

Effect of Ivermectin on Pulmonary Aspergillosis in Racing Pigeons
Rafah Oday Hussain

Effect of Ivermectin on Pulmonary Aspergillosis in Racing Pigeons

Rafah Oday Hussain
Baqubah Technical Institute
M.Sc. Microbiology

Received 10 November 2014 ; Accepted 17 December 2014

Abstract

Aspergillosis is considered one of the most common, causes of respiratory disease in pet birds. It is caused by infection with a fungus of the genus *Aspergillus*. The species of this organism that most frequently causes respiratory disease in pet birds is *Aspergillus fumigatus*. Two aqueous solutions contain 0.04% and 0.08% ivermectin which supplied commercially by VAPCO company for pharmaceutical production are used in treatment of forty birds infected by aspergillosis orally.

Results reveals that presence of significant correlation ($p < 0.05$) in time of clearance of the lesion between the treated group by 0.08% concentration and control group which is treated by 1% clotrimazole were be 8-10 days and more 45 days respectively. Also there is significant elevation in monocyte rates result in immunepotentiation for all treated group by ivermectin share in killing the causative agents.

The rate of cure was 100% in all treated birds which were treated by ivermectin but in different periods.

Keywords: Ivermectin, pulmonary Aspergillosis, Pigeons

تأثير الأيفرمكتين على داء الرشاشيات الرئوي في الحمام الزاجل

رفاه عدي حسين

المعهد التقني / بعقوبة

الخلاصة

يعتبر الأسبرجيلوسس واحداً من أكثر الأسباب لأمراض الجهاز التنفسي في الطيور الأليفة سببها إصابة فطرية بجنس *Aspergillus* وأن المسبب الأكثر تكراراً لالتهابات الجهاز التنفسي هو نوع *Aspergillus fumigatus*. يعتبر داء الرشاشيات الأكثر شيوعاً، ويسبب أمراض الجهاز التنفسي الشائعة في الطيور الأليفة. وهو ناتج عن الإصابة بنوع من

Effect of Ivermectin on Pulmonary Aspergillosis in Racing Pigeons Rafah Oday Hussain

الفطريات من جنس *Aspergillus*. الأنواع من هذه الكائنات التي تسبب أمراض الجهاز التنفسي الأكثر شيوعاً في الطيور والحيوانات الأليفة هي *Aspergillus fumigatus* تم استخدام اثنين من المحاليل المائية تحتوي على 0.04% و 0.08% الإيفرميكتين التي زودت تجارياً من قبل شركة VAPCO لإنتاج الأدوية المستخدمة في علاج أربعين طيراً مصابة بالرشاشيات فموياً. أظهرت النتائج وجود ارتباط معنوي ($p < 0.05$) في زمن التخلص من الآفة بين المجموعة التي تلقت العلاج بواسطة تركيز 0.08% والمجموعة الضابطة التي تعامل بنسبة 1% كلوتريمازول كان 8-10 أيام وأكثر من 45 يوماً على التوالي. كما تبين انه هناك ارتفاع كبير في معدلات *monocytes* يؤدي إلى *immunepotiation* لكافة المجموعة التي عولجت بواسطة الإيفرميكتين في قتل العامل المسبب. وكانت نسب شفاء الطيور المعاملة بالإيفرميكتين 100% في جميع الطيور وعلى فترات مختلفة.

الكلمات المفتاحية : الإيفرميكتين ، داء الرشاشيات الرئوي ، الحمام الزاجل

Introduction

Aspergillosis is a respiratory disease of birds caused by *Aspergillus* spp., which is found almost everywhere in the environment. *Aspergillus. fumigatus* is the most common species of the fungus causes disease, although *Aspergillus flavus*, *Aspergillus niger*, and others can also cause problems. *Aspergillus fumigatus* is a ubiquitous saprophytic mold that forms conidia (Latge, 1999). Ongoing studies suggest that *A. fumigatus* conidia may be able to resist killing by the avian respiratory macrophage (Van Waeyenberghe *et al.*, 2012).

