

Hematological and Histopathological Effects of Ivermectin in Treatment of Ovine Dermatophytosis in Diyala Province-Iraq

Ghassan H. Jameel¹, Tareq R. Minnat², Anas A. Humadi³, Ali Ibrahim Ali Al-Ezzy⁴

¹Department of Microbiology, College of Veterinary Medicine, University of Diyala, Iraq

²Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Diyala, Iraq

^{3,4}Department of Pathology, College of Veterinary Medicine, University of Diyala, Iraq

Correspondence: Ali Ibrahim Ali Al-Ezzy³, Department of pathology³, College of Veterinary Medicine, University of Diyala, Iraq

Abstract: This study conducted to determine the hematological and histopathological effect of ivermectin in treatment ovine dermatophytosis in Diyala province. Fifty scraping samples were collected from sheep (ewes, lambs) which showed clinical signs of skin diseases from some farms in different areas of Diyala province. Out of 50 skin scraping of sheep 40 (80.0%) were positive for ringworm. Two species of dermatophyte sp. were isolated *Trichophyton verrucosum* and *Trichophyton mentagrophytes*. The highest incidence of infection was recorded in March (100.0%), February (87.5%) compared with (66.66%) in November and December. Recovery was observed after 13 days of single subcutaneous injection of 200 mg/kg ivermectin. Subcutaneous was administered to sheep started by scales dropping and appearance of the hairs with significantly important ($p < 0.05$) when compared with local application of iodine preparation and recovery was observed after 30 day. The hematological effect of ivermectin show elevation in the eosinophil, monocytes and neutrophils rate when compared with normal values. The histopathological effect of ivermectin show that the affected area of the skin after treatment reflects by presence of perivascular cuffing; appear of keratinization; presence of few amount of fibrous connective tissue; presence of large amount of macrophages & eosinophil and neutrophils. In conclusion, ivermectin can be used successfully and effectively in the treatment of ovine dermatophytosis, minimizing the duration and the cost of treatment in addition to other uses in treatment of mange, skin lice and some endoparasites.

Keywords: Hematology, Histopathology, Ovine dermatophytosis, Ivermectin

1. Introduction

Dermatophytosis (ringworm) in sheep is a zoonotic skin infection of keratinized tissues caused by several types of fungus. The fungus causing ringworm is likely most often transmitted when infected sheep are closely sheared releasing spores attached to wool shafts into the air. Nearby lambs with no wool, little lanolin, and irritated skin are very susceptible. Ringworm can also be spread by contact with animals, clipping, brush, cards, lamb tubes and blankets, fence posts, wire, and the hands of handlers[1]. For each animal species, the dermatophytes involved depend on the host studied and on the geographical and environmental conditions. *Trichophyton verrucosum* and *Trichophyton mentagrophytes* are the usual zoophilic dermatophyte involved in sheep ringworm throughout the temperate regions of the world[2]. The animal age and trauma are important predisposing factors of disease[3]. Sheep ringworm mainly occurs in lambs and is rapidly spread in the herd via infected propagates, example hyphae, and specialized fungal spores named arthrospores.[4]. The disease occurs worldwide and *T. verrucosum* is the almost exclusive etiologic agent [5]. Spores may survive in the environment for 2 to 3 years [6]. Aside from animal involvement, several human outbreaks of *T. mentagrophytes* infection have been reported in the face and the body, so far by direct contact with infected animals or indirect contact with infectious propagates in the environment [7, 8]. It has been reported that animals housed in close proximity to each other for long periods and the

presence of infected debris in buildings considered as the main causes of the infection[9].

One to four weeks after coming into contact with the fungus, sheep will first show signs of disease. The fungus affects both the outer layers of skin and the wool. The fungus spreads outward from the center causing a more or less circular area of wool loss. The ears, head, loin and neck are the most likely areas to be affected[10]. The first sign of ringworm is often a raised area where the wool is clumped and feels stiff. These areas are more easily felt than seen. Once the wool comes out, a circular area of wool loss 2 to 2 inches in diameter is seen[6]. Often, this area is covered by a gray-white scab. Itching is not usually present. Almost all animals with ringworm will recover in 1-4 months, though a spot of darker wool may result. It is sometimes difficult to diagnose club lamb fungus in animals in fleece; therefore, shearing assists in accurate diagnosis [4].

