

## Sero-prevalence of hypodermosis in cattle in Mosul city, Iraq

D.G. Alhamdany<sup>1</sup> and N.S. Alhayali<sup>1</sup>

Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

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#### Correspondence:

N.S. Alhayali

[nadias.alhayali@gmail.com](mailto:nadias.alhayali@gmail.com)

### Abstract

Bovine hypodermosis has been known as a veterinary disease that mainly caused by *Hypoderma bovis* and *Hypoderma lineatum*. This study aimed to determine sero-prevalence of cattle grubs in Mosul, Iraq utilizing indirect ELISA as well as effect of some risk factors on prevalence of hypodermosis. A total of two hundred blood specimens were collected from healthy and infested cattle through direct palpation. All samples were collected from cattle at Teaching Veterinary Hospital, Mosul abattoir, Kokjali and Bazwaya region during October 2020 to March 2021. Outcomes of iELISA showed 40.5% as an infestation rate of hypodermosis. Notably, infestation intensity has distributed as light, moderate and severe 11, 18.5 and 70.3% respectively. Interestingly, highest seroprevalence of hypodermosis was recorded in October 67.7%, and the lowest was recorded in February and March 22.2%, 25% respectively. Observations of current study also recorded the infection rate in age was 54.7% in animals less than 3 years old, and the lowest was 16% in animals more than 3 years old. Moreover, breed showed a significant risk factor 53.8% for imported cattle and 14.7% for local breed. Importantly, risk factor concerning area recorded the highest rate in Kokjali 52.2%. In contrast, the lowest rate recorded in Teaching Veterinary Hospital 14.8%. The present study is the first investigation to identify cattle hypodermosis by iELISA in Mosul, Iraq. iELISA has been approved as the best diagnostic tool for early detection of hypodermosis during the migrating phase. Interestingly, age, breed and months of study were significant risk factors.

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### Introduction

*Hypoderma* has been defined as an obligate ectoparasite belonging to the family Oestridae infesting cattle which migrates and feed on tissues of living cattle in subcutaneous tissues and internal organs during larval stages of *Hypoderma*. It has been reported that the most important species causing warbles are *H. bovis* and *H. lineatum* (1). Following hatching, larvae (L1) penetrates healthy skin within six hours that induces by enzymatic secretions of salivary glands. Thereafter, migration begins in subcutaneous connective tissue (2). Previous work has revealed that life cycle of *Hypoderma* species differs according to temperature and region (3). In addition, it has been demonstrated that warble flies would lead to economic

losses, for instance reduce host physiological functions, reduce milk yield, weight loss and destroy host tissues, among all these losses, the hide damage is the most important one (4). Diagnosing hypodermosis by visual palpation of warbles in the back of animals has been considered for many years were insufficient and only detect infestation in last stages (5). Recently, the sero-surveillance for cattle hypodermosis and other diseases has been employed in different countries including Iraq (6-8). It has been reported that 4 to 8 weeks following infestation, specific IgG antibodies profoundly develops during migration of L1, persist 3-4 months after molting to L3, and approximately 16 to 20 weeks post infestation show increase in concentration, that followed with a clear decrease within larvae arrival to the back region (9).

Numerous investigations have demonstrated that ELISA is widely used in different countries for early diagnosis of hypodermosis before the larvae arrive in the back and before warble formation for proper treatment (10). Thus, most frequently ELISA is necessary to prevent economic losses associated with this myiasis (1). Since underly and insufficient data concerning bovine hypodermosis in Mosul. The current study has carried out to keep in view the relationship between risk factors and prevalence of this myiasis in cattle. As we as to provide and evaluate the usefulness of ELISA in detecting grub infested cattle.

**Materials and methods**

**Samples' collection**

In the present study, from (October 2020 to March 2021) two hundred blood specimens were collected, 5-10 ml for each sample. All samples collected randomly from healthy and infested animals through palpation on the back regions of animals. Notably, Teaching Veterinary Hospital, different flocks from Kokjali and Bazwalya are the main regions for sample collection in Mosul. All data sorted considering date, age (less than 3, and more than 3 years) and breed (local or cross breed).

**Indirect ELISA technique (iELISA)**

The diagnostic commercial kit indirect ELISA assay ID.vet, 310, rue Louis Pasteur- Grabel - France, has been utilized to diagnose *Hypoderma* spp. for IgG antibodies in bovine serum which also has been applied by (11,12). Microplates coated with hypodermine A, B and C from *Hypoderma* spp. Importantly, samples were processed following instructions in kit. The O.D. recorded at 450 nm.

