

Treatment of pigeon (*Columba livia domestica*) infected with *Hadjelia truncata* by ethanolic suspension of *Calvatia craniiformis* in comparison with ivermectin

Ghassan H. Jameel¹, Amer M.A.AL.Amery², Maisaa G. Taher³ and Zahid I. Mohammed⁴

¹Department of Microbiology, ²Department of Parasitology, ⁴Department of Public health, College of Veterinary Medicine, ^{1,4}Diyala University, ²Baghdad University ³Department of Pathology, College of Medicine, Diyala University, Iraq.

E-mail: M.murhum@yahoo.com

Received: 11/10/2015; Accepted: 1/3/2016

Summary

This study aimed at evaluating the efficacy of different concentrations of *C. craniiformis* mushroom suspension in pigeon flock infected by *Hadjelia truncata* worms in Diyala province in comparison with 0.1% ivermectin. Thirty pairs of pigeons were brought to the veterinary clinic with case history of progressive weight loss, weakness, and some birds have died suddenly. In the necropsy of 3 cases the gizzards contained some nematodes, located under the koilin layer of the gizzard, which was identified microscopically as *Hadjelia truncata* which was one of the important parasites of the digestive system of poultry. Three different concentrations of suspension were prepared from *Calvatia craniiformis* mushroom powder (0.25, 0.5 and 1mg/ml) and that tested for antiparasitic effect in comparison with ivermectin as common broad spectrum anthelmintics drug. All the concentrations of the suspension showed active effect against the parasites but, ivermectin showed a better effect.

Keywords: *Hadjelia truncata*, Ivermectin, *Calvatia craniiformis*, Pigeon.

Introduction

Pigeons are seen in more regions of the world. They live side by side with humans and other animal species in the nature and they are bred as a source of food, as a hobby, as a symbol and for experimental aims (1). Their interaction with man and other domestic and wild birds portends it as a potential carrier of zoonotic parasite (2). They have a role in spreading some zoonosis diseases to people as well as being a reservoir of many parasitic diseases for poultry (3). Various parasites significantly impede pigeon growth, development and productivity; they at times lead to death, especially the sqabs.

Hadjelia truncata is a nematode from the family Habronematidae that lives in the gizzard of a number of birds in Europe and Asia; including pigeon (*Columba livia domestica*), and hoopoe (*Upupa epops*). It causes lesions in the gizzard lining of pigeons, which may even lead to death (4). The life cycle of these parasites has not been definitively identified, though it is speculated that an arthropod acts as an intermediate host similar to that of other members of the superfamily *Spiruroidea* (5). The lesser meal worms, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), also known as the darkling beetles or litter beetles are

common cosmopolitan pests in poultry houses (6). Both adults and larvae are scavengers consuming chicken food, feces, dead birds, and sometimes attacking live birds (7). The most common clinical signs are nonspecific and include weight loss, poor feed consumption, poor feathering, diarrhea, and increased mortality. In one of the flocks, the percent of affected birds was approximately 10%. Diagnosis is currently based on the presence of worms under the koilin layer of the gizzard during necropsy (5 and 8).

This study was aimed to evaluate the efficacy of different concentration of *C. craniiformis* mushroom suspension in a pigeon flock infected by *Hadjelia truncata* worms in Diyala province in comparison with 0.1% ivermectin.

Materials and Methods

Thirty pairs of pigeons were selected for evaluation and treatment based on the producer's concern of weight loss, which was determined by pectorals muscle atrophy, in at least 1 bird of two pairs and post mortem findings was done before and revealed the presence of threatened nematodes inserted in the glandular surface of the crop. The identification of the parasite seen in the cage floor carry out with feces after administration

of the medications in less number because, the parasite was not excreted with feces and inserted under lining of the crop and the gizzard till death occurred. The diagnosis was based on its morphological peculiarities. To certify the diagnosis, some worms had been sent to the Iraq Natural History Research Centre and Museum, University of Baghdad, Iraq (9). The birds were divided into two groups, in each group thirty birds were treated with different substance.