Animals cases of ringworm infection have been successfully treated by different antifungal agents such as azole compounds, iodine preparation as local application, and griseofulvine orally[11]. Commercial vaccines containing an attenuated strain of *T. verrucosum* (LTF-130 or CCM 8165) have been used successfully for the control of bovine dermatophytosis in Europe and Russia [12]

Ivermectin is macrocyclic lactones are products or chemical derivatives of soil microorganisms belonging to the *Streptomyces avermitilis* fungus. The main uses of ivermectin in treatment of intestinal helminthes infections as strongylo-

diasis, onchocerciasis and heart worm, also it is active agents in treatment of ectoparasites like ticks and lice [13]. Limited studies on sheep ringworm have been published in Iraq and the disease is considered to be common in most farms of our country specially newborn therefore the aims of the present study were to determine the prevalence of ovine ringworm, the distribution of *T. verrucosum*, *T. mentagrophytes* as causative agents and using Ivermectin in ovine dermatophytosis treatment and finally to determine reverse transmission to the owners and other animals.

2. Materials and Methods

2.1 Samples Collection and Culture Procedure

After having approval of ethics committee in College of Veterinary medicine, Diyala University, Fifty samples were collected from the sheep that showed clinical signs of skin disease for isolation and identification of the causative agent. These scraping samples were taken from the peripheral or edge of the lesion, then collected into sterile Petri dish and transmitted to the laboratory under aseptic conditions. The specimens are treated with 10% KOH to dissolve tissue material, leaving the alkali-resistant fungi intact, or stained with special fungal stains [14, 15].

The medium was prepared and poured into sterile Petri-dishes for isolation or kept in slant screw capped bottles (universals) for maintaining the isolates. Some of prepared Petri-dishes had been taken and by sterilized disposable syringe; ten, twenty and forty ml of 0.2 mg/100 ml ivermectin solution were pulled and added to culture medium to obtain three concentrations 0.001%, 0.02% and 0.04% and serves in refrigerator till use.

Each sample was inoculated directly on two Sabouraud dextrose agar (SDA) (PH 6.9) with the addition of 0.05g/liter chloramphenicol and 0.4g/liter cycloheximide which incubated in the incubator at 37°C to assist growth of moulds for (1-4) weeks before discarding to ensure the appearance of slow growing dermatophytes, with intermittent observation of the fungal growth and when the growth appeared and completed the identification test was done. After colony identification, and to demonstrate the effect of ivermectin *in vitro*; small parts from one of the causative fungi colony (*T. mentagrophytes*) under aseptic condition were taken and cultured in the center of the media mixed ivermectin and incubated in the incubator at 37°C for (1-4) weeks.

2.2 Skin Biopsy

Under local anesthesia, a small plug of skin called a punch biopsy is removed from affected area before treatment and after recovery then fixed in (10%) formalin for histopathology.

2.3 Identification of Fungi

In this test the colonies morphology, shape, color, consistency, texture and reverse plate color and other apparent characteristics were examined. One drop of lactophenol cotton blue stain was put on the slide and then mixed with part of the colony by using sterile forceps, then covered with a cover

slide and examined under 40X lens to determine the shape of mycelium and spores (macro, microspores) and Chlamydospores.

2.3.1 Ivermectin

Is manufactured by Merco Vet. Company\ Belgium and used by two ways; the first is injected to the infected animals subcutaneously and second, added to the culture medium.

2.3.2 Animal groups

Infected animals were divided into two groups:

- Group: (Ivermectin treatment)** 25 sheep (at different age and sex) were treated at a dose 200 micrograms /Kg. B. Wt. by subcutaneous injection; the treatment depends upon the clinical signs. So the common dose was 200 micrograms /Kg. B.W [16] (Bogan and McKellar, 2001). The dose is repeated after 11 days according to the information which had been taken from the manufacture corporation if need.
- Group: (Iodine treatment)** 25 sheep (at different age and sex) were treated by 2% iodine ointment by local application once daily carefully conducted using gloves, because of a possible infestation of the worker [17].

2.3.3 Collection of blood sample

After disinfected area of jugular vein, five milliliters of blood was collected before and after of ivermectin treatment from group (a) animals and they are reserved in EDTA tube to determine the hematological changes of blood parameter.