Aspergillus grows readily in warm and moist environments. The microscopic spores of the fungus become airborne, and poor ventilation, poor sanitation, dusty conditions, and close confinement increase the chance the spores will be inhaled. Usually, the fungus does not cause disease, however, if a bird does not have a healthy immune system, it can cause illness. Predisposing factors include other illnesses, stress, poor nutrition, poor husbandry or unsanitary conditions, another injury to the respiratory system, and prolonged use of certain medications such as antibiotics or corticosteroids (Rupley, 1997).

Aspergillosis is mostly caused by *Aspergillus fumigatus* but *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus glaucus*, *Aspergillus nidulans*, and other *Aspergillus* species or mixed infections can play a role in the disease (Joseph, 2000). The reason why *A. fumigatus* is the predominant species of airborne fungal infections might be that the spores are much smaller than the spores of other *Aspergillus* species (Richard and Thurston, 1983; Alias *et al.*, 2009).

Effect of Ivermectin on Pulmonary Aspergillosis in Racing Pigeons
Rafah Oday Hussain

The fungus grows quite readily on ordinary laboratory culture media at room temperature, at 37C⁰, and higher. Czapek's agar or Sabouraud's agar are used. The colonies are green to bluish green at first and darken with age so as to appear almost black. The colonies vary from velvety to floccose. The conidiophores are short, up to 300μ long by 2-8μ in diameter, the vesicles are apical flask-shaped up to 20-30μ in diameter, the sterigmata are 6-8 by 2-3μ in diameter, in chains forming solid columns up to 400 by 50μ (Thom and Church, 1926). Inhalation is considered the main infection route for *A. fumigatus* in birds (Oglesbee, 1997), and because *A. fumigates* spores are too small to be trapped completely in the nasal cavity or trachea, some are able to reach the lungs and air sacs (Fedde, 1998). The air sacs are usually the primary infection sites, since inhaled air reaches the posterior thoracic and abdominal air sacs prior to contacting epithelial surfaces in the lungs (Nardoni *et al.*, 2006). In the lung parenchyma, spores get embedded in the atria and parts of the infundibula in the parabronchus and are engulfed by (surface) phagocytic epithelial cells (Maina, 2002). When there are too many spores or the bird has an impaired immune response, the innate defence mechanisms do not succeed in eliminating infection at the site of the air capillaries. This may lead to the development of loosely attached plaques, which may or may not become overgrown by connective tissue of the host. These plaques or necrotic debris in the respiratory tract can obstruct the trachea or bronchi and/or fill up the air sacs (Oglesbee, 1997). Occasionally, sporulation occurs in the lungs and air sacs (Nardoni *et al.*, 2006; Cacciuttolo *et al.*, 2009). Hyphae containing fruiting bodies can fill the lumen and may penetrate the air sacs, causing serositis and superficial necrosis in the adjacent organs (Tsai *et al.*, 1997). Besides direct extension of the infection through the air sac wall, disseminated mycosis also occurs by haematogenous spread. Hyphae, which are known to be tissue and angio-invasive (Dahlhausen *et al.*, 2004), as well as host cells play a role in this spreading mechanism. Macrophages in the respiratory tract ingest spores and find their way through the interstitium into the blood and lymphatic stream and thus to other organs (Richard and Thurston, 1983). Aspergillosis can follow one of two courses acute or chronic. Birds with acute aspergillosis have severe difficulty breathing, decreased or loss of appetite, frequent drinking and urination, cyanosis (a bluish coloration of mucous membranes and/or skin), and even sudden death. The fungus generally affects the trachea, syrinx (voice box), and air sacs Leenders and

Effect of Ivermectin on Pulmonary Aspergillosis in Racing Pigeons
Rafah Oday Hussain

Belkum , 1999. The lungs may also be involved. Diagnosis is generally made through a post-mortem examination. Chronic aspergillosis is much more common, and unfortunately, much more deadly due to its insidious nature. The bird may not become symptomatic until the disease has progressed too far for a cure. The respiratory system is the primary location of infection. White nodules appear and ultimately erode through the tissue, and large numbers of spores enter the bloodstream. The spores then travel throughout the body, infecting multiple organs including kidneys, skin, muscle, gastrointestinal tract, liver, eyes, and brain(Kearns,2003).Antifungal drugs such as itraconazole and amphotericin B may be administered orally, topically, by injection, or nebulizing, depending upon the drug. There are several reports that itraconazole may be more toxic to African grey parrots, when compared to other species. Therapy needs to be continued for weeks to months and more than one antifungal drug may be used. Supportive care such as oxygen, supplemental heat, tube feeding, and treatment of underlying conditions are often needed. Unfortunately, the prognosis is always guarded(Walsh and Stevens ,2011).

