REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

Clinical and Serological Diagnosis of Bovine Hypodermosis in Wasit Province

Hasanain A. J. Gharban

Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, University of Wasit, Wasit, Iraq
Email: hgirban@uowasit.edu.iq

Abstract

The present study was conducted on cattle of some areas related to Wasit province during the period of May 2015 to July 2016, and included 536 animals of different ages, both sexes and of local and cross breeds, which examined clinically by manual skin palpation, and then submitted for collection of venous blood samples for serological testing by indirect-ELISA. The overall prevalence was 21.83% clinically and 36.19% serologically. Among of 117 (21.83%) cattle were positive by clinical examination, the seropositives by indirect-ELISA were 115 (21.46%) ones. Regarding to season factor, a higher increasing of warble infestations was reported at April month (58.49%), whereas the highest seroprevalence were detected at December month (69.05%). Relating to age factor, the high incidence rates of hypodermosis were noticed clinically in younger cattle (1-3 years), (33.22%); while serologically, it's more prevalent in older cattle (>6 years), (73.81%). On sex factor base, the significant increasing (P>0.05) of warbles infestation was reported in females (24.04% and 39.23%) more than males (11.58% and 22.11%) by both clinical and serological tests, respectively. In breed factor, the clinical examination was revealed on a high morbidity rate of infestation in local-breed (33.48%) in comparison to cross-breed (13.27%); while serologically, the high levels of specific anti- hypodermosis antibodies were reported in both, local (35.68%) and cross (36.57%), breeds without significant differences (P≤0.05).

Keywords: Cattle, *Hypoderma bovis*, *Hypoderma lineatum*, Serology, Iraq.

Introduction

Cattle hypodermosis is a myiasis parasitic disease caused by larvae of *Hypoderma bovis* and *Hypoderma lineatum* (Diptera: Oestridae), which characterized by presence of warbles (grubs) under the skin of infested animal (1). Among most parasitic diseases, *Hypoderma* spp. are representing a major problem to the livestock, which affecting on wild and domesticated animals as cattle, sheep and goats as well as in humans (accidently) (2). In frequent, cattle are sustaining different types of conditions as wounds and food contamination that appears to provide a suitable entrance for maggot particularly in open field (3). Although, the control measures against bovine hypodermosis are focused, mainly, on administration of effective broad spectrum macrocyclic lactone compounds (as avermectins and milbemycins); the flies continue to infest the cattle in all continents of the northern hemisphere like Asia, Northern Africa, Europe and North America (4, 5). *Hypoderma* spp. can be inducing, if not treated early, high economic losses by reducing the nutritive value of cattle carcasses for human consumption, damaging of infested animal hides, increasing the meat trim and reducing of milk production (6, 7).

In past, cattle grub was detected entirely in relied either on palpation of the warbles on the back and flank regions or through the visual examination of carcasses in slaughterhouses, and in both methods, the infestations were diagnosed at very late stage when most of the damage

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

has already been done (8, 9). However, the early sensitive and reliable diagnosis is required through detection of specific antibodies against first instars (L1) at the beginning of migratory phase, which allowing for systemic treatment and to reduce the losses associated with this disease (10). Hence, the immunological techniques such as the enzyme-linked immunosorbent assay (ELISA) have been developed, modified and used widely in last twenty years in several countries (11). The enzyme of collagenase hypodermin C, which secreted by the first instars (L1) during their migration within the host, is considered a useful diagnostic antigen for detection of specific antibodies that start to appear usually within 4-8 weeks after infestation during the migration of L1 and persist positives for 3-4 months post-emergence of L3 (12). The main advantage of ELISA's kit that depended on this enzyme, as antigen, is that detecting the presence of active infestations in sera of previously infested cattle as earlier as 6 weeks after infestation; whereas, the cross-reaction between both *Hypoderma* spp. (*bovis* and *lineatum*), still the main disadvantage of this kit (6, 13).