2.4 Statistical Analysis

The differences are compared by using (F-Test) at $p < 0.05$ and Chi-Square (version 12.) [18]

3. Results

Fifty skin scrapings of sheep 40 (80.0%) were positive for ringworm. The highest percentage of infection was seen in March (100.0%), February (87.5%). While the lowest percentage seen in November and December (66.66%) as in table (1).

The present study shows high percentage of infection recorded in young sheep (lambs) than adult sheep (ewes) with significant at ($P < 0.05$) as in table (2). Recovery was observed after 13 days from single injection of ivermectin at a dose of 200 mg/kg. S/C. was administered to sheep started by scales dropping and appearance of the hairs with significantly important ($p < 0.05$) when compared with iodine preparation administered locally so the recovery was observed after 30 days, as in figure (1). The clearance of lesion period differed between two groups of treatment with significant difference $P < 0.05$ as in table (3). The hematological changes show decrease in Hb, PCV value and significant difference ($P < 0.05$) in differential white blood cells as in table (4). Two species of dermatophyte *sp.* were isolated *Trichophyton verrucosum* and *Trichophyton mentagrophytes*, as in figure (2). The result of inhibitor test shows significant difference between the concentrations of ivermectin on *T. mentagrophytes* growth *in vitro* as in table (5) and figure (3).

Histopathological effect of ovine dermatophytosis show that the affected area of the skin before treatment by ivermectin which reflects three tissue changes, the first is hyper keratinization of the epidermis, the second is the presence of few inflammatory cells resemble by neutrophils and monocytes and the third is few fibrous connective tissue. Whereas, the affected area of the skin after treatment by ivermectin which reflects four tissue changes; the first is incomplete healing of the epidermis; the second is the presence of high number of inflammatory cells resemble by eosinophils, monocytes and neutrophils; the third is too much fibrous connective tissue and prevascular cuffing cells as in figure (4 a-b).

Table 1: Frequency distribution of ovine dermatophytosis according to months of study

Months	No. of Examined samples	No.(%) of Positive samples
November 2013	6	4(66.66%)
December 2014	6	4(66.66%)
January 2014	8	6(75%)
February 2014	8	7(87.5%)
March 2014	8	8(100%)
April 2014	8	6(75%)
May 2014	6	5(83.33%)
Total	50	40(80%)

$X^2 = 3.906$; $P < 0.05$, N.S.D

$X^2 =$ Chi-Square, NSD = No Significant difference

Table 2: Frequency distribution of sheep with ovine dermatophytosis lesions according to age

Age	No. of Examined samples	No.(%) of Positive samples
2-12 month	30	27 (90%)
2-5 years	20	13 (65%)
Total	50	40 (80%)

$X^2 = 4.688$, $P < 0.05$, S.D.

$X^2 =$ Chi-Square, SD = Significant difference

Table 3: Type of treatment and the time of clearance of ovine dermatophytosis lesions

Animal groups	treatment Dosage	Period of clearance
Ivermectin treatment	1 % Ivermectin	13-24 Days*
Iodine treatment	2 % Iodine ointment	30-40 Days

Table 4: Heamatological change of sheep with ovine dermatophytosis treated by ivermectin

The parameters	Normal range	Before treatment M± S.D	After 11 days of treatment M± S.D
Pocked cell volume (PCV) (%)	24-45	23 ± 2.68	22 ± 1.26
Hemoglobin (Hb) (g/dL)	8-15	7.6± 0.087	7.4± 0.196
Platelets (Per/ μ L)	250000 - 750000	795000 ± 1.34	865000± 1.46
Red blood cell (RBC) ($\times 10^6 / \mu$ L)	9.0-15.0	8,755,250± 1.22	7,883,350± 1.32
Mean corpuscular volume (MCV) (fl)	28-40	26± 1.41	27±0.632
Mean corpuscular hemoglobin (MCH) (Pg)	8.0-12.0	8 ± 1.264	9 ± 1.032
Mean corpuscular hemoglobin concentration (MCHC) (g/L)	31.0-34.0	33± 0.632	33.6 ± 0.752
White blood cell count (WBC) (Per/ μ L)	4000-12.000	5125± 0.418	5.138 ± 0.422
Neutrophils %	20-42	43± 0.894	45 ± 1.560
Lymphocytes%	40-75	39± 1.366	24 ± 0.830*
Monocytes %	0-6	8± 1.264	10 ± 0.152*
Eosinophils %	0-10	9± 0.632	20 ± 1.092*
Basophils %	0-3	1 ± 0.582	1± 0.582