This study was carried out to study the effect of Ivermectin.

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 strongyloidiasis , onchocerciasis and heart worm ,also it is active agents in treatment of ectoparasites like ticks and lice (Yates and Wolstenholme , 2004; Omura and Crump,2004).

Materials and Methods...

1. Sample Collection and Culturing:

Sixty swabs were collected from the birds which showed clinical signs of respiratory disease from the throat of lived birds and from the lung and air sacs from killed birds for isolation and identification of the causative agent. These swabs transmitted to the laboratory under aseptic conditions (Cheesbrough , 1992).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

Effect of Ivermectin on Pulmonary Aspergillosis in Racing Pigeons
Rafah Oday Hussain

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 Sabouraud Dextrose Agar media 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. Sabouraud agar was developed to support the studies of dermatophytes, which require long incubation periods (weeks) (Odds, 1991). After colony identification, and to demonstrate the effect of ivermectin *in vitro*; small parts from one of the causative fungi colony (*A. fumigatus*) under aseptic condition were taken and cultured in the center of the media mixed ivermectin and incubated in the incubator at 25±2°C for (1-4) weeks.

2- solutions:

0.02% ivermectin orally.

0.04% ivermectin orally.

3- Sick birds:

This study was conducted in special veterinary clinic in Hibhib city during period between April 2013 to April 2014. Sixty sick birds were examined (40 male and 20 females), with age range from (1-3) years, full history was taken to regard the duration of the disease, proven treatment and ensured that every bird had stopped any treatment at least one month before starting the present therapy. The sick birds were divided into 3 groups:-

Group A :- include 20 birds represent the treatment group, they advised to use 0.02% ivermectin solution (0.25 ml to each bird) orally once daily for 1 month.

Group B :- include 20 birds represent the treatment group, they advised to use 0.04% ivermectin solution (0.5 ml to each bird) orally once daily for 1 month.

Group C :- represent the control group which includes 20 birds, were advised to use the control treatment 1% Itraconazole (0.2 ml to each bird) daily for 1 month (Wanamaker and Massey, 2004; Wikimedia, 2012).

One drop of blood have been taken from each bird in all groups and blood smear was done to estimate the differential white blood cell count (Seybold *et al.*, 1980).

Statistical Analysis:

Effect of Ivermectin on Pulmonary Aspergillosis in Racing Pigeons
Rafah Oday Hussain

The differences are compared by using (F-Test) at $p < 0.05$ (Zar,1984).

Results

Most of the isolates were given positive result for *Aspergillus fumigatus* and some of their were negative and the figure (1) was revealed the shape of the isolated colonies of on Sabouraud Dextrose Agar media which is characterized by usual fungus spreading , velvety appearance , dark green to little black color.



Figure-1: Colonies on Sabouraud Dextrose Agar media are usually spreading, velvety, dark green to bluish green at first and darken with age so as to appear almost black.

The Table (1) is revealed the effect of our treatment with different doses in group A and B also shown the completed clearance of the clinical signs in a period about 15 days and 8 days respectively while, the clinical signs were decreased but doesn't disappeared in control group.

Table(1): revealed the number of sick birds and the dose of drugs and time of recovery.

Birds group	The dose to each sick bird daily	Period of clearance
20 Birds (treated)	0.25 ml of ivermectin	15-20 Days*
20 Birds (treated)	0.5 ml of ivermectin	8 -10Days*
20 Birds(control group)	0.2ml 1% Itraconazole (Sporanox®)	No clearance of signs till 15 Days($p < 0.05$) *

($p < 0.05$)*

Effect of Ivermectin on Pulmonary Aspergillosis in Racing Pigeons
Rafah Oday Hussain

Table(2): Type and differential count of WBC in different groups of birds.