This study was designed for determining the prevalence of bovine hypodrmosis, clinically, by the manual palpation for detection of the dermal grubs and, serologically, through detection of specific antibodies in sera by indirect ELISA. Also, the study discussed the association of the positive samples to some epidemiological factors such as the season, age, sex, and breed.

Material and method

Ethical approval and study animals

This study was licensed by the Scientific Committee of Department of Internal and Preventive Veterinary Medicine in the College of Veterinary Medicine (University of Wasit / Wasit), and carried out across a number of areas in Wasit province during the period of May 2015 to June 2016, and included a totally of 536 cattle. The animals were examined, clinically, through the manual palpation of the back and hides, and then, submitted for 5 ml of jugular venous blood sample collection into an EDTA vacutainer tubes (*AFMA, Jordan*). The blood samples were centrifuged at 4000 rpm / 15 minutes and the sera were packaged into numbered 1 ml eppendorf microtubes (*China*), and kept at 20°C until used (14). Also, the cattle owners were questioned for obtaining of the main data that related to study's epidemiological factors, and divided into 12 monthly age groups, three age groups (1 - ≤3, 3 - ≤5 years and ≥ 5 years), two sex groups (females and males) and two breed groups (local and cross-breed).

Serological testing

Indirect enzyme-linked immunosorbent assay (ELISA) kit (*Institut POURQUIER, France*) was used in this study for detection of specific antibodies against bovine hypodermosis (*H. bovis* and *H. lineatum*). According to manufacturer instruction, the sera samples were tested and the optical densities were read at an optical density (OD) of 450 nm using the microplate ELISA reader (*Bio Tek-USA*), and the results were considered validated where the positive control had 0.350 minimal mean OD, and 3.5 minimal OD ratio between positive and negative controls. The samples seropositivity (S/P) were calculated by using the formula ($S/P = 100 \times OD_{Sample} - OD_{Negative\ Control} / OD_{Positive\ Control} - OD_{Negative\ control}$) and the results were interpreted as following: if the S/P ≤ 45%, this mean that the sample is negative, where, if the S/P ≥ 55, this mean that the sample is positive.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

Statistical analysis

To detect the significant differences (P≤0.05) between the positive cattle clinically and serologically, and among the different epidemiological factors (season, age, sex and breed), the *Chi-square* (*x2*) test was applied by using of a computerized Microsoft Office Excel (*2007*) and IBM/SPSS (*v23*) programs (15).

Results

Out of 536 cattle, the clinical examination of skin for detection of hypodermosis grubs showed that 117 (21.83%) of cattle were positives, while the serological ELISA test was revealed on 194 (36.19%) seropositive cattle (Table 1).

Table (1): Results of clinical and serological diagnosis of hypodermosis in 536 cattle

Total No.	Test	Positives	Negatives
536	Clinical examination	117 (21.83 %) ^{B}	419 (78.17%)
	ELISA	194 (36.19%) ^{A}	342 (63.81%)

Variation in small letters, vertically, referred to significant differences at level of P≤0.05

The cross-classification results for clinical and serological identification of bovine hypodermosis were discussed in (Table 5) that revealed on 115 (21.46%) and 340 (63.43%) of cattle were, respectively, positives and negatives by both test; whereas, 2 (0.37 %) of cattle were positives, clinically, and negatives, serologically; and 79 (14.74%) of cattle were negatives, clinically, and positives, serologically.

Table (2): Cross – classification results of clinical and serological tests in 536 cattle

	Clinical examination		
ELISA	Positives	Negatives	Total No.
Seropositive	115 (21.46%) ^{Aa}	79 (14.74%) ^{Bb}	194 (36.19%)
Seronegative	2 (0.37%) ^{Bb}	340 (63.43%) ^{Aa}	342 (63.81%)
Total No.	117 (21.83 %)	419 (78.17%)	536

Variation in large and small letters, horizontally and vertically, refers to significant differences at level of P≤0.05

Association of positive results to epidemiological risk factors; season, age, sex and breed were discussed among the clinical and serological positive results (Table 3).