Preparation of mushroom suspension: Fruiting body of *Calvatia craniiformis* mushroom was dried and crushed in sterile Petri dish to obtain a yellow – brown powder. Then 10 gm from the powder was dissolved in 10 ml ethanol 70% and mixed perfectly. The concentration become (1000 mg/ 1ml) and considered as stock solution (10). From which (0.1, 0.05, 0.025 ml) were completed by distill water to 10 ml to obtain (1, 0.5, 0.25% of each) concentrations and given to each bird at a dose of 10 ml\bird from each concentration.

Ivermectin 0.1% (1mg\ ml) (w/v) as oral drench was manufactured by veterinary and agricultural products manufacturing company (VAPCO) Jordon (active constituent: 0.1 mg/ ml) with a dose (0.2 mg/ 1 Kg B. wt.). Because of the weight of infected bird ranging from 250 to 400 grams, the dose was determined between 0.25, 0.5 and 0.75 ml (ivermectin)\bird. One mg/ ml was diluted ten times to get concentration of 0.1 mg/ ml in order to be given at a dose of 25, 0.5 and 0.75 ml (ivermectin)\bird .

Thirty pairs of pigeon (*Columba livia domestica*) have been assigned into two groups (15 pairs/ group) and treatment orally as follows: Group T1 divided into three sub group (5 birds/ group) and treatment with ivermectin at dose of 0.25, 0.5 and 0.75ml/ bird, respectively. Group T2 also divided as in group T1 and treatment with alcoholic suspension of mushroom (*Calvatia craniiformis*) at dose of 10, 5 and 2.5 mg/ bird, respectively.

Results and Discussion

The diagnosis and identification of the Parasite depending on the morphology of the parasites which was consistent with key

identification for characteristics *H. truncate* reported previously (11-13). Crop and gizzard (*Hadjelia truncate*) worm isolation and identification were carried out by light microscopy examination after the post mortem findings (Fig. 1). Males and females length measured 6.5-9 mm and 12-16.5 mm, respectively. The body was slender, straight and white in color. The mouth in the cephalic region of male and female was surrounded by two lateral lips that were trilobed and had a cylindrical pharynx (Fig. 2).



Figure, 1: Gross section reveals the presence of *Hadjelia truncate* parasite located under the koilin layer of the gizzard (x10).

Figure, 2: Reveals the anterior end of the male and female of *Hadjelia truncate* parasite (x20).

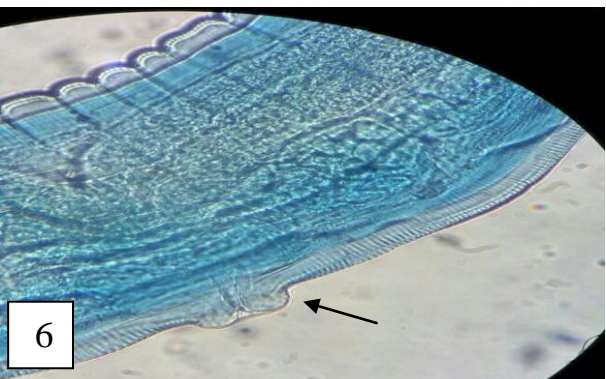
The posterior end of the male was spirally coiled at the region of the caudal alae, and the spicule was situated at 0.05 mm from the posterior end when the excretory pore was present (Fig. 3 and 4). In female, the excretory pore was situated at 0.1 mm from the blunted posterior end (Fig. 5). The vulva was located in the anterior part of the body (Fig.6). The lips of the vulva were protruded above the body surface. The embryonated eggs come out from the vulva (Fig. 7).



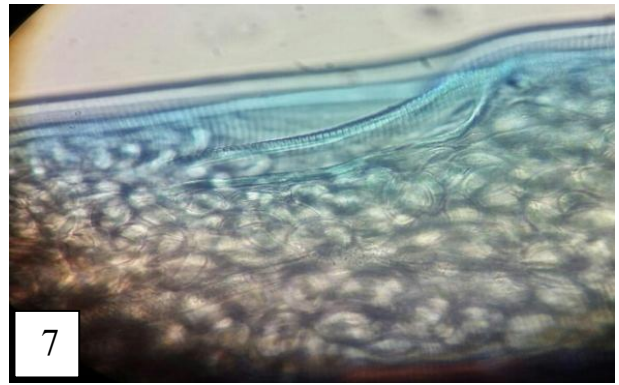
Figure, 3 and 4: Posterior end of the male with appearance of the spicules and the excretory pore respectively (x20).