Differential count of WBC	Treatment group by 0.25 ml of ivermectin	Treatment group by 0.5 ml of ivermectin	Control group by 0.2 ml of 1% Itraconazole (Sporanox®)
Hetrophils	56%	59%	41%
Lymphocytes	27%	20%	49%
Monocytes	14%	17%	7%
Eosinophils	2%	3%	2%
Basophils	1%	1%	1%

Figure (2): Shows the effect of three concentration of Ivermectin (1) was mean the concentration 0.001% and number (2) means the concentration 0.02% and number (4) means the concentration 0.04%.

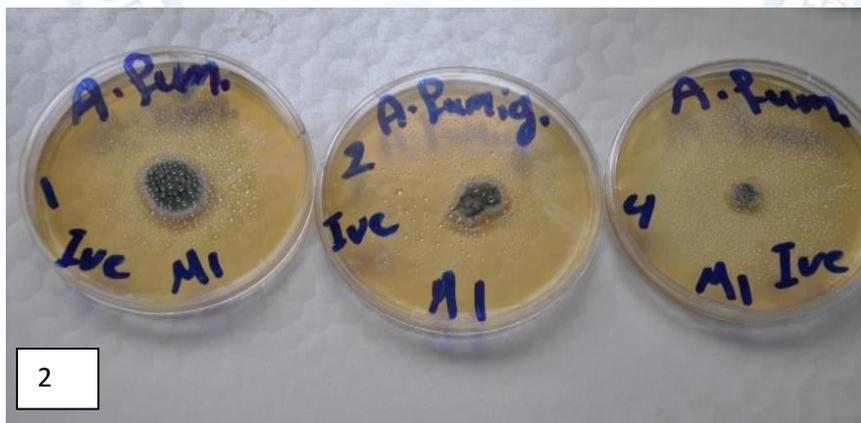


Figure-2: Reveals the inhibitor effect of ivermectin on *Aspergillus fumigatus* growth *Invitro*. The petri dish which carry number (1) was mean the concentration 0.001% and number (2) means the concentration 0.02% and number (4) means the concentration 0.04%

Effect of Ivermectin on Pulmonary Aspergillosis in Racing Pigeons
Rafah Oday Hussain

Discussion

Pulmonary aspergillosis is an invasive type that is a serious infection with pneumonia that can spread to other parts of the body and *A. fumigatus* is the most common the causative agent (Bozkurt *et al.*, 2008). This infection almost always occurs in people with a weakened immune system due to cancer, Acquired Immunodeficiency Syndrome (AIDS), leukemia, an organ transplant, chemotherapy, or other conditions or medications that lower the number or function of normal white blood cells or weaken the immune system.

The color of the growing colonies of the causative agent *Aspergillus fumigatus* is black. The black color of the isolated fungus due to the advanced age of the colonies (Thom and Church, 1926).

The differences between clearance time of the treatment groups and control group was statistically significant ($p < 0.05$) also, there is significant difference in monocytes rate in treated group by 0.5ml of ivermectin when compared with other groups so it reaches to (17%) and the normal value was (0-10%). No significant difference was observed in the other white blood cells.

Most of the medications previously used in treatment of fungal infections includes azol compounds; no details were seen about using of ivermectin in treatment of this disease else previous study was done by Kirmizigulet *et al.* (2012) which proved the ability of ivermectin in treatment of ringworm disease in cattle which caused by *Trichophyton verrucosum* fungus.

The effect of ivermectin maybe due to its toxic effect on the cell membrane of the fungus which was similar to the effect of imidazole derivatives in inhibiting the biosynthesis of ergosterol, the main sterol in membranes of fungi (Foiani *et al.*, 1994). These agents also affect the synthesis of triglycerides and phospholipids. Changes in oxidative and peroxidative enzyme activities, leading to an intracellular buildup of toxic concentrations of hydrogen peroxide, may contribute to the observed deterioration of subcellular organelles and to cell necrosis. The imidazole derivatives inhibit the transformation of blastospores of *Candida albicans* into the invasive mycelial form. This inhibition probably facilitates the task of host defense cells and may be the principal factor leading to clearance of infection (Borgers, 1980).