**Results**

Observations of the present study have shown that the warbles were diagnosed on the back region. They appeared randomly in different numbers ranged from 1 to 30 on either one or both sides of the infested animal, extraction of larvae manually is shown in (Figure 1). Detection of larvae in the hide of slaughtered animals is shown in (Figure 2), while figure 3 shows pus and blood attached with larvae 3 after extraction. Furthermore, figure 4 displays larvae 3 of *Hypoderma* species following isolation. We found that the total percentage of hypodermosis by iELISA was 40.5% (81/200) (Table 1). Interestingly, the infection rate with hypodermosis by iELISA in cattle was distributed between light, moderate and heavy 11, 18.5, and 70.3% respectively (Figures 5 and 6).

Outcomes of our work have revealed that the highest infestation rate recorded at October was 67.7% at  $P \leq 0.05$ , while the lowest rate in February and March were 22.2 and 25% respectively at  $P \leq 0.05$  (Table 2).

Results of current investigation also revealed that the risk factor of sex was non-significant between males and

females 44 and 30.9% respectively. Consistently, grazing pattern was also non-significant factor and recorded outdoor 37% and indoor 47%. Interestingly, age has been indicated as a significant risk factor that recorded high infested rate reached to 54.7% for animals less than 3 years, and the lowest infested rate was 16% for animals with age more than 3 years old. Furthermore, breed factor was also considered as a significant risk factor influencing infestation that recorded the highest rate 53.8% for imported cattle, while the lowest rate was 14.7% for local breed at  $P \leq 0.05$  (Table 3). Additionally, we also found that the area is an effective risk factor for infection with hypodermosis. The highest infestation rate was recorded in Kokjali 52.2% and in Bazwalya 49.6%. However, the lowest infestation rate recorded in Teaching Veterinary Hospital 14.8% at  $P \leq 0.05$  (Figure 7).



Figure 1: Manual extraction of larvae. (A and B) Shows warble located on back region of living animal. (C) Extraction of larvae by squeezing the warble. (D) Shows larva 2. (E) Shows whole larvae 3 following extraction

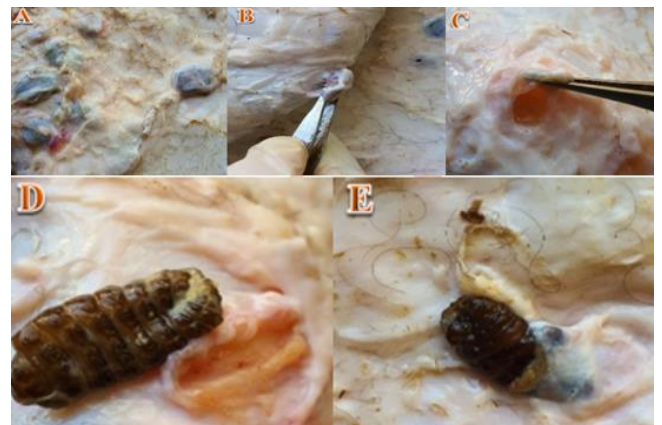


Figure 2: Mature Larvae 3 extraction from dermis of back region of slaughtered cattle. (A and B) Shows larvae 3 in its cyst in dermis. (C) Reveals to the fibrous cyst after extraction of larvae. (D) Larvae 3 with the cyst E larvae 3 during extraction.



Figure 3: Larvae 3 with cyst after extraction. Notably pus in A and blood in B.



Figure 4: Displays some isolated larvae samples.

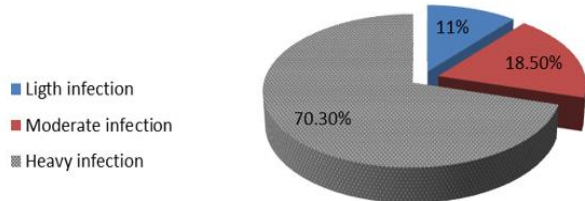


Figure 5: Intensity of infestations with hypoderma species in cattle by iELISA.

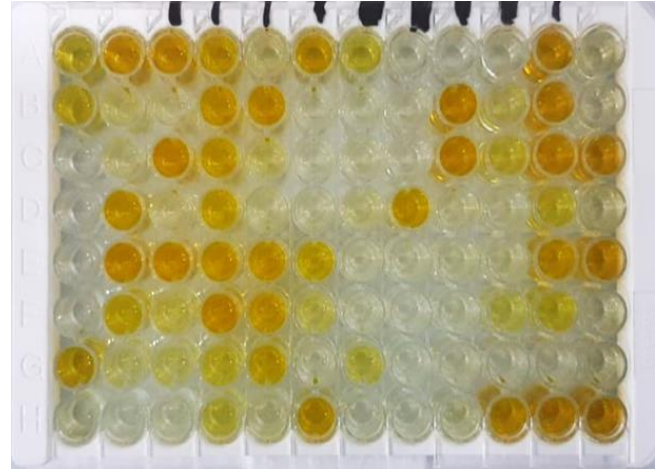


Figure 6: Results of iELISA different degrees in colors reflect intensity.

