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Research Article

Bovine Hypodermosis in the Maghreb: Sero-epidemiological Study in Algeria by Indirect ELISA

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Abstract

During May-July 2014, a total of 837 cattle serum samples were collected from 4 departments in northern Algeria and tested for the presence of *anti-Hypoderma* antibodies by indirect ELISA. Warble counts and some intrinsic (age, breed, sex) and extrinsic factors (location, husbandry system) were also considered. The overall seroprevalence by indirect ELISA was 49.8%, whereas by clinical inspection only 25.7% of the animals were infested. Except for the sex, all the factors included in the study were significantly associated with seroprevalence. The husbandry system was identified as the factor with more influence in seroprevalence, so cattle kept under extensive (79%) or semi-extensive (50.6%) husbandry systems have 74.10 and 18.13 more risk than those under an intensive system (5.8%), respectively. Both the seroprevalence and the intensity of infection are inversely related with the age of the animals. So, the youngest animals (<3 years) are less frequently infested but display the highest intensities of infection. The seroprevalence detected in northern Algeria reflects a high exposition of cattle to *Hypoderma* spp. To minimize the economic losses due to this myiasis it is essential to establish control programs based on serological surveillance prior to the administration of macrocyclic lactones.

Keywords: *Bovine hypodermosis, Maghreb, Algeria, Indirect ELISA, Seroprevalence, Risk factors*

Cezayir - Mağrip'te Bovine Hipodermozisi: İndirek ELİSA İle Sero-epidemiyolojik Bir Çalışma

Özet

Mayıs - Temmuz 2014 tarihleri arasında Kuzey Cezayir'de 4 bölgeden toplam 837 sığır serum örneği indirek ELİZA ile anti-Hipoderma antikorlarını belirlemek amacıyla toplandı. Çıban sayımları ve bazı iç (yaş, tür, cinsiyet) ve dış (lokasyon, barınma koşulları) faktörler dikkate alındı. İndirek ELİZA ile seroprevalans %49.8 olarak tespit edilirken klinik incelemede hayvanların %25.7'i enfeste olarak belirlendi. Cinsiyet hariç diğer tüm faktörlerin anlamlı derecede seroprevalans ile ilişkili olduğu tespit edildi. Barınma koşullarının seroprevalansı en fazla etkileyen faktör olduğu belirlendi. Eksentif (%79) veya yarı-eksentif (%50.6) şartlarda barındırılan hayvanlarda intesif şartlara oranla (%5.8) risk sırasıyla 74.10 ve 18.13 olarak tespit edildi. Hem seroprevalans hem de enfeksiyon yoğunluğu hayvanların yaşı ile ters orantılıydı. Genç hayvanlar (<3 yaş) daha az sıklıkla infeste olurken en şiddetli enfeksiyonu gösterdiler. Kuzey Cezayir'de belirlenen seroprevalans sığırlarda yüksek düzeyde *Hipoderma* spp. maruziyetini göstermektedir. Bu miyazise bağlı ekonomik kayıpları minimalize etmek için makrosiklik lakton uygulamalarından önce serolojik taramalar ışığında kontrol programlarının geliştirilmesi önemlidir.

Anahtar sözcükler: *Bovine hipodermozis, Mağrip, Cezayir, İndirekt ELİSA, Seroprevalans, Risk faktörleri*

INTRODUCTION

Cattle warble fly infestation (WFI), caused by *Hypoderma bovis* and *H. lineatum*, is a common myiasis found in all continents of the northern hemisphere. Despite the absence of ostensive clinical signs, *Hypoderma* spp. can cause economic losses by reducing milk production, increasing the meat trim and damaging the hides [1].

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Currently, control measures against bovine hypodermosis are mainly focused on the administration of broad-spectrum macrocyclic lactone compounds, involving some potential environmental risks [2]. Despite effective chemotherapy, warble flies continue infecting cattle in North America [3,4], some countries in Europe [5,6], northern Africa [7] and Asia [8,9].

There are several diagnostic methods designed to detect the disease including larval detection by grub monitoring or the examination of carcasses at slaughter. However, by using the traditional method of grub detection by back palpation, the infestation is diagnosed at very late stages when most of the damage has already been done; thus an earlier diagnosis is needed to minimize the economic losses associated to this myiasis. The detection of antibodies against first instars (L1) at the beginning of the migratory phase allows systemic treatment, avoiding the damage caused by the parasite on the host tissues [10]. The Enzyme Linked Immunosorbent Assay (ELISA) has been widely used to monitor the occurrence of hypodermosis in Britain [11] and other European countries for a number of years [12] and has been recommended for surveillance when clinical diagnosis becomes impractical because of low infestation levels [3,13]. Anti-*Hypoderma* antibodies appear within 4-8 weeks post infection during the migration of L1 and persist at positive levels for 3 or 4 months after the emergence of the third instar larvae [13,14].