Table (3): Prevalence of bovine hypodermosis among the epidemiological factors

Risk Factors		Groups	Total No.	Clinical P+	Serological P+
		January	39	14 (35.9%) ^{c}	24 (61.54%) ^{b}
		February	42	18 (42.86%) ^{b}	17 (40.48%) ^{d}
		March	54	25 (46.3%) ^{b}	8 (14.81%)

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

1	Season	April	53	31 (58.49%) [a]	9 (16.98%)
		May	62	13 (20.97%)	17 (27.42%)
		June	29	3 (10.34%)	6 (20.69%)
		July	40	2 (5%)	11 (27.5%)
		August	43	0 (0%)	13 (30.23%)
		September	41	0 (0%)	15 (36.59%)
		October	43	0 (0%)	19 (44.19%) [d]
		November	48	4 (8.33%)	26 (54.17%) [c]
		December	42	7 (16.67%)	29 (69.05%) [a]
2	Age (Year)	1-3	283	94 (33.22%) **[a]**	47 (16.61%) **[c]**
		> 3 - 6	169	18 (10.65%) **[b]**	85 (50.3%) **[b]**
		> 6	84	5 (5.95%) **[c]**	62 (73.81%) **[a]**
3	Sex	Female	441	106 (24.04%) **[a]**	173 (39.23%) **[a]**
		Male	95	11 (11.58%) **[b]**	21 (22.11%) **[b]**
4	Breed	Local-breed	227	76 (33.48%) **[a]**	81 (35.68%) **[b]**
		Cross-breed	309	41 (13.27%) **[b]**	113 (36.57%) **[b]**

Variation in small letters, vertically refers to significant differences at level of P≤0.05

In season factor, the highest prevalence of bovine hypodermosis by clinical examination was reported at April (58.49%) and March (46.3%) with absence of grubs detection during 3 months (August, September and October), (Figure1-A); whereas, the highest prevalence of anti-*Hypoderma* antibodies by serology were showed at December (69.05%) and January (61.54%), and the lowest at March (14.81%) and April (16.98%), (Figure1-B). Based on age factor, the highest prevalence of infection was detected, clinically (Figure 2-A), in a group of 1-3 years (33.22%); and serologically (Figure2-B), in a group of >6 years (73.81%). In sex factor, the bovine hypodermosis in females, (24.04%) and (39.23%), was more than showed in males, (11.58%) and (22.11%); clinically (Figure 3-A) and serologically (Figure 3-B), respectively. Among breed factor, the local-breed reported a high prevalence (33.48%) in comparison with cross-breed, (Figure4-A); whereas serologically, the significant differences does not reported between both local-breed (35.68%) and cross-breed (36.57%), (Figure 4-B).