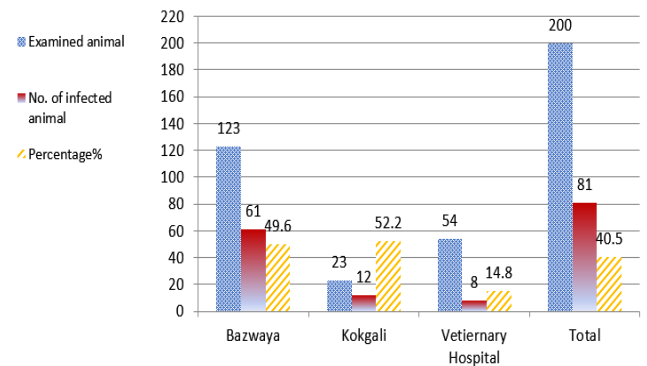


Figure 7: Relationship of infested cattle with hypoderma by iELISA with study areas.

Table 1: Serological prevalence of *Hypoderma* spp. by an indirect ELISA

Total No.	Positive	Negative
200	81 (40.5) %	119 (59.5) %

Table 2: Relationship of infested cattle with hypoderma by iELISA with months of study

Months of study	Examined	Infested	%
October 2020	31	21	67.7 <sup>a</sup>
November	32	14	43.75 <sup>b</sup>
December	32	17	53.12 <sup>abc</sup>
January 2021	33	12	36.36 <sup>bcd</sup>
February	36	8	22.2 <sup>d</sup>
March	36	9	25 <sup>d</sup>
Total	200	81	40.5

a, b, c, d vertically different mean there is a significant difference at level  $P \leq 0.05$ .

Table 3: Relationship of infested cattle with hypoderma by iELISA with risk factors

Risk factor	n examined	n infested(%)
Sex	Male	145
	Female	55
Grazing	Out	132
	In	68
Age	>3 years	126
	<3 years	74
Breed	Imported	132
	Local	68

a, b, c, d vertically different mean there is a significant difference at level  $P \leq 0.05$ .

## Discussion

Aforementioned studies have demonstrated that hypodermosis is a cosmopolitan and endemic myiasis that profoundly affecting. Thus, leading to different economic casualties particularly in the quality of hide due to holes caused by larvae 2 and 3 of *Hypoderma* species (1). In Iraq, more specifically in Mosul, there is shortage knowledge accompanied with less focus on this myiasis, except few numbers of studies in Babil (13) and Wasit (14). In this study, visual diagnostic method has been mainly conducted depending on palpation on the back region of cattle to detect warbles in spring and summer during the last stage of the disease. However, this direct method is laborious, as well as needs more experience. Additionally, it doesn't give real prevalence on hypodermosis for many reasons. For instance, the migrating stage (winter stage) starts from the hatching of eggs to L1 then start penetrating host tissues, this stage lasts approximately 3 to 4 months, thus there is a high possibility of failure to expand sample population during this period. Moreover, hair density makes it difficult to notice and extract larvae, these outcomes have been strongly supported by previous investigations (9,15).

All above mentioned reasons encouraged us to look for alternative test in our study. It has been reported that the sero-prevalence method by ELISA is sensitive and more reliable in different countries for early detection of initial migration of L1 stage of hypodermosis. It enables researchers to get more accurate prevalence of the disease that is essential to apply proper programs for treatment, eradication and control of the disease before it inflicts damage in the host tissues (10,14,15).

In fact, according to the sero-prevalence of *Hypoderma* spp. IgG antibodies by indirect ELISA showed that the total infestation rate was 40.5% which is the first study in Mosul, Iraq. Importantly, the observations were in agreement with previous studies in Wasit, Iraq (14), and Pakistan (16). However, it was higher in comparison to data from study in East Turkey (15). Indeed, geographical locations, climatic differences and samples populations, all factors that may lead to the outcome's variation. Our investigation has

confirmed that ELISA is one of the tools as it is cheap, easy and available for early diagnosing of hypodermosis during the migrating stage of larvae. Since it provides ability of detection before antibodies levels stop or decrease when larvae1 molt into larva2 and larvae3 because hypodermin C stops and loses its function and secretion. Strikingly, 40.3% the highest antibody titers elevated during the study period in winter coincided with the migration (winter resting phase) of larvae1, this data agreed with previous study (17). Moreover, numerous researches have revealed that the hypodermin C levels (*Hypoderma* antigen) decreases rapidly with the arrival of larvae 1 to the back region and molting to larvae two (16,18), thus strongly supported our data. In the same line of thought, our study period (October to March) considered as an ideal sampling period for sero-diagnostic and sero-epidemiological studies of cattle hypodermosis. Furthermore, it also recorded the highest prevalence was in October, November and December 67.7%, 43.75% and 53.12% respectively, while the prevalence declined in January, February and March (16,17).