Bovine hypodermosis is a major economic problem in the Maghreb, particularly affecting the leather industry [15]. In Morocco, Sahibi *et al.*[16] reported a global seroprevalence of 49.5%. Jemli *et al.*[17], in northern Tunisia found by clinical examination a herd and individual prevalence of 90% and 30%, respectively. Northern Algeria is in the southern limit of distribution of hypodermosis. Up to date, little is known about the distribution of WFI in this country and all the published data make reference to clinical studies based on warble counts, with prevalences ranging from 18.1% [7] to 84% [18]. Unfortunately, in Algeria, like in other Maghrebian countries cattle breeders are confronting with severe economic losses in the absence of warble management programs.

Bearing in mind the importance of ELISA for the early detection of hypodermosis in cattle, the present study was designed to determine the seroprevalence of this myiasis in Algerian cattle by using indirect ELISA. In addition, relationships between seroprevalence and intensity of infection with some intrinsic and extrinsic risk factors such us age, breed, sex, husbandry system and location were also analyzed.

MATERIAL and METHODS

Study Area

Northern Algeria is in the temperate zone and has a mild Mediterranean climate. However, its broken topography provides sharp local contrasts in both temperatures and precipitations. This area, comprising from the coast to the Tell Atlas, is inhabited by more than the 90% of Algeria´s population, because it is the most fertile region in the country. The geographic distribution of cattle, very scarce in southern Algeria, follows almost the same pattern as human population.

The present study has been conducted in 4 departments from 2 bioclimatic areas of northern Algeria: The humid area (Departments of Tizi Ouzou and Boumerdes), with mild temperatures and annual precipitations around 1.000 mm, and the Semi-arid area (Bouira and Bourdj Bou Arreridj) with scarce precipitations and large differences between high and low temperatures [19].

Animals and Samples

During May-July 2014, a total of 837 bovine blood samples were randomly collected from northern Algeria. Simultaneously, clinical examination by back palpation of the animals was carried out to assess the intensity of infection.

Different factors (age, sex, breed, location, grazing pattern) were recorded during the sampling in order to analyze their influence on warble fly prevalence. Variables were grouped and categorized for statistical analysis as follows:

Age groups: 1 (<3 years), 2 (3-5 year), 3 (>5 year).

Climatic area: 1 (Humid area), 2 (Semi arid area).

Department: 1 (Tizi Ouzou), 2 (Boumerdes), 3 (Bouira), 4 (Bourdj Bou Arreridj).

Breed: 1 (Montbéliard), 2 (Crossbreed), 3 (Atlas Brown).

Husbandry system: 1 (Intensive), 2 (Semi-extensive), 3 (Extensive).

Sex: 1 (Male), 2 (Female).

Indirect ELISA Protocol

Vinyl flat bottom microtiter plates (Thermo Scientific) were coated with a total extract, obtained from L1 of *H. lineatum* collected at the slaughterhouse. The antigen was added at a concentration of 5.5 µg/mL in phosphate-buffered saline (PBS, pH 7.5) and incubated at 37°C for 30 min in agitation. Unspecific unions were blocked for 30 min at 37°C with PBS containing 0.2% Tween-20 (PBS-T) and 1% skimmed milk powder as dilution buffer (PTL). Serum samples were tested in duplicate at 1:10. A positive and negative pooled serum was used as standards in each plate. Following the addition of sera, plates were incubated for 1 h at 37°C. After washing, Immuno-Conjugate (Horseradish peroxidase rabbit antibovine IgG (H + L); Bio Rad Laboratories) was used at 1:800 dilution in PT and the incubated for 30 minutes at 37°C. Finally, the reaction was revealed with *O*-phenylenediamine (SigmaFast OPD tablets; Sigma-Aldrich) and stopped by the addition of

100 µL of 3N H2SO4. After 2-5 min the absorbance was measured at 492 nm using a spectrophotometer (680XR; Bio-Rad Laboratories).