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

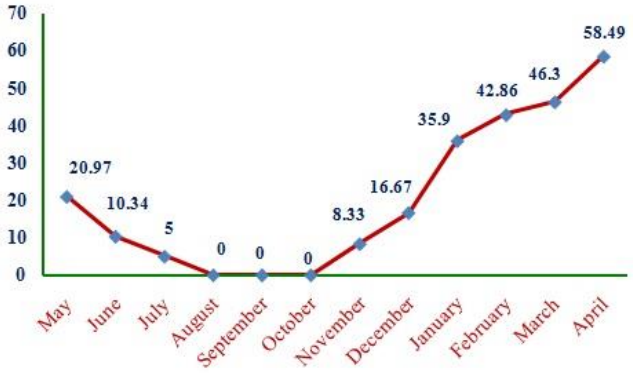

Figure (1-A): Clinical prevalence of bovine hypodermosis among season factor

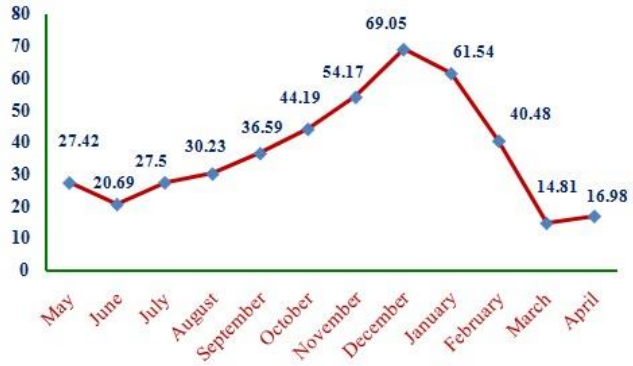

Figure (1-B): Serological prevalence of bovine hypodermosis among season factor

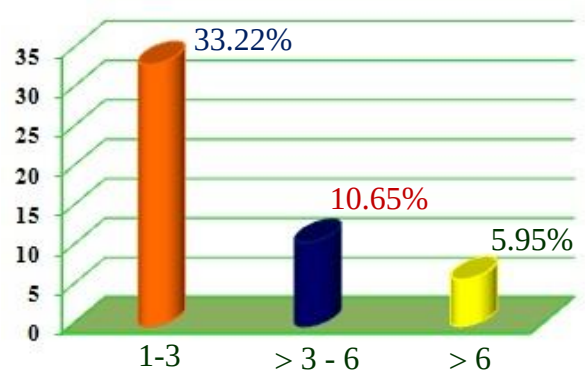

Figure (2-A): Clinical prevalence of bovine hypodermosis among age factor

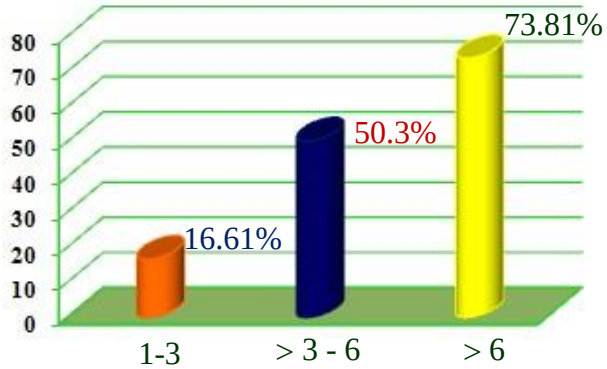

Figure (2-B): Serological prevalence of bovine hypodermosis among age factor

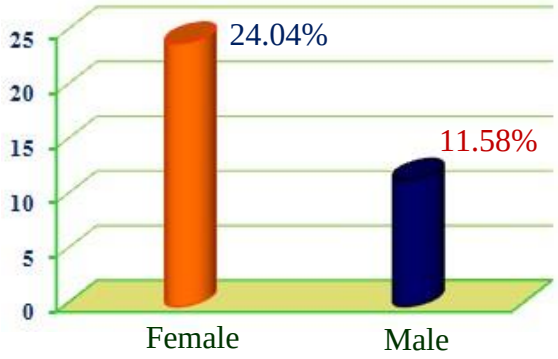

Figure (3-A): Clinical prevalence of bovine hypodermosis among sex factor

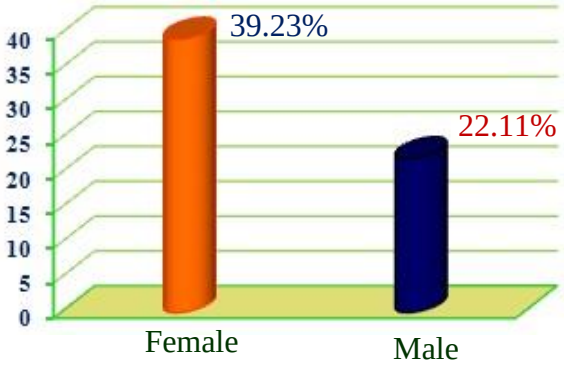

Figure (3-B): Serological prevalence of bovine hypodermosis among sex factor

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

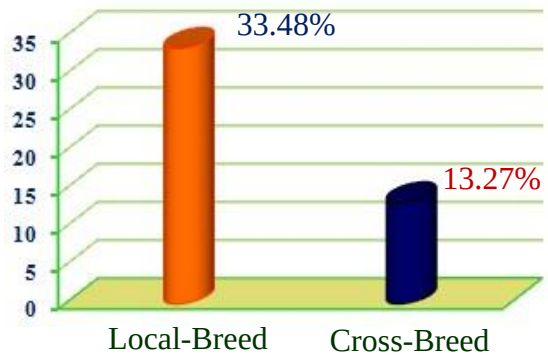

Figure (4-A): Clinical prevalence of bovine hypodermosis among breed factor

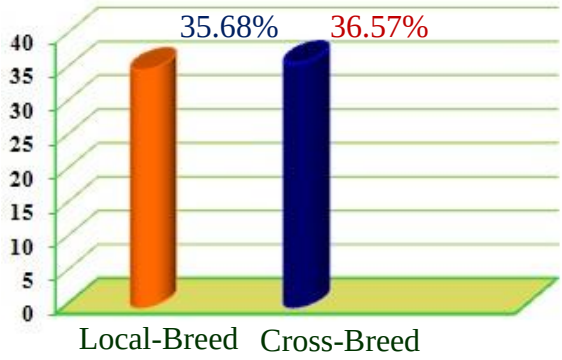

Figure (4-A): Clinical prevalence of bovine hypodermosis among breed factor

Discussion

In live cattle, the diagnosis of hypodermosis can be achieved either by direct clinical detection of warbles during a period of several weeks post-infestation when the 2nd and 3rd larval stages are persist as skin grubs on animal's back and/or hides, or by using of indirect serological techniques to detect the specific anti-*Hypoderma* antibodies in a period of 4-8 weeks post-infestation when the 1st larval stage migrates within the animal's tissue (16, 17). In this study, the results reported that 21.83% and 36.19% of overall examined cattle were positives clinically and serologically, respectively. Bovine hypodermosis is quit widespread in several regions in Iraq, and the average of infestation rate could reach 22.21% by clinical examination (18) and 52.31% by serological indirect-ELISA (19). In fact, although the using of chemotherapy against adult fly and first larval stage could reduce the economic impacts of hypodermosis, significantly, and even eradicate the disease from large areas as demonstrated in several studies, it's still spread in poor socioeconomic settings, mainly, in Asiatic countries where the routine