Previous studies have demonstrated a variation in infestation rate in relative to animal genders. The infestation rate was higher in females than males (14,19), the higher rate of infestation in males than the females (20). Importantly, both mentioned studies disagreed with our observations that referred to sex factor as nonsignificant risk factor. Our interpretation, that both genders exposed to same environmental conditions, as well as had same management procedures.

We also found that there were nonsignificant differences recorded between grazing patterns, indoor or outdoor, 37%, 47% respectively. In contrast, we found a significant difference between breed, imported or local, 53.8% and 14.7% respectively. This might explain that imported breed is more susceptible for hypodermosis, thus have been infested from their origin countries such as (Georgian cattle and Turkey) raised on open grazing lands. Consistently, within different ages, it was evidence that younger animals, less than 3 years old, 54.7% higher than older animals, more than 3 years old, 16% (14,20,21) Our interpretation, the softer skin in younger animals which enable L1 to penetrate the skin easily than thicker skin in older animals, and that exactly what have been reported by (22). Alternatively, repeated exposure to hypodermosis would develop immunity by increasing IgG intensity after frequent exposure to infestation (23). Therefore, larvae1 might die in previously infested cattle due to skin defense mechanisms, which has been illustrated by previous investigations (2,24).

Finally, differences in locality recorded significant rates; Kokjali 52.2%, Bazwaya 49.6%, with less than in Teaching Veterinary Hospital 14.8%. These outcomes might relate to overcrowded flocks in Bazwaya, as well as bad management programs followed. Other reasons behind this variation are age and gender differences. Importantly,

these results in agreement with previous study that added host specify, environmental conditions, temperature, villosity, humidity, rainfall and wind as a risk factors that induce exposure to this disease (25).

## Conclusion

In summary we can conclude that hypodermosis significantly diagnosed for the first time in Mosul, Iraq. As well as, it shows that iELISA is the tool for early detection of hypodermosis, and it is better than palpation for detection warbled during spring and summer only. In addition, the study showed that age, breed and months are significant risk factors on hypodermosis.

## Acknowledgments

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## Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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للمدة ما بين تشرين الأول ٢٠٢٠ ولغاية آذار ٢٠٢١ ومن مناطق مختلفة، المستشفى البيطري التعليمي وكوكجلي وباروايا ومجزرة الموصل. بلغت نسبة الخمج الكلية باستخدام اختبار الممتز المناعي غير المباشر ٤٠,٥% وتوزعت شدة الإصابة بين الخفيف والمتوسط والشديد ونسب ١٨,٥ و ٧٠,٣% على التوالي. سجلت اعلى إصابة بالنغف الجلدي في شهر تشرين الأول بنسبة ٦٧,٧% واقلها في شباط و آذار ونسب ٢٢,٢% و ٢٥% على التوالي. كما أظهرت نتائج الدراسة أن لعامل العمر تأثيرا معنويا حيث سجلت اعلى نسبة خمج ٥٤,٧% في الأعمار اقل من ٣ سنوات واقلها في الأعمار أكبر من ٣ سنوات بنسبة ١٦%. كما اظهر عامل السلالة تأثيرا معنويا أيضا على نسبة الخمج حيث بلغت اعلى نسبة خمج في السلالات المستوردة ٥٣,٨% منها في السلالات المحلية ١٤,٧%. كما أشارت نتائج الدراسة أن اعلى نسبة خمج كانت في كوكجلي ٥٢,٢% واقلها في المستشفى التعليمي البيطري ١٤,٨%. كشفت الدراسة الحالية عن داء نغف جلد الأبقار باختبار الممتز المناعي غير المباشر وبينت الدراسة انه من أكفأ الاختبارات الحساسة للكشف المبكر عن الخمج خلال الأطوار المهاجرة علما أن العمر، نوع السلالة وأشهر السنة من العوامل المؤثرة على نسبة الخمج.

## الكشف المصلي لداء نغف الجلد في الأبقار في مدينة الموصل، العراق

دعاء غاتم الحمداني و نادية سلطان الحيالي

فرع الأحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

### الخلاصة

النغف الجلدي في الأبقار هو من الأمراض الطفيلية البيطرية التي يحدث بسبب نوعي دودة المواشي الشمالية. هدفت الدراسة إلى الكشف المصلي للنغف الجلدي في جلد الأبقار في الموصل باستخدام اختبار الممتز المناعي المستضد غير المباشر وتأثير بعض عوامل الخطورة على نسبة انتشار الخمج. تم جمع ٢٠٠ عينة دم من الأبقار السوية والخمجة سريريا باستخدام الجس على ظهر الحيوان. وجمعت العينات