Cut-off Estimation

The cut-off value was determined by means of the receiver operating characteristic (ROC) analysis on sera from positive and negative control populations. Positive sera (n=27) came from naturally infected cattle which have palpable grubs and negative sera (n=51) from cattle with no previous exposure to *Hypoderma*. Levels of sensitivity were plotted against 1-specificity at each cut-off point on an ROC curve [20]. The area under the ROC curve is a simple and well suited overall measure of diagnostic test accuracy. In this study, the optimal combination of sensitivity and specificity was determined as 92.3% and 94.1% respectively, for a cut-off value of absorbance 0.740 *(Fig. 1)*.

Statistical Analysis

In order to assess the influence of some intrinsic (age, sex and breed) and extrinsic factors (husbandry system and location) in both seroprevalence and clinical prevalence of WFI, a logistic regression algorithm was applied. The dependent variable was the presence of warbles in each animal. Factors indicated previously were introduced in a backward conditional method and removed from the model one by one (on the basis of the highest p-value) until the best model was built [21]. Next, all pairwise interactions that were biologically plausible were evaluated. Odds ratio were computed by raising e to the power of the logistic coefficient over the first category of each factor, not over the last.

A multifactorial ANOVA over positive animals was used for the examination of the intensity of infestation; the dependent variable –number of nodules counted in animals– has been analyzed after having verified the variance homogeneity. Tukey HSD post hoc test was used to detect the differences between pairs.

Chi-squared automatic interaction detector has been performed to stratify risk factors in order of importance. CHAID algorithm identified factors that divide cattle in subgroups with different positive/negative ratio. CHAID is a tool to identify mayor factors using as criteria the significance of a Chi-squared test and successively splitting data in increasingly homogeneous nodes in relation to dependent variable (warble presence) until the classification tree is fully grown.

Spearman rank correlation was applied to measure the strength of a potential association between the number of warbles and the optical density corresponding to each warbled animal.

Statistical analyses were done using R statistical package v. 3.2.0 (22). CHAID algorithm was performed with CHAID function [22].

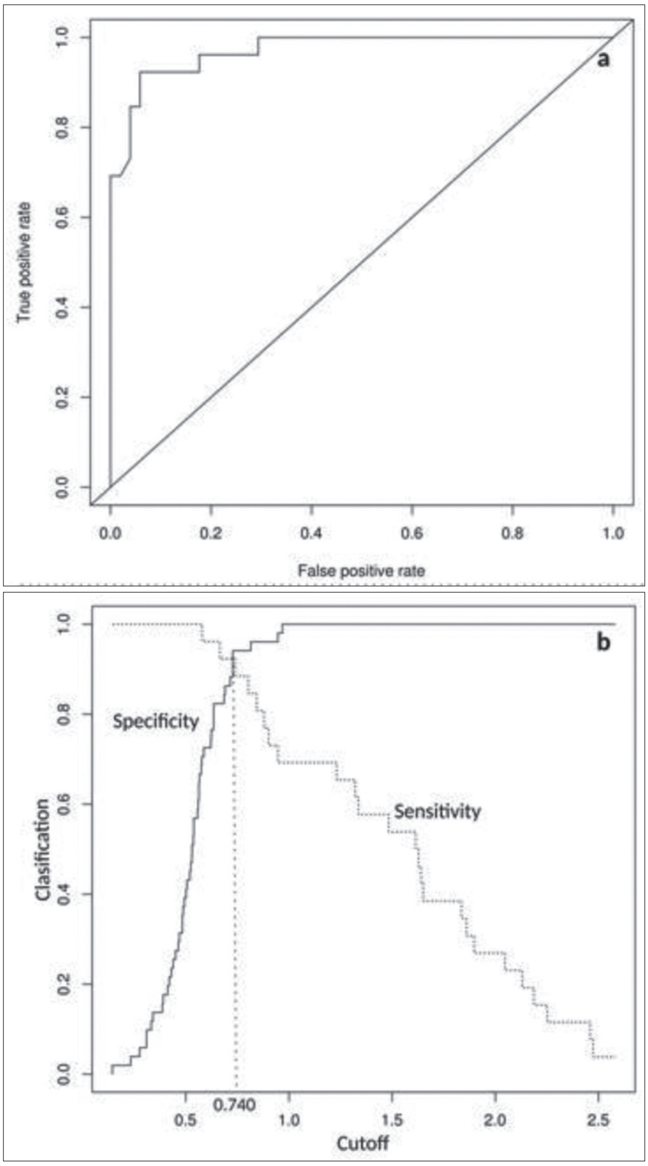

Fig 1. (a) Relative operating characteristic curve analysis of the results achieved with the indirect ELISA (b) Representation of the sensibility and specificity of the ELISA by using *Hypoderma* positive and negative control populations to obtain the cutoff value