Figure, 5: Posterior end of the female with appearance of the anal pore ventrally (x10)



Figure, 6: Reveals the lips and orifice of the vulva in the ventral aspect (x40).



Figure, 7: Reveals the aggregated eggs in the oviduct (x40).

The efficacy of treatment with suspension and ivermectin showed that Ivermectin was very effective in treatment of the infection which is certified by our observation in expulsion of some worms from the birds in the cage after 24 hours from administration of drug at a dose volume of 0.5 and 0.75 ml\bird. Mostly the worms were expelled from the crop passing through the inactive gizzard because, the worms in the lining of the gizzard would die from depression which lead to starvation due to the effect of ivermectin followed by improvement of birds health at interval of two weeks (Table, 1).

Ivermectins inhibit the gamma amino butyric acid (GABA) neurotransmission at two or more sites in nematodes (14), blocking inter neuronal stimulation of excitatory motor neurons and thus leading to a flaccid paralysis (8 and 15). Other authors (16) showed that Ivermectin bind with high affinity to glutamate-gated chloride channels which occurred in invertebrate nerve and muscle cells, causing an increase in the permeability of the cell membrane to chloride ions with hyperpolarization of the nerve or muscle cell. Hyperpolarization results in paralysis and death of the parasite either directly or by causing the worms to starve. Whereas (17) revealed that there were little information published on the treatment or antemortem diagnosis of these parasites and they used two effective drugs as ivermectin and levamisol in treatment of the infection and achieved good results. While, (18) pointed that the efficacy of ivermectin in both domestic and wild pigeons was 100%. These results were in close agreement with (19) who found the efficacy of ivermectin to be 95% in pigeons. The findings of present study were also in complete

agreement with those of (20) who reported 100% efficacy of ivermectin in pigeons. Also (21) has been found that 0.1% injectable ivermectin used in treatment of cattle and hog is the best which given orally to the pigeons at 1/10 is about 3 drops.

Table, 1: Reveals the groups of the birds and the type of the medication.

Groups of the birds	No.	Type of the medications	Period of expulsion of the worms	Period of health improvement
Treated Group 1	30	Ivermectin	24 hours	2 weeks
Treated Group 2 Subgroup 1	10	2.5 mg\bird of <i>C.craniiiformis</i> mushroom	After 72 hours from 2 nd treatment	1 month
Subgroup 2	10	5 mg\bird of <i>C.craniiiformis</i> mushroom	5 days	3 weeks
Subgroup 3	10	10 mg\bird of <i>C.craniiiformis</i> mushroom	72 hours	2 weeks

Analysis of the powder of mushroom proved the presence of three components; the first is calvatic acid which has chemical formation P-carboxyphenyl-azoxycarbonitrile (22). The second components from chemical analysis and spectroscopic means of the mushroom powder is hydroxyphenylazoformamide derivatives which has three chemical compounds, 4-hydroxyphenyl-1azoforamid, 4-hydroxyphenyl-ONN-azoforamid and 2-methylsulfonyl-4-hydroxy-6-methylthiophenyl-1-azoforamid, it is known craniformin (phenolic tautomer of rubroflavin). The hydroxyphenyl-1 azoforamid derivatives or craniformin have phenolics in its formation which are endowed with interesting biological activities as abroad spectrum bactericidal and fungicidal effect represented by *Candida albicans*, *Aspergillus niger* (23). Also the chemical analysis of mushroom powder which was done in White Fields Company for Chemical and Engineering Studies and Consultations in Baghdad – Iraq proved the presence of different medicinal materials as gallic acid and others as lectin, statin, L-ergothionine. Gallic acid is a trihydroxy-benzoic acid, a type of phenolic acid (24). The two analysis of the mushroom powder had

been proved presence of phenolics .The mode of action of phenolics is protein coagulation. They destroy selective permeability of cell membranes and leakage of cell constituent's results. They are effective against bacteria, fungi, and some viruses. When repeated may cause accumulation in tissue and eventual toxic effects , such as neurotoxicity (25).This neurotoxicity result in paralysis status in the worms and fell down from the intestinal mucosa to pass out with feces .