M= Mean, SD= Stander division, Significant difference ($P < 0.05$)

Table 5: Evaluation of different concentrations of ivermectin in *T. mentagrophytes* as growth in vitro

No.	Concentrations	T- test value (1,2)	T- test value (1,3)	T- test value (2,3)
1	0.001%	26.77*	87.42*	40.0*
2	0.02%			
3	0.04%			

*significant difference at ($P < 0.05$)



Figure 1: Reveals the efficacy of ivermectin in treatment of ovine dermatophytosis and show the clearance of the lesions in the lambs with hair regrown. A: before treatment, B: After treatment



Figure 2: A: The shape of completely grow colony of *T. verrucosum* fungi on (SDA) at 37C°. B: The shape of completely grow colony of *T. mentagrophytes* fungi in (SDA) at 37C°



Figure 3: Shows the inhibitory effect of three concentrations of ivermectin on *T. mentagrophytes* as growth *in vitro*.
Plate No.1, the concentration mean = 0.001%
Plate No.2, the concentration mean= 0.02%
Plate No.4, the concentration mean= 0.04%.

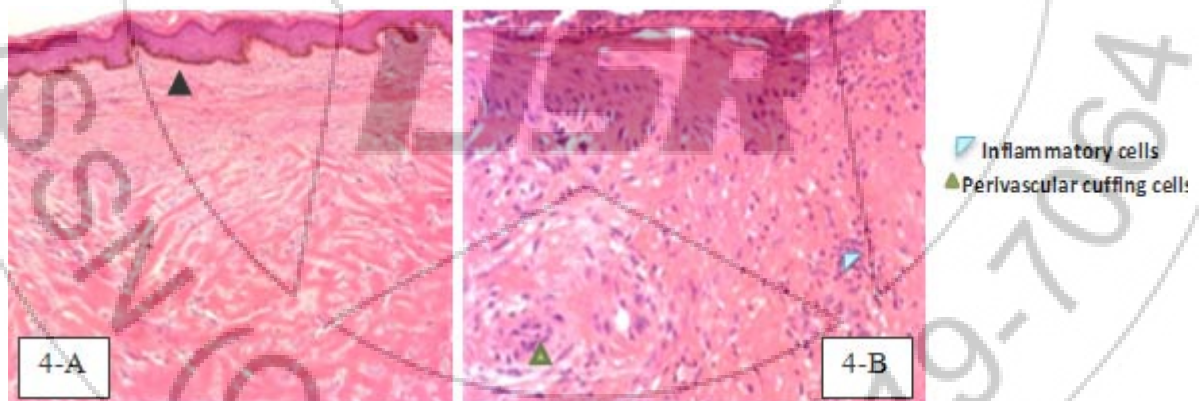


Figure 4(a): Revealed the histopathological section of affected area of the skin before treatment by ivermectin. (20 X).
Figure 4(b): Revealed the histopathological section of affected area of the skin after treatment by ivermectin.(40X)

4. Discussion

The high percentage of ovine dermatophytosis 80% may indicate endemic nature, environmental conditions, overcrowding and continuous exposure to the causative agent which agree with opinion of others [19, 20]. Higher percentage of infection in young sheep than adult sheep may attributed to animals age, sucking of lamb from infected ewes. This opinion comes in concordance with [3], they reported the age and trauma are important predisposing factors of disease and the infection mainly occurs in lambs and is rapidly spread in the herd via infected propagates.

After culture and examination, *T. verrucosum* represent the majority of isolates characterized by chlamydo spores in chain; rare macroconidia. *T. mentagrophytes* represents the minor isolates that reveals the presence of chlamydo spores, microconidia and macroconidia. These results make us to say, the *T. verrucosum* is the usual cause of ringworm in sheep that agree with [21] who proved that *T. verrucosum* isolated from 70% of ram lambs. *T. mentagrophytes* and *T. verrucosum* equally cause ringworm infection of sheep [19].

In the present study we used the injectable ivermectin as biological source of treatment more benefit and less side

effect, and better than chemical drugs which are used locally. [22] elicits the side effects of some topical antifungal drugs as amphotericin B and ketoconazole and miconazole which cause anorexia, vomiting, seizures, and cardiac arrest, which have been reported with the use of amphotericin B. Ketoconazole may produce hepatotoxicity. Miconazole may produce tachycardia, arrhythmias, fever, nausea, and thrombophlebitis after intravenous administration. [13] were ensures that mammals can ingest ivermectin with a high degree of safety.