Şekil 1. (a) İndirek ELİZA ile elde edilen sonuçların karakteristik analiz eğrisi (b) Hipoderma pozitif ve negatif kontrol popülasyonları kullanılarak elde edilen eşik değeri ile belirlenen ELİZA'nın duyarlılığı ve özgüllüğü

RESULTS

The overall seropositivity by *Hypoderma* spp. in cattle from northern Algeria was 49.8% (417 out of 837); when considering warble counts only the 25.7% of the animals were infested.

The results of the serological survey are summarized in *Table 1*. WFI was detected in all the departments included in the study; the highest prevalence was recorded in Tizi Ouzou (63.9%) located in the humid area and the lowest in Bourdj Bou Arreridj (42.4%) placed in the semiarid area.

***Table 1.** Seroprevalence and intensity of infection by Hypoderma spp. in cattle from northern Algeria*
***Tablo 1.** Kuzey Cezayir'de sığırlarda Hipoderma spp. seroprevalansı ve enfeksiyon yoğunluğu*

Factor	Levels	Number of Animals	Seroprevalence (%)	95% Confidence Interval	Intensity of Infection * Mean (SD)
Location	Tizi Ouzou	183	63.9	56.5–70.8	9.0(3.30)
	Boumerdes	217	44.6	37.9–51.6	9.58(4.38)
	Bouira	194	52.0	44.8–59.2	8.93(3.77)
	B. B. Arreridj	243	42.4	36.1–48.9	9.22(4.79)
Age	<3 years	205	42.4	35.6–49.5	11.6(3.07)
	3-5 years	371	47.4	47.4–52.7	7.8(4.05)
	>5 years	261	59.0	52.8–65.0	7.5(3.20)
Sex	Male	161	50.9	43.0–58.8	8.8(3.71)
	Female	676	49.6	45.7–53.4	9.3(4.07)
Husbandry System	Intensive	52	5.8	1.5–16.9	1.0(0)
	Semi extensive	708	50.6	46.8–54.3	8.6(4.09)
	Extensive	77	79.2	68.2–87.3	11.3(2.44)
Breed	Monbéliard	56	53.6	39.9–66.8	10.2(3.90)
	Crossbreed	396	52.2	47.5–57.5	9.2(3.60)
	Atlas Brown	385	47.7	42.2–52.4	9.0(4.33)

** Over warbled cattle*

***Table 2.** Last step of the Logistic regression analysis when considering ELISA data*
***Table 2.** ELİSA verisi dikkate alındığında Lojistik regresyon analizinin son basamağı*

Factors	Estimate	S.E.	Z Value	P	OR*	Lower Upper 95% CI for OR	
B.B. Arreridj							
Bouira	0.2814	0.2845	1.377	0.1684	1.3249	0.8878	1.9773
Boumerdes	0.1071	0.2008	0.528	0.5976	1.1118	0.7500	1.6479
Tizi Ouzou	1.1071	0.2408	4.599	<0.001*	3.0257	1.8875	4.8501
Monbeliarde							
Crossbreed	0.6045	0.4333	1.761	0.0783	1.8303	0.9339	3.5870
Local breed	0.9609	0.3582	2.683	0.0073*	2.6139	1.2955	5.2743
< 3 years							
3-5 years	0.4594	0.1915	2.399	0.0164*	1.5833	1.0877	2.3842
> 5 years	0.8623	0.2038	4.230	<0.001*	2.3685	1.5884	3.5316
Intensive							
Semi extensive	2.8978	0.6096	4.754	<0.001*	18.1338	5.4904	59.8929
Extensive	4 .3055	0.6714	6.412	<0.001*	74.1085	19.8763	276.3126

** Odds ratio were computed by raising e to the logistic estimation over the first category of each factor*

Analysis of ELISA results by logistic regression *(Table 2)* showed that cattle from Tizi Ouzou are 3.02 times more likely to acquire this myiasis than cattle from Bourdj Bou Arreridj. This binary logistic test indicated that in addition to location, other factors like age, breed and husbandry system were also significantly associated with seroprevalence. So, respect to the age cattle older than 5 years are 2.36 times more likely to be seropositive than cattle younger than 3 years. Likewise, the likelihood of being infected is 2.61 times higher for the local breed, Atlas Brown, than for the Montbéliard. Finally, the risk to get infested in cattle kept under extensive or semi-extensive husbandry systems is 74.10 and 18.13 times higher than in those under an intensive system, respectively.