treatment are not carried out (20). Worldwide, the clinical prevalence of bovine hypodermosis was 40.17% in China (6), 59.9% in Ethiopia (21), 14.71% in Iran (22), 23.5-35.5% in Pakistan (23), 16.21% in Romania (9) and 18.2% Spain (24); whereas, the serological prevalence was 38.6-41.3% in Albania (1), 38.6% in Turkey (25), 49.8% in Algeria and 49.5% in Morocco (26). Hence, the wide and large variation in prevalence of bovine hypodermosis that seen among herds, regions and countries, might be attributed to the type of applied diagnostic methods, using of insecticide, husbandry management, host sensitivity and breed, and differences in environmental conditions that inter-penetrate the life cycle of *Hypoderma* parasite such as wind velocity, rainfall, season, humidity, temperature and the topographical nature of region (18, 27).

However, the positive correlation between circulating antibody levels and the warble burdens at different time points throughout the course of infection, confirmed the usefulness of ELISA in large sero-epidemiological hypodermosis surveys, especially, in regions where the infestation is unknown. Nonetheless, the differences between the clinical and serological obtained results could be explained by the recent destruction of migrating larvae in some animals, persistence of parasite residuals that act as antigens resulting in positive reactions, or

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

due to the continual concurrent exposures without actual infestation especially in old age cattle (11, 28).

In this study, the high prevalence of warbles was noticed, clinically, from January to April, reached the peak at April, and absence during August, September and October months; whereas serologically, the seropositive prevalence was among all months of the year and the high prevalence was showed during the months of October to February, which reached the peak at December. As reported by several studies, the high titer of antibodies was occurred during the migratory phase of L1 *Hypoderma*, where the skin damage had not yet and represented the optimal period for collection of sera and diagnosis of infestation, then for treating of infested animals (29, 30).