Effect of Ivermectin on Pulmonary Aspergillosis in Racing Pigeons
Rafah Oday Hussain

The second effect of ivermectin is maybe due to the immunopotentiating effect on the leukocytes especially the monocytes. Monocytes are a granulocytes enter the blood from the bone marrow and circulate for about 72 hours. Then enter the tissues and become tissue macrophages (Ganong,2005). Monocytes and their macrophage and dendritic-cell progeny serve three main functions in the immune system. These are phagocytic, antigen presentation, and cytokine production includes Tumor Necrosis Factor (TNF), Interleukin-1 and Interleukin-2. Monocytes can perform phagocytosis using intermediary (opsonising) proteins such as antibodies or complement that coat the pathogen, as well as by binding to the microbe directly via pattern-recognition receptors that recognize pathogens (Swirskiet *al.*, 2009). This illustration was supported by Bozza *et al.*, (2002) which had been proved the ability of dendritic cells to phagocytose or engulf the two proliferative forms of *Aspergillus fumigatus* the conidia and hyphae.

This Result was in agreement with the findings of Schvit, (1979) who is refer to the role of the circulating monocytes of the host against fungi.

No significant effect of 1% Itraconazole on the infection because, the clinical signs does not disappeared and, may be needs more time of treatment to give it's goal.

References

1. Alias, S.A., Omar, S. and Azlina, A.W. 2009. Microfungi of Windmill Island, Antarctica: Diversity and ultrastructure studies of Soil Fungi. Malaysian Journal of Science, 28(1): p. 25-32. (SCOPUS-Cited Publication).
2. Borgers M. 1980. Mechanism of action of antifungal drugs, with special reference to the imidazole derivatives. Rev Infect Dis.;2(4):520-34.
3. Bozkurt MK, Ozcelik T, Saydam L, Kutluay L. 2008. [A case of isolated aspergillosis of the maxillary sinus]. Kulak Burun Bogaz Ihtis Derg; 18(1): 53-5.
4. Bozza S., Gaziano R., Sperca A., Bscei A., Montagnoli C., Francesco P. and Roman L. 2002. 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.* 168, 1362-1371. [Cacciuttolo, E.](#), [Rossi, G.](#), Nardoni, S., [Legrottaglie, R.](#) and Mani, P. 2009.
5. Anatomopathological aspects of avian aspergillosis. Veterinary Research Communications, 33: 521–527.

Effect of Ivermectin on Pulmonary Aspergillosis in Racing Pigeons
Rafah Oday Hussain

6. Cheesbrough M., 1992. Medical Laboratory Manual for Tropical Countries. Volume 2. Tropical Health Technology, Butterworth-Heinemann, Great Britain, pp. 371-385.
7. Dahlhausen B., Abbott R. and VanOverloop P.2004. Rapid detection of pathogenic Aspergillus species in avian samples by real-time PCR assay: a preliminary report.In.
8. E.Bergman(Ed.).Proceedings of the 25th Annual Conference and Expo of the Association of Avian Veterinarians 37.New Orleans. LA.USA.
9. Fedde M.R.1998.Relationship of structure and function of the avian respiratory system to disease susceptibility. Poultry Science,77:1130- 1138.
10. Foiani, M.; Marini, F.; Gamba, D.; Luchini, G. and Plevani, P.1994. Mol. Cell Biol.14(2):923-933.
11. Ganong W.F.2005. White Blood Cells. Review of Medical Physiology. (22),ed. Lange. PP: 516-520.
12. Joseph V.2000. Aspergillosis in raptors. Seminars in Avian and Exotic Pet Medicine.9:66-74.
13. Kearns, K.S. 2003. Avian aspergillosis. In Kearns KS; Loudis B (eds.). Recent Advances in Avian Infectious Diseases. International Veterinary Information Service, Ithaca NY.
14. Kirmizigul A.H., Gokce E., Sahin M., Kiziltepe S., Buyuk F. and Erkilic E.E. 2012.Clinical Effectiveness of Ivermectin on Bovine Dermatophytosis. *Kafkas Univ Vet Fak Derg 18(3): 523-526.*
15. Latge J. P. 1999. Aspergillus fumigatus and aspergillosis. Clin. Microbiol. Rev. 12:310-350.
16. Leenders A.C. and Belkum A.V. 1999. Density and Molecular Epidemiology of Aspergillus in Air and relationship to outbreaks of Aspergillus infection. J Clin Microbiol ;1752-7.
17. Maina J.N.2002.Some recent advances on the study and understanding of the functional design of the avian lung: morphological and morphometric perspectives. Biological Reviews of the Cambridge Philosophical Society,77:97-152.
18. Nardoni S., Ceccherelli R.,Rossi G. and Mancianti F. 2006.Aspergillosis in Larus cachinnans micaellis: survey of eight cases. Mycopathologia,161:317-321.
18. Odds F. C. 1991. Sabouraud('s) agar. *J. Med. Vet. Mycol. 29:355–359.*
19. Oglesbee B.L. 1997. Mycotic Diseases. Altman, RB; Clubb, SL; Dorrestein, GM; Quesenberry, K. (eds.). Avian Medicine and Surgery. W.B. Saunders Co. Philadelphia, PA.
20. Omura S. and Crump A.2004. The life and times of ivermectin- a success story. Timeline-Tropical Infectious Diseases. Vol.(2),PP:984-989.