Significant difference ($p < 0.05$) was recorded in duration of treatment (13-24 days) with ivermectin compared with topical antifungal such as iodine compound which needs more time for clearance between 30-40 days and needs high cost [17]

The hematological changes of ivermectin in blood parameter show significant elevation ($P < 0.05$) in the rate of eosinophils and monocytes in addition to activation of the neutrophils after treatment by ivermectin which may attributed to the effect of ivermectin on stimulation of cellular immune response of the animal. This explanation come in concordance with [23]. They recorded these cytotoxic materials which released by stimulated eosinophils may be cause damage in the cell membrane of the fungus and facilitate the drug entrance inside the target cells with its cytotoxic effect [24], mentioned that eosinophil granules cationic proteins induce tissue damage and dysfunction. [25] had been proved the ability of dendritic cells to phagocytose or engulf the two proliferative forms of *Aspergillus fumigatus* the conidia and hyphae.

This study agree with [26] which refer to immunopotentiating effect of ivermectin on treated animals when the dose was duplicated because, the potent cause which interfere with disease cure is the good immunity. Other studies were refers to the role of cellular branch of immune system in protective immunity against dermatophyte infections [27, 28]. The idea is supported by other study which reflect the ability of the ivermectin at a dose 300 mg /Kg. B.W to enhance antibody production and lymphocytes number results in treatment the early formed bovine warts not in advance stages [29]. This result didn't has any side effects on the animals health along the period of treatment because the ivermectin is nationally used in treatment of the endo and ectoparasites in ruminants. This study was agree with [15] which proved the ability of ivermectin in treatment of ringworm infection in cattle. This study disagree with [30] mentioned that ivermectin have no antibacterial or antifungal activities although it is structurally similar to macrolide antibiotics and antifungal macrocyclic polyenes. This structural similarity of macrolides may leads to this effect on the causative agent. Our study was supported by *in vitro* experiment that revealed the effect of inoculated ivermectin to *Trichophyton mentagrophytes* fungus culture. These findings proved that ivermectin could be used successfully and effectively in the treatment of ovine dermatophytosis, minimizing the duration and the cost of treatment in addition to other uses in treatment of mange, skin lice and some endoparasites.

References

- [1] Singh G, Kumar P, Joshi SC: Treatment of dermatophytosis by a new antifungal agent apigenin™. Mycoses 2014.
- [2] Quinn PJ, Markey BK, Leonard FC, Fitz-Patrick ES, Fanning S, Hartigan PJ: Dermatophytosis in Cattle. Veterinary Microbiology and Microbial Disease. , second edition edn; 2011.
- [3] Oborilova E, Rybnikar A: Experimental dermatophytosis in calves caused by *Trichophyton verrucosum* culture. . Mycoses 2005(48):187-191.
- [4] Al-Saidy HAM, Jameel GH, Sultan AA: Evaluation of *Calvatia Craniformis* Mushroom Activity in Treatment of Ringworm Disease Ion Cattle. Journal of Biology, Agriculture and Healthcare 2014, 4(19):1-5.
- [5] Anupama A: Isolation, Identification And Antifungal Susceptibility Of Dermatophytes From Clinical Samplesâ€œOne Year Study. KLE University, Belgaum, Karnataka; 2014.
- [6] Brioudes AI, Warner J, Hedlefs R, Gummow B: A review of domestic animal diseases within the Pacific Islands region. Acta tropica 2014, 132:23-38.
- [7] Taylor C, Gurr S: Fungal pathogenesis: Past, present and future. Fungal Biology Reviews 2014, 28(1):24-28.
- [8] Agnetti F, Righi C, Scoccia E, Felici A, Crotti S, Moretta I, Moretti A, Maresca C, Troiani L, Papini M: *Trichophyton verrucosum* infection in cattle farms of Umbria (Central Italy) and transmission to humans. Mycoses 2014.
- [9] Akbarmehr J: The prevalence of cattle ringworm in native dairy farms of Sarab city (East Azarbayjan province), Iran. African Journal of Microbiology Research 2011, 5(11):1268-1271.
- [10] EL-DIASTY EM, AHMED M, OKASHA N, MANSOUR SF, EL-DEK SI, EL-KHALEK HMA, YOUSSEF MH: ANTIFUNGAL ACTIVITY OF ZINC OXIDE NANOPARTICLES AGAINST DERMATOPHYTIC LESIONS OF CATTLE. Rom J Biopsy 2013, 23(3):191-202.
- [11] Ghodasara S, Murthy K, Gajbhiye P, Savalia K, Ahlawat A: Comparative Evaluation of Chemotherapeutics for Ringworm Management in Gir calves. Intas Polivet 2013, 14(2):336-339.
- [12] Quinn PJ, Markey BK, Leonard FC, Hartigan P, Fanning Sa, FitzPatrick E: Veterinary microbiology and microbial disease: John Wiley & Sons; 2013.
- [13] Omura S: Ivermectin: 25 years and still going strong. International journal of antimicrobial agents 2008, 31(2):91-98.
- [14] Cheesbrough M: District laboratory practice in tropical countries: Cambridge university press; 2006.
- [15] KIRMIZIGÜL AH, GÖKÇE E, ŞAHİN M, Kiziltepe Ş, BÜYÜK F, ERKILIÇ EE: Clinical Effectiveness of Ivermectin on Bovine Dermatophytosis. Kafkas Univ Vet Fak Derg 2012, 18(3):523-526.
- [16] Bogan JA, Mckellar QA: The pharmacodynamics of Ivermectin in sheep and cattle . Br J Pharmacol 2001, 132(6):1247 - 1254.
- [17] Radostits GM, Gay CC, Blood DC, Hinchcliff KW: Veterinary medicine. A textbook of the disease of cat-