Chi-squared automatic interaction detector stratified the husbandry system as the most influencing factor for warble fly seroprevalence, followed by the age *(Fig. 2)*.

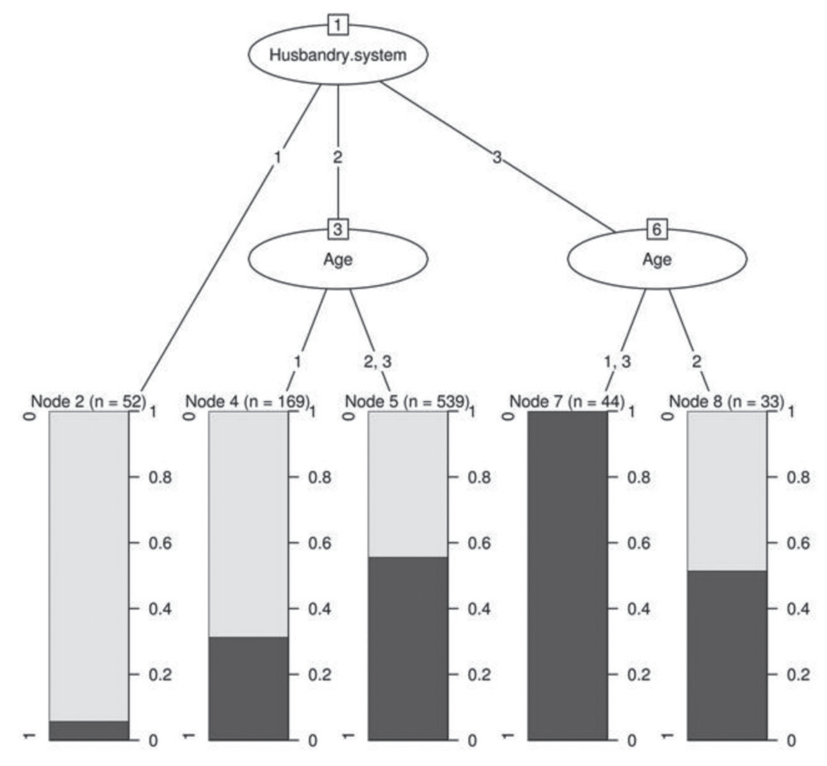

Fig 2. Classification tree produced by the CHAID algorithm when considering the different factors included in the study

Şekil 2. Çalışmada kullanılan farklı faktörler dikkate alınarak CHAID algoritması ile oluşturulan sınıflandırma ağacı

Table 3. *Last step of the Logistic regression analysis when considering clinical data*
Table 3*. Klinik veri dikkate alındığında Lojistik regresyon analizinin son basamağı*

Factors	Estimate	S.E.	Z Value	P	OR*	Lower Upper 95% CI for OR	
B. B. Arreridj							
Bouira	0.5416	0.2501	2.165	0.0383*	1.7188	1.052	2.8064
Boumerdes	0.5324	0.2504	2.126	0.0335*	1.7030	1.0424	2.7820
Tizi Ouzou	1.2641	0.2716	4.654	<0.001*	3.5397	2.0786	6.0276
< 3 years							
3-5 years	-0.7391	0.2096	-3.527	<0.001*	0.4775	0.3166	0.7201
> 5 years	-0.9060	0.2291	-3955	<0.001*	0.4041	0.2579	0.6331
Monbeliarde							
Crossbreed	0.3973	0.3852	1.031	0.3823	1.4877	0.6993	3.1652
Local breed	0.8755	0.4034	2.178	0.0299*	2.4000	1.0005	5.2913
Intensive system							
Semi extensive	1.9234	0.7351	2.616	0.0088*	6.844	1.6201	28.9120
Extensive	3.6715	0.7712	4.761	<0.001*	39.3109	8.9713	178.2122

** Odds ratio were computed by raising e to the logistic estimation over the first category of each factor*

Analysis of the presence or absence of warbles by logistic regression *(Table 3)* showed the location, age, breed and management system as risk factors for the presence of warbles. The results were in accordance to those obtained with the ELISA, with exception of the age of the animals. In this case, oldest cattle have less risk to present warbles on the back than animals under 3 years of age.