Regarding to age factor, the higher incidence of warbles was detected clinically in a group of younger cattle (1-3 years); while serologically, it's more prevalence in a group of old age cattle (> 6 years). The lower prevalence rate of grubs in old age cattle might be due to the fact that their skin are too hard and thick for larval penetration or due to the development of high immunity as a result of previous infestations (31). The persistence of live or dead larvae with persistence of granulomatous reaction around them in subcutaneous tissues could indicate the development of immunity that play an important role in reducing or fighting of larval migration and increasing of host resistance (32). It has been evaluated that the repeated exposure to larval antigens can result in progressing of acquired immunity against bovine hypodermosis, and this immunity is depending on host's age, severity of previous exposures and the number of invaded larvae (33).

Based on sex factor, the study showed that the bovine hypodermosis was more prevalent in females than males by both clinical and serological examinations with significant differences ($P>0.05$). These results were in agreement with those reported by (19, 31) and disagreement with (18, 30). In field conditions and during the season of fly activity, the wounds were very prone to be occurred in the shoulder areas, vulva and navel, and the females appeared to be more frequent affected than males (34, 35). In fact, cows are received generally a less-required attentions than bulls that kept in stables, and pastured in semi extensive or extensive management systems (26, 36).

Relating to breed factor, although the clinical results showed a high significant prevalence of hypodermosis in a group of local-breed when compared to a group of cross-breed; it's not detected serologically, ($P\leq0.05$). These results might because of local breeds were more sensitive for hypodermosis than cross-breed, frequent exposures for sources of infestation, pastured in inappropriate conditions and not received the required level of protection or therapy (11, 25).

Conclusion

This study was the first one compared between clinical and serological detection of *Hypoderma* infestation in cattle, which demonstrated significant reliability of serological test when compared to clinical detection of warbles. Additionlly, this study found that the prevalence of bovine hypodermosis might be changed obviously during different seasons as well as between different ages, sexes and breeds. However, furthermore studies using of

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

serological and molecular assays can provide a basic epidemiological data about the actual incidence of parasite.

References

1. Otranto D., Zalla P., Testini G., and Zanaj, S. (2005). Cattle grub infestation by *Hypoderma* sp. in Albania and risks for European countries. *Veterinary parasitology*, *128*(1), 157-162.
2. Hassan MU., Khan MN., Abubakar M., Waheed HM., Iqbal Z., and Hussain, M. (2010). Bovine hypodermosis-a global aspect. Tropical animal health and production, 42(8), 1615-1625.
3. Slansky, F. (2007). Insect/mammal associations: Effects of cuterebrid bot fly parasites on their hosts. Annu. Rev. Entomol., 52, 17-36.
4. Deepak Mathur, N. K. V. . (2022). Analysis & Prediction of Road Accident Data for NH-19/44. International Journal on Recent Technologies in Mechanical and Electrical Engineering, 9(2), 13–33. https://doi.org/10.17762/ijrmee.v9i2.366
5. Lumaret JP., Errouissi F., Galtier P., and Alvinerie M. (2005). Pour-on formulation of eprinomectin for cattle: Fecal elimination profile and effects on the development of the dung-inhabiting Diptera Neomyia cornicina (L.) (Muscidae). Environmental toxicology and chemistry, 24(4), 797-801.
6. Hagemoen RI., and Reimers, E. (2002). Reindeer summer activity pattern in relation to weather and insect harassment. Journal of Animal Ecology, 71(5), 883-892.
7. Guan G., Luo J., Ma M., Yang D., Wang Y., Gao J., and Boulard, C. (2005). Sero-epidemiological surveillance of hypodermosis in yaks and cattle in north China by ELISA. Veterinary parasitology, 129(1), 133-137.
8. Gupta, D. J. . (2022). A Study on Various Cloud Computing Technologies, Implementation Process, Categories and Application Use in Organisation. International Journal on Future Revolution in Computer Science &Amp; Communication Engineering, 8(1), 09–12. https://doi.org/10.17762/ijfrcsce.v8i1.2064
9. Kara M., Arslan MO., and Gicik, Y. (2005). The prevalence of bovine hypodermosis in Kars province, Turkey. Tropical animal health and production, 37(8), 617-622.
10. Karatepe M., and Karatepe, B. (2008). Hypodermosis in cattle slaughtered in Nigde province, Turkey. Tropical animal health and production, 40(6), 383-386.
11. Gorcea FC., Călescu N., Gherman CM., Mihalca AD., and Cozma, V. (2011). Diagnostic values of clinical, pathological and serologic findings in cattle hypodermosis in Peştişani, Gorj County Romania. Sci Parasitol, 12, 173-6.
12. Ahmad H., Bushra KM., Waseem S., and Mazhar, Q. (2011). Seroprevalence of Hypodermosis (Hypoderma lineatum) in the cattle of Potohar region, Pakistan. Int J of Cell and Mol Bio, 2(2), 497-510.
13. Balkaya I., Simsek S., and Saki, C. E. (2010). A serological and molecular survey of cattle hypodermosis in east-Turkey. Veterinary parasitology, 173(3), 287-291.
14. Panadero-Fontán R., Lopez-Sández C., Parra-Fernández F., Morrondo-Pelayo P., Díez-Baños P., and Colwell, D. D. (2002). Detection of circulating hypodermin C: an antigen