Effect of Ivermectin on Pulmonary Aspergillosis in Racing Pigeons
Rafah Oday Hussain

21. Richard J.L. and Thurston J.R.1983. Rapid hematogenous dissemination of *Aspergillus fumigates* and *A. flavus* spores in turkey poult following aerosol exposure. *Avian Diseases*, 27:1025-1033.
22. Rupley, AE. *Manual of Avian Practice*. W.B. Saunders Co. Philadelphia, PA; 1997.
23. Schvit K.E.1979. Phagocytosis an intracellular killing of pathogenic yeast by human monocytes and neutrophils. *Infect. Immun.* 24(3),932.
24. Seybold I.M. , Goldston R.T. and Wilkes R.T.1980. Laboratory evaluation of the blood smear. *The basic clinical pathology*,75(5); 781-9.
25. Swirski F.K., Nahrendorf M., Etzrodt M., Wildgruber M., Cortez-RetamozoV., Panizzi P., Figueiredo J-L., Kohler R.H., Chudnovskiy A., Waterman P., Aikawa E. , Mempel T.R. Libby P. Weissleder R. and Pittet M.J. 2009. Identification of Splenic Reservoir Monocytes and Their Deployment to Inflammatory Sites. *Science*, 325: 612-616.
26. Thom C. and Church MB. 1926. *The Aspergilli* . Williams & Wilkins Co, Baltimore. Cited by Dr. Tara Nath Gaire: Department of Veterinary Medicine and Public Health Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan, Nepal.
27. Tsai S.S., Park J.H. , Hirai K. and Itakura C.1992. Aspergillosis and candidiasis in psittacine and passeriforme birds with particular reference to nasal lesions. *Avian Pathology*, 21:699-709.
28. Van Waeyenberghe L, Pasmans F, D'Herde K, Ducatelle R, Favoreel H, Li SJ, Haesebrouck F, Martel A. 2012. *Vet. Res.*; 43:32.
29. Walsh TJ.and Stevens DA. 2011. Aspergillosis. In: Goldman L, Schafer AI, eds. *Goldman's Cecil Medicine*. 24th ed. Philadelphia, Pa: Elsevier Saunders; chap 347.
30. Wanamaker B.P. and Massey K.L.2004. *Applied Pharmacology for the Veterinary Technician*. Third Edition.Saunders , Elsevier(USA).
31. Wikimedia. 2012. [Composition comprising Itraconazole for oral administration](#). Fresh Patents.com. 26 October 2006.
32. Yates D.M. , Wolstenholme A.J., 2004 . An Ivermectin – sensitive Glutamate gated chloride channel subunit from *Dicrofilaria immitis* .*Int. J. parasitol* . 43(9) :1075 – 81 .
33. Zar J.H., 1984. *Biostatistical analysis*, 2nd ed., prentice Hall Inc., Englewood, N.J.S.