As regard to the intensity of infection *(Table 1)*, the number of warbles ranged from 1 to 24 nodules. Multifactorial ANOVA showed significant differences in the intensity of infection when considering the sex (*P*=0.002) and the age of the animals (*P*<0.001); with females and animals younger than 3 years showing the highest burdens. Husbandry system also had a significant influence in the number of warbles/animal (*P*<0.001). Post hoc Tukey's test revealed significant differences between all the categories both of age and husbandry system.

The Spearman rank correlation test (rho= 0.4579, <0.001) applied over warbled animals, revealed a very high association between OD values obtained by ELISA and warble counts.

DISCUSSION

The overall seroprevalence of hypodermosis in cattle from northern Algeria was 49.8%, which means that almost a half of those animals exposed to the parasite. This percentage was very similar to the seroprevalence (49.5%) detected by Sahibi *et al*.[16] in Morocco, and slightly higher than those recorded in other Mediterranean countries such as Turkey (38.6%) [23] and Albania (41.28%) [24].

The prevalence obtained by ELISA is higher than that resulting in the clinical examination. The diagnosis based on grub detection is laborious and relatively insensitive, resulting in an underestimation of infestation levels unless animals were inspected regularly throughout the emergence period [25]. In addition, it detects the infestations when larvae have finished their migrations, i.e. when the damage is done. On the contrary, the ELISA method is more valuable than grub monitoring for epidemiological surveys, and it was used in many countries in the diagnosis of WFIs, because, it has the advantage that it can be applied to many animals rapidly and easily, is also relatively cheap and it can used either milk or serum samples [26]. Moreover, ELISA provides a sensitive indication of how many individuals actually are exposed to infestation and reveals the number of animals that will harbor larvae that would die in the host prior to reaching the back where they are clinically detectable [3].

In this study the results showed that WFI prevails in all the studied departments of Northern Algeria, although in humid areas have more risk to become infected than in semi-arid areas, indicating more favorable conditions for the development and survival of pupal and adult stages [27]. Similar results were recorded by Sahibi *et al*.[16] in Morocco who found higher seropositivity in sub-humid areas (60%) in relation to semi-arid areas (50%).

The outcome of the statistical analysis is consistent with the biological cycle of *Hypoderma* spp. and confirms the free grazing as a major risk factor for WFI. Indeed, the husbandry system is known to exert a major effect on both prevalence and intensity of infection in bovine hypodermosis since extensive husbandry systems are the most favorable for the occurrence of this myiasis, because flies have more chances to contact the animal. This fact agrees with several previous studies [7,9,27-30]. Moreover, the autochthonous local breed Atlas Brown which is raised under semi-extensive and extensive husbandry systems presented higher seroprevalence than dairy breeds like the Montbéliard that is mostly kept intensively.

Both the seroprevalence and the intensity of infection are inversely related to the age of cattle. So, the youngest animals (<3 years) were less frequently infested but displayed the highest intensities of infection. This finding can be easily explained, because this category consisted of a great proportion of calves younger than one year, with no chances to be in contact with the parasite. In contrast, animals younger than 3 years were most heavily infested because the intensity of infection decline with age after repeated infestations [14,31]. According to these authors, cattle develop acquired resistance after repeated exposures to *Hypoderma* larval antigens. This resistance is recognized as an important factor in controlling grub populations and depends on the host's age and the number of larvae invading the host [32].

In addition to husbandry system and age, multifactorial ANOVA also showed significant differences in the intensity of infection when considering the sex of the animals; with females, young and free grazing animals showing the highest grub burdens. This result could be explained by the fact that cows in Northern Algeria are generally raised in semi-extensive and extensive management systems, whereas bulls are mainly kept in stables, decreasing the risk of infection.

Both Pearson's and Spearman's Rank correlations applied over clinically positive animals identified a high positive relationship between OD values and warble burden. Although Pruett and Barrett [33] reported an absence of correlation between circulating antibody levels and the number of grubs achieving their biological cycle, Panadero *et al*.[6] found a positive correlation between IgG levels and warble burdens at different time points throughout the course of the infection.

In conclusion, the seroprevalence detected in northern Algeria reflects a high exposition of cattle, especially those maintained under extensive conditions, to *Hypoderma* spp. To minimize the economic losses due to this myiasis, it is crucial to establish control programs based on serological surveillance to be conducted prior to the administration of macrocyclic lactones.

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Conflict of Interest

The authors declare that they have no competing interests.