- tle, sheep, pigs, goat and horses., ninth edition edn; 2000.
- [18] J.H. Z: Biostatistical analysis, second edition edn: prentice Hall Inc., Englewood, N.J.s. ; 1984.
- [19] Gupta AK, Cooper EA: Update in antifungal therapy dermatophytosis. *Mycopathologia* 2008, 166(5): 353-367.
- [20] Webber R: Communicable disease epidemiology and control: A global perspective: Cabi; 2009.
- [21] Sargison N: ringworm in sheep. In.; 2014.
- [22] Wanamaker BP, Massey KL: Polyene Antifungal Agents. *Applied Pharmacology for the Veterinary Technician*, third edition edn: Saunders.; 2004.
- [23] Gleich G, Adolphson C: The eosinophilic leukocyte: structure and function. *Adv Immunol* 1986, 39:177-253. .
- [24] Young J, Peterson C, Venge P., Cohn Z . Mechanism of membrane damage mediated by human eosinophil cationic protein. *Nature* 1986, 321 (6070): 613-616. .
- [25] Bozza S, Gaziano R, Sperca A, Bscei A, Montagnoli C, Francesco P, Roman L: Dendritic cells transport conidia and hyphae of *Aspergillus fumigatus* from the airways to the draining lymph nodes and initiate disparate Th response to the fungus. . *J Immunol* 2002, 168:1362 -1371.
- [26] Sajid MS, Iqbal Z, Muhammad G, Sandhu MA, Khan MN, Saqib M, Iqbal MU: Effect of ivermectin on the cellular and humoral immune responses of rabbits. *Life sciences* 2007, 80(21):1966-1970.
- [27] Kaaman T: Current Topics in Medical Mycology., vol. 1. New York: Springer-Verlag.; 1985.
- [28] Sohnle PG. In: *Fungal infections and Immune Response*. New York, Plenum; 1993: 27 -47.
- [29] Ghassan H J, Saba J J, Thekra A E, Wasan A M: Study of Ivermectin effect on the bovine warts Diyala journal for pure sciences 2011, 7 (2):117-126.
- [30] Taylor HR, Greene BM: The status of ivermectin in the treatment of human onchocerciasis. *Am J Trop Med Hyg* 1989, 41:460-466.

Author Profile

Ghassan H. Jameel, Assistant Professor, Department of Microbiology, College of Veterinary Medicine,, University of Diyala, Iraq.

Tareq R. Minnat, Assistant Lecturer, Department of internal and preventive medicine, College of Veterinary Medicine, University of Diyala, Iraq.

Anas A. Humadi, Assistant Lecturer, Department of pathology, College of Veterinary Medicine University of Diyala, Iraq.

Ali Ibrahim Ali Al-Ezzy, Assistant Professor, Department of pathology, College of Veterinary Medicine, University of Diyala, Iraq.