermosis risks cows and the leather industry, affecting the country's economy. Moreover, detecting hypodermosis effectively be achieved by testing milk for antibodies.

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Introduction

Cattle are affected mainly by the obligatory myiasis known as hypodermosis, carried by the larvae of the genus *Hypoderma* larval stages of three *Hypoderma* spp.; *Hypoderma bovis*, *Hypoderma lineatum*, and *Hypoderma sinense* cause hypodermosis in cattle and other bovine species (1). The life cycle of the *Hypoderma* spp. consists of adult flies laying eggs on hosts' hair in the summer, first-stage larvae hatching from the eggs, burrowing through the skin of the host in *H. bovis*, while in *H. lineatum* and *H. sinense* migrating to the tissue of the esophagus where they spend the winter. The larvae reach the back's subcutaneous tissue the following spring, progressing into the second and third larval stages and producing distinctive subcutaneous pimples known as warbles (2). Hypodermosis is responsible for significant global economic losses to the livestock sector due to hiding damage, decreased milk and meat production, and severely underweight animals. Additionally, people may accidentally become infected with bovine *Hypoderma* species, leading to a severe illness (3,4). Most of the northern hemisphere's nations, including those in North America, Europe, Africa, and Asia, as well as Iraq, have endemic cases of hypodermosis (5-7). Cattle with hypodermosis are diagnosed by palpating the warbles on the back and flank areas of the animal or by visually examining the carcasses in slaughterhouses. In both cases, the infestations were identified very late, which point most of the damage had already been done (8). To reduce the losses related to this disease and enable systemic treatment, dependable identification is necessary by detecting specific antibodies against first instars at the beginning of the migratory stage (9). Thus, over the past years, countries have created, modified, and extensively utilized immunological

procedures such as the enzyme-linked immunosorbent test (ELISA) (10-12). With this approach, a diagnosis can be made before the spring warbles appear, and cattle can be treated with active chemicals as soon as the larvae are still moving and haven't yet resulted in financial losses (13).

In this investigation, bovine hypodermosis will be diagnosed clinically by manual skin palpation to detect cutaneous warbles and indirect ELISA to detect specific antibodies in the milk. The study also covered the links between the positive samples and some risk factors like the breed, age, month of sampling, and grazing pattern (indoors and outdoors).

Materials and methods

Ethical approve

The sample collecting methods were authorized in September 2022 with approval issue number UM.VET.2022.086.

Study areas and period

The study was carried out in several regions of the Nineveh governorate, including Bazwaia, Alubbor, Kokjali, Bab Shamash, Yarimja Alsharqia, Veterinary Teaching Hospital, Ashuwa'yrat, and Qayyarah, between October 2022 and March 2023.

Clinical assessment

Hand palpation was used in the field to perform the clinical assessment. The skin and subcutaneous tissues of the afflicted cows' backs, sides, and hump were investigated to find evidence of *Hypoderma* spp. larvae.

Milk sampling

190 cow's milk samples were taken in sterile 10 ml plain tubes and preserved at -20ºC until analysis. Breed (local, imported), age (five years or less, greater than 5 years), month of sampling, and grazing pattern (indoors, outdoors) were documented with each sample of cow's milk.

Identification of antibodies against *Hypoderma* spp.

The indirect ELISA kit (Montpellier, France) is used to identify *Hypoderma* spp. antibodies in bovine milk were used. The detection procedure was followed (7). The test was set up, incubated (Memmert®, Germany), and then washed (Biochek, USA). An ELISA microplate reader was used to detect the optical density at 450 nm. The S/P value of each sample was calculated according to the manufacturer's guidelines.

Statistical analysis

Considering all the examined data, the Chi-square test was performed with the SPSS V25 program at the significance level of $P \leq 0.05$ (14).

Results

Clinical findings

Out of the 190 cows examined by skin palpation in the field, seven (3.68%) showed hypodermosis. The number of warbles present on the animals ranged from 1-3. These warbles were present on the back, flank, and hump regions of the animal. Clinical observations at the larval entry site revealed inflammatory and yellowish-colored exudate (Figure 1A). The warbles were hard and elevated above the surrounding skin (Figure 1B). Every warble has a hole where the second-stage larvae were found (Figure 1C). After being extracted from infected cows, complete larvae 3 were shown (Figure 1D). Moreover, table 1 summarizes the relation between the evaluated cows' clinical and ELISA findings. The findings revealed that seven (3.68%) of the total number of cows examined had hypodermosis. However, out of the milk samples analyzed, 29 (15.26%) had positive ELISA findings.

Figure 1: *Hypoderma* spp. warbles in the back of infested cows A: point of larvae penetration with inflamed and yellowish exudate B: firm and raised warbles above the skin C: the second-stage larvae D: whole larvae 3 following extraction from infected cows

Percentages of antibodies against *Hypoderma* spp. according to the study, regions

Twenty-nine (15.26%) of 190 milk samples were positive for *Hypoderma* spp. antibodies ranged from 6.66 to 36.36% for the regions studied. The highest percentage (36.36%) was reported in Bazwaia, while the lowest 6.66% was reported in the Veterinary Teaching Hospital, Ashuwa'yrat, and Qayyarah. The remaining percentages fell within these two ranges, and table 2 shows substantial regional differences at the $P \leq 0.05$ level of significance.