REFERENCES

1. Drummond RO: Economic aspects of ectoparasites of cattle in North America. **In,** Leaning, WHD, Guerrero J (Eds): The Economic Impacts of Parasitism in Cattle. 9-24, *Proc. MSD AGVET Symposium, 23rd World Vet Congr,* Montreal, 1987.

2. Strong L: Overview: The impact of avermectins on pastureland ecology. *Vet Parasitol,* 48, 3-17, 1993. DOI: 10.1016/0304-4017(93)90140-I

3. Colwell DD: Out of sight but not gone: Sero-surveillance for cattle grubs, *Hypoderma* spp., in western Canada between 2008 and 2010. *Vet Parasitol*, 197, 297-303, 2013. DOI: 10.1016/j.vetpar.2013.07.009

4. Rehbein S, Holste J E, Smith L L, Lloyd, J L: The efficacy of eprinomectin extended-release injection against *Hypoderma* spp. (Diptera: Oestridae) in cattle. *Vet Parasitol*, 192, 353-358, 2013. DOI: 10.1016/j.vetpar.2012.11

5. Haine D, Boelaert F, Pfeiffer D U, Saegerman C, Lonneux, J F, Losson B, Mintiens K: Herd-level seroprevalence and risk-mapping of bovine hypodermosis in Belgian cattle herds. Prev *Vet Med*, 65, 93-104, 2004. DOI: 10.1016/j.prevetmed.2004.06.005

6. Panadero R, Vazquez L, Colwell, DD, Lopez C, Dacal V, Morrondo P, Diez-Banos P: Evaluation of an antigen captures ELISA for the early diagnosis of *Hypoderma lineatum* in cattle under field conditions. *Vet Parasitol*, 147, 297-302, 2007. DOI: 10.1016/j.vetpar.2007.04.004

7. Saidani K, López C, Mekademi K, Díaz P, Díez-Baños P,, Benakhla A, Panadero R: Bovine hypodermosis in North-Central Algeria: Prevalence, intensity of infection and risk factors. *Kafkas Univ Vet Fak Derg,* 20, 871-876, 2014. DOI: 10.9775/kvfd.2014.11197

8. Guan G, Luo J, Ma M, Yang D, Wang Y, Gao J, Sun H, Liu Z, Liu A, Dang Z, Boulard C, Yin H: Sero-epidemiological surveillance of hypodermosis in yaks and cattle in north China by ELISA. *Vet Parasitol*, 129, 133-137, 2005. DOI: 10.1016/j.vetpar.2004.12.021

9. Ahmed H, Khan MR, Panadero R, Lopez C, Asif S, Irfan M, Mazhar Q: Influence of epidemiological factors on the prevalence and intensity of infestation by hypoderma spp. (Diptera: Oestidae) in cattle of Potowar region, Pakistan. *Pakistan J Zool*, 45, 1495-1500, 2013.

10. Boulard C, Argente G, Hillion E: Hypodermose bovine: 2e partie: diagnostic et traitement. *Point Vét*, 20, 17-27, 1988.

11. Tarry DW, Sinclair IJ, Wassall DA: Progress in the British hypodermosis eradication programme: the role of serological surveillance. *Vet Rec,* 131, 310-312, 1992. DOI: 10.1136/vr.131.14.310

12. Boulard C, Argenté M, Argenté G, Languille J, Paget L, Petit E: A successful, sustainable and low cost control-programme for bovine hypodermosis in France. *Vet Parasitol*, 158, 1-10, 2008. DOI: 10.1016/j.vetpar.2008.07.026

13. Webster KA, Dawson C, Flowers M, Richards MS: Serological prevalence of *Hypoderma* species in cattle in Great Britain (1995-1996) and the relative value of serological surveillance over clinical observation. *Vet Res,* 41, 261-263, 1997.

14. Boulard C: Durably controlling bovine hypodermosis. *Vet Res*, 33, 455-464, 2002. DOI: 10.1051/vetres:2002032

15. Benakhla A, Jemli M, Sahibi H, Boulard C: Bovine hypodermosis in Maghreb. **In:** Improvements in the Control Methods for Warble fly in Livestock. 34-37, European Commision, COST 811, Brussels, 1998.

16. Sahibi H, Rhalem A, Boulard C: Epidemiology of bovine hypodermosis and its control in Morocco. **In,** Improvement of means of Control of Warblefly in Cattle and Goats. Proc. XIII European COST 811. Parma, Italy, Sept. 56, 1996.