capture ELISA for diagnosis of cattle grub (Diptera: Oestridae) infestations. Veterinary parasitology, 108(1), 85-94.

15. Colwell DD., López C., Diez-Banos P., Morrondo P., and Panadero, R. (2008). Impact of previous infestation on dynamics of circulating hypodermin C in cattle artificially infested with Hypoderma lineatum (Diptera: Oestridae). Veterinary parasitology, 154(1), 114-121.
16. Agarwal, A. . (2022). Co-Integrable Sets and Probability. International Journal on Recent Trends in Life Science and Mathematics, 9(2), 41–46. https://doi.org/10.17762/ijlsm.v9i2.134
17. Duncan JR. and Prasse, KW. (2003). Veterinary Laboratory Medicine: Clinical Pathology, 4th edition. Ames: Blackwell, Pp: 3 -45.
18. Petrie A., and Watson, P. (2006). Statistics for Veterinary and Animal Science, Second Edition. Ames: Blackwell Publishing, Pp: 24-112.
19. Sager H., and Elsheikha, HM. (2011). Diseases Caused by Insects. Essentials of Veterinary Parasitology, 8 (1), 115-117.
20. Ahmed H., Afzal MS., Mobeen M., and Simsek, S. (2016). An overview on different aspects of hypodermosis: Current status and future prospects. Acta tropica, 162, 35-45.
21. A'aiz NN., Kashash KH., and Al-Kaabii, NA. (2011). Prevalence of bovine hypodermosis in Babylon province in Iraq. Al-Anbar J. Vet. Sci., 4 (2), 99-102.
22. Al-Sarray AH., and Khalaf, GJ. (2017). Early Serodiagnosis of Hypoderma Genus in Cattle by ELISA Test. Journal of the College of Education. 28 (1), 118-129.
23. Yadav A., Katoch R., Khajuria JK., Godara R., and Agrawal, R. (2013). Prevalence of Hypoderma lineatum in cattle of Jammu region. Journal of parasitic diseases, 37(2), 196-198.
24. Gebremedhin, EZ. (2011). Prevalence of ovine and caprine oestrosis in Ambo, Ethiopia. Tropical animal health and production, 43(1), 265-270.
25. Radfar MH., and Hajmohammadi, V. (2012). Prevalence of goat warble fly, Przhevalskiana silenus in southeastern of Iran. Sci Parasitol, 13, 73-76.
26. Khan MN., Zafar I., Khan IA., Chaudhry SA., and Sajid, M. S. (2008). Surveillance of cattle hypodermosis in district Chakwal, Pakistan. International Journal of Agriculture and Biology, 10(3), 337-339.
27. Ana Carolina Abrantes, João Serejo, Madalena Vieira-Pinto. (2022). Drought as a Risk Factor to the Occurrence of Tuberculosis-like Lesions Detected during Red Deer Post-Mortem Inspection: A Pilot Study. Revista Electronica De Veterinaria, 01 - 06. Retrieved from https://www.veterinaria.org/index.php/REDVET/article/view/155
28. Panadero R., Fernández M., Vázquez L., López C., Dacal V., Cienfuegos S., and Diez-Banos, P. (2007a). Occurrence and larval growth of Hypoderma lineatum in the oesophagi of cattle from northwest Spain: influence of geographical and climatic conditions. Medical and veterinary entomology, 21(3), 225-230.
29. Simsek S., Utuk AE., Koroglu E., and Dumanli, N. (2008). Seroprevalence of hypodermosis in cattle in some provinces of Turkey. Research in veterinary science, 84(2), 246-249.

Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

30. Saidani K., Lopez-Sandez C., Diaz-Fernandez P., Cabanelas-Dopazo E., Perez-Creo A., Morrondo-Pelayo, P. and Benakhla, A. (2016). Bovine Hypodermosis in the Maghreb: Sero-epidemiological Study in Algeria by Indirect ELISA. Kafkas Universitesi Veteriner Fakultesi Dergisi, 22(5), 757-763.
31. Boulard, C. (2002). Durably controlling bovine hypodermosis. Veterinary research, 33(5), 455-464.
32. Panadero R., Vázquez L., Colwell DD., López C., Dacal V., Morrondo P., and Díez-Baños, P. (2007b). Evaluation of an antigen capture ELISA for the early diagnosis of Hypoderma lineatum in cattle under field conditions. Veterinary parasitology, 147(3), 297-302.
33. Colwell DD., Panadero-Fontan R., López-Sandez C., Parra-Fernandez F., Paz-Silva A., Sánchez-Andrade R., and Dıez-Baños, P. (2003). Effect of treatment on the dynamics of circulating hypodermin C in cattle naturally infested with Hypoderma lineatum (Diptera: Oestridae). Veterinary parasitology, 113(3), 263-272.
34. Jan S., Lateef M., Abbas F., Maqbool A., Jabbar MA., Razzaq A., and Kakar, E. (2014). Epidemiology and Early Detection of Goat Grub, Przhevalskiana silenus, in Northern Mountainous Region of Balochistan, Pakistan. International Journal of Agriculture and Biology, 16(2).
35. Ahmed H., Khan MR., Panadero R., Lopez C., Asif S., Irfan M., and Mazhar, Q. (2013). 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(6), 1495-1500.
36. Otranto, D. (2001). The immunology of myiasis: parasite survival and host defense strategies. TRENDS in Parasitology, 17(4), 176-182.
37. Vázquez L., Dacal V., López C., Díaz P., Morrondo P., Díez-Baños P., and Panadero, R. (2012). Antigen-specific antibody isotypes, lymphocyte subsets and cytokine profiles in cattle naturally infested by Hypoderma sp. (Diptera: Oestridae). Veterinary parasitology, 184(2), 230-237.
38. Sarker MS., Sen A., Ahaduzzaman M., Sayeed MA., Bupasha ZB., Ferdous J., and Uddin, N. (2016). Occurrence, risk assessment and wound healing approach of myiasis in cattle and goat in Chittagong, Bangladesh. Res. J. Vet. Pract, 4(4), 60-65.
39. Colebrook E., and Wall, R. (2004). Ectoparasites of livestock in Europe and the Mediterranean region. Veterinary parasitology, 120(4), 251-274.
40. Rahman MA., Hossain MA., and Alam, MR. (2009). Clinical evaluation of different treatment regimes for management of myiasis in cattle. Bangladesh Journal of Veterinary Medicine, 7(2), 348-352.