Table 1: Relation between the evaluated cows' clinical and ELISA findings

Test	Positive	Negative
Skin palpation	7 (3.68%) a	183 (96.31%) a
Indirect ELISA	29 (15.26%) b	161 (84.73%) b

At p≤ 0.05 level, a significant difference is indicated by a vertical difference between a and b.

Table 2: Antibody percentages against *Hypoderma* spp. according to the study, regions

Region	Number		% Positive
	Tested	Positive	
Bazwaia	11	4	36.36 a
Alubbor	15	5	33.33 a
Kokjali	34	6	17.64 b
Bab Shamash	51	8	15.68 b
Yarimja Alsharqia	19	2	10.52 c
VTH	30	2	6.66 d
Ashuwa'yrat	15	1	6.66 d
Qayyarah	15	1	6.66 d
Total	190	29	15.26

VTH: Veterinary Teaching Hospital. Vertical difference between letters significant at P<0.05.

Percentages of antibodies against *Hypoderma* spp. according to some risk factors

The percentage of antibodies against *Hypoderma* spp. was correlated with the age of the cows. For those who were five years of age or less, the rate was 20.32%; for those who were older, it was 5.97% (Table 3). When an animal breed is considered, the local breed had the most significant rate 16.07% compared to the imported breed 9.09% with a statistically significant difference. Additionally, there was a statistically significant difference found between the groups of cows based on the grazing pattern; antibodies against *Hypoderma* spp. were found in 27 (28.72%) of the cows who were fed outside, which is higher than the 2.08% found in the cows that were fed indoors (Table 3).

Table 3: Percentages of antibodies against *Hypoderma* spp. according to some risk factors

Factor	Groups	Number		%
		Tested	Positive	
Age	≤ 5 years	123	25	20.32 a
	> 5 years	67	4	5.97b c
Breed	Local	168	27	16.07 a
	Imported	22	2	9.09 b
Grazing	Indoors	96	2	2.08 c
	Outdoors	94	27	28.72 d
Total		190	29	15.26

Vertical difference between letters significant at P<0.05.

Additionally, by the month of sampling, October 2022 had the highest recorded percentage of antibodies against *Hypoderma* spp. 23.33%. On the other hand, December 2022 was 9.37% had the lowest antibody percentage. The remaining percentages fell within these two ranges, there were notable changes across the months (Figure 2).

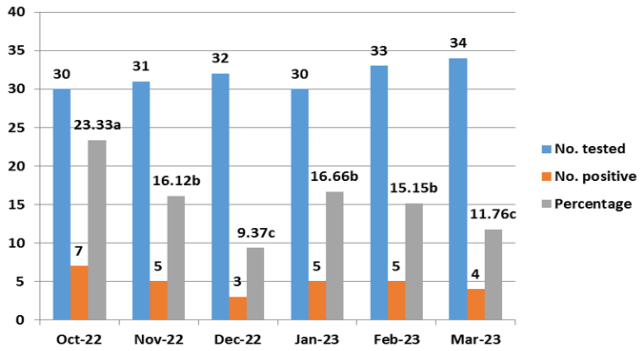

Figure 2: Antibody percentages against *Hypoderma* spp. depending on the month of sampling. Vertical difference between letters significant at P<0.05.

Discussion

Diagnosing hypodermosis is essential for establishing a treatment strategy and attempting to eradicate the infection from the affected area. When warbles appear on an animal's back, it is typically possible to recognize them as an infestation of warble flies through direct clinical examination. Additionally, immuno-diagnosis has been utilized to identify diseases early in many countries. An essential first step in the immunological diagnosis is finding specific antibodies against *Hypoderma* spp. in animal serum or milk (15,16). The findings revealed a difference between the clinical and ELISA findings for the investigated cows. The superiority of ELISA versus clinical examination is supported by Gharban (17). Using a single sample for examination is an advantage of immunological diagnosis over clinical investigation, which requires a much more extensive examination period (17-19). The low clinical findings may also because the warbles only remain with their host for 1-2 months, after which they spend 3-4 months leaving it (20-22).

When the region's prevalence was considered, the cows in Bazwaia 36.36% were significantly more infested than all other regions, and the overall percentage of antibodies against *Hypoderma* spp. was 15.26%. It is low in comparison to earlier research that found prevalence rates of 40.5% in Mosul city, Iraq (7), and 52.31% in Wasit, Iraq (23), or with other countries (24-28). The variations in the prevalence rates among areas and countries could result from several environmental factors influencing the warble's growth. Further variables that may affect the incidence include host

specificity, breeds, husbandry, and the usage of insecticides (29,30). This study has documented many factors influencing the degree of Hypoderma invasion, including age, breed, and grazing pattern. There are notable differences in the frequency of bovine hypodermosis between the two age groups of cows. The study's findings made it clear that younger had a higher percentage of hypodermosis than older ones. These findings are consistent with the higher prevalence of hypodermosis in young animals, which may be caused by their softer skin, which makes it easier for the early instars of *Hypoderma* to penetrate, as previously documented (31,32). The reason behind this could be attributed to several factors, such as the thicker skin of older animals that prevents larvae from penetrating, the host's immune systems suppressing the development of larvae, or the formation of resistance from recurrent exposure to larvae (6,33). Similar patterns in the age-specific prevalence of hypodermosis have also been documented by Pruett and Kunz (34) and Papadopoulos (26).