17. Jemli M H, Malek N, Chaouch M: Investigation of hypodermoses in Tunisia: Result of pilot survey. **In,** Puccini V, Giangaspero (Eds): Improvements in the Control Methods for Warble Fly in Livestock. 71-79, COST 811, European Commission, Italy, 1996.

18. Benakhla A, Boulard C, Sedraqui S, Oussaid F: L'hypodermose bovine: Approche épidémiologique et caractérisation du cycle biologique en vue de l'établissement d'un plan de prophylaxie dans le nord est Algérien. *Rev Med Vet,* 144, 693-700, 1993.

19. Saidani K, López C, Díaz P, Díez-Baños P, Benakhla A, Panadero R: Effect of climate on the epidemiology of bovine hypodermosis in Algeria. *Kafkas Univ Vet Fak Derg*, 22, 147-154, 2016. DOI: 10.9775/kvfd.2015-14122

20. Zou KH, O'Malley AJ, Maur L: Receiver-Operating characteristic analysis for evaluating diagnostic tests and predictive models. *Circulation*, 115, 654-657, 2007. DOI: 10.1161/CIRCULATIONAHA.105.594929

21. R Core Team R: A language and environment for statistical computing. R foundation for Statistical Computing, Vienna, Austria, 2015. URL http://www.R-project.org.

22. The FoRt Student project Team, CHAID: chi-squared Automated Interaction detection R package version 0.1-2, 2015.

23. Simsek S, Utuk A E, Koroglu, Dumanli N: Seroprevalence of hypodermosis in cattle in some provinces of Turkey. *Res Vet Sci*, 84, 246-249, 2008. DOI: 10.1016/j.rvsc.2007.05.007

24. Otranto D, Zalla P, Testini G, Zanaj S: Cattle grub infestation by *Hypoderma* sp. in Albania and risks for European countries. *Vet Parasitol,* 128, 157-162, 2005. DOI: 10.1016/j.vetpar.2004.11.016

25. Argenté G, O'Brien D: What is a warble-clean area? **In,** Improvement in the Control Methods for Warble-fly in Farm Livestock. Proc. XII European COST 811. Kinsale, Ireland, Sept. 21-23, 1-5, 1995.

26. Otranto D, Testini G, Sottili R, Capelli G, Puccini V: Screening of commercial milk samples using ELISA for immuno-epidemiological evidence of infection by the cattle grub (Diptera: Oestridae). *Vet Parasitol*, 99, 241-248, 2011. DOI: 10.1016/S0304-4017(01)00463-0

27. Tarry D: Warble fly infestation and climate. *Vet Rec,* 106, 559-560, 1980. DOI: 10.1136/vr.106.26.559

28. Panadero R, López C, Díez N, Paz A, Díez P, Morrondo P: Influence of internal and environmental factors on the distribution and occurrence of *Hypoderma* (Diptera: Oestridae) in cattle in Galicia (Northwest of Spain). *J Med Entomol*, 37, 27-28, 2000. DOI: 10.1603/0022-2585-37.1.27

29. Ahmed H, Khan MR, Panadero R, López C, Iqbal MF, Naqvi SMS, Qayum M: Geographical distribution of Hypodermosis *(Hypoderma* sp.) in Northern Punjab, Pakistan. *Kafkas Univ Vet Fak Derg,* 18 (Suppl-A): A215-A219, 2012. DOI: 10.9775/kvfd.2012.6533

30. Zalla P, Shoshi N, Bejo B, Postoli R, Rapti D: Impact of management system on the presence of hypodermosis in cattle. *Maced J Anim Sci*, 2, 235-240, 2012.

31. Gingrich RE: Acquired resistance to *Hypoderma lineatum*: Comparative immune response of resistant and susceptible cattle. *Vet Parasitol*, 9, 233-242, 1982. DOI: 10.1016/0304-4017(82)90069-3

32. Baron RW, Weintraub J: Lymphocyte responsiveness in cattle previously infested and uninfested with *Hypoderma lineatum* (de Vill.) and *H. bovis* (L.) (Diptera: Oestridae). *Vet Parasitol,* 24, 285-296, 1987. DOI: 10.1016/0304-4017(87)90050-1

33. Pruett JH, Barrett CC: Kinetic development of humoral anti-*Hypoderma lineatum* antibody activity in the serum of vaccinated and infested cattle. *Southwest Entomol*, 10, 39-48, 1985.