The results also showed significant differences between local and imported breeds. The highest positivity was reported in local breeds 16.07% compared with imported cows 9.09%. The current finding may be explained by the local breed's higher sensitivity to hypodermosis than the imported cows. Similar patterns in the breed prevalence of hypodermosis have additionally been recorded by Balkaya *et al*. (35) and Ayvazoglu *et al*. (36). Based on the grazing pattern, the statistical analysis revealed significant variations between the groups of cows. Of the cows fed outside, 27 (28.72%) had antibodies against *Hypoderma* spp.; this is greater than the 2.08% reported in the cows fed indoors. The high rate of hypodermosis may have resulted from management techniques used in the research area, which made it possible for female animals to graze in fields or pastures where they were more likely to become infected with the disease (37-39). These results agree with those of Otranto *et al* (1), who looked into the possibility that a significant contributing cause of hypodermosis positive is the practice of free grazing.

The percentage of antibodies against *Hypoderma* spp. varied significantly according to the month of sampling. Higher positivity was found in October 2022 than in December 2022. The current finding may be explained by the life cycle pattern of *Hypoderma* spp., which shows that adult flies are only active in warm weather and are not active below 18°C (40). Contrary to our findings, the peak of infection was noted between January and March (41), and the infestation rate peaked in March and dropped to its lowest in June. This is due to the significant differences in fly biology worldwide (42-44).

Conclusion

This study showed significant variation between the indirect ELISA test and the clinical assessment of hypodermosis. It has been found that the indirect ELISA test on milk samples is a practical, affordable, and simple diagnostic method for bovine hypodermosis in a variety of geographic regions. Additionally, the ELISA results demonstrated how breed, age, month of sampling, and grazing pattern affected the prevalence of positive *Hypoderma* infestations. The study's findings suggest that hypodermosis spreads, and those early preventive measures should be taken.

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Conflict of interest

The authors state that they have no conflicts of interest with the publication of this work.

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التحري عن الأجسام المضادة لأنواع الهايبوديرما في حليب الأبقار في بعض مناطق محافظة نينوى، العراق

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الخلاصة

هدفت الدراسة إلى الكشف عن النغف الجلدي باستخدام الفحص السريري واختبار الاليزا غير المباشر. أجريت الدراسة للفترة من تشرين الأول/٢٠٢٢ ولغاية آذار/٢٠٢٣ في عدد من مناطق محافظة نينوى. تم إجراء الفحص السريري على ١٩٠ بقرة ومن ثم جمعت عينات الحليب منها وفحصت باستخدام العدّة التجارية لاختبار الاليزا. سبعة (٣،٦٨%) من أصل ١٩٠ بقرة كانت مصابة بالنغف الجلدي بعد فحصها حقلياً. بالمقابل، كانت تسعة وعشرين بقرة ١٥،٢٦٪ موجبة بامتلاكها الأجسام المضادة عند فحص عينات الحليب. أعلى نسبة سُجلت في بازوايا ٣٦،٣٦% في حين أقل نسبة ٦،٦٦% كانت في المستشفى التعليمي البيطري والشويرات والقياره. كما أظهرت الأبقار التي أعمارها خمس سنوات أو أقل أعلى نسبة ٢٠،٣٢% لتواجد الأجسام المضادة بالمقارنة مع التي أعمارها تزيد عن خمس سنوات ٥،٩٧%. كما أظهرت النتائج أن أعلى نسبة كانت في السلالة المحلية ١٦،٠٧% بالمقارنة مع السلالة المستوردة ٩،٠٩%. كما إن الأبقار التي تتبع نظام التغذية الخارجية لديها أعلى نسبة ٢٨،٧٢% من الأجسام المضادة مقارنة مع التي تتبع نظام التغذية الداخلية ٢،٠٨%. فضلاً عن ذلك، بأن شهر تشرين الأول/٢٠٢٢ سَجل أعلى نسبة ٢٣،٣٣% من الأبقار المصابة مقارنة بشهر كانون الأول/٢٠٢٢ ٩،٣٧%. استنتجت الدراسة إلى أن النغف الجلدي يشكل خطراً على الأبقار وصناعة الجلود، وكلاهما لهما تأثير على اقتصاد البلد. فضلاً عن إن فحص الحليب للكشف عن الأجسام المضادة الخاصة بالنغف الجلدي ذو قيمة في تشخيص الإصابة.