The excessive consumption of tannins, which are polyphenolic compounds, has been associated with a reduction of food intake and food digestibility, impairment of rumen metabolism and mucosal toxicity (26-29). This mechanism results in deforming due to intestinal mucosa toxicity. Present results agree with (30) who refer to the condensed tannins have been shown to reduce gastrointestinal parasite loads in goats by reducing worm fertility, eliminating adult worms, and retarding the establishment of incoming larvae. Also (31) showed the role of plant phenolic compounds in controlling parasitic nematodes of small ruminants. *Calvatia craniiiformis* mushroom was an edible species (32). Young puffballs with a firm, white gleba have a mild odor and pleasant taste (33). The doses of mushroom suspension which were used did not show any side effect in treated pigeon indicating its safety. The evaluation of medications efficacy there for it could be say that of alcoholic suspension of mushroom was based on clinical improvement and observation if the worms were expelled out or not.

In conclusion alcoholic suspension of mushroom was safe when used orally at a dose (2.5, 5 and 10 mg) to each bird but, may need high dose from suspension to be more effective.

References

1. Harlin, R.W. (1994). Pigeons. The Veterinary Clinics of North America Small Anim. Pract., 24:157-173.
2. Adang, K. L.; Oniye, S. L.; Ezealor, P. A.; Abdu, A. U. and Ajanusi, O. J. (2008). Ectoparasites of dsomestic pigeon (*Colamba Livia domestic* Linnacus) in Zaria Nigeria. Res. J. Parasitol., 3:79-84.

3. Piasecki, T. (2006). Evaluation of urban pigeon (*Colomba Livia Furban*) health status in relation to their threat to human's health. *Medycyna Weterynaryna*, 62:531-535.
4. Senties-Cue, C. G.; Charlton, B. R.; Anthenill, L.; Naem, S.; McDougald, L. and Bland, M. (2011). Parasitic ventriculitis caused by *Hadjelia truncate* in California rock pigeons (*Columba livia*). *J. Vet. Diag. Invest.*, 23(6):1243-1246.
5. Anderson, R. C. (2000). The super family Habronematoidea. In *Nematode Parasites of Vertebrates: Their Development and Transmission*, 2nd ed. CABI Publishing, Wallingford, Oxfordshire, UK. Pp: 432-433.
6. Francisco, O. and Prado, A. P. (2001). Characterization of the larval stages of *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) using head capsule width. *Rev. Bras. Biol.*, 61:125-131.
7. Pfeiffer, D. G. and Axtel, R. C. (1980). Coleoptera of poultry manure in caged layer houses in North Carolina. *Environ Entomol.*, 9(2):21-28.
8. Taylor, M. A.; Coop, R. L. and Wall, R. L. (2007). Parasite taxonomy and morphology. In *veterinary parasitology*, 3rd ed. Blackwell Publishing Ltd., Oxford, UK. Pp: 8-9.
9. Al-Moussawi, A. A. (2008). First record in Iraq of two nematode parasites from the blue-cheeked bee-eater *Merops superciliosus persicus* Pallas, 1773. *Bull. Iraq Nat. Hist. Mus.*, 10(3):1-7.
10. Hamao, U.; Tomio, T.; Hironobu, I. and Osamu, T. (1976). Production of a new antibiotic, calvatic acid. (United States Patent 3980522).
11. Appleby, E.C.; Gibbons, L. M. and Georgiou, K. (1995). Distortion of the gizzard in Cyprus pigeons (*Columba livia*) associated with *Hadjelia truncate* infestation. *Vet. Rec.*, 1(36):561-564.
12. Razmi, G. R.; Kalidari, G. A. and Maleki, M. (2007). First report of the *Hadjelia truncate* infestation in pigeons in Iran. *Iranian J. Vet. Res.*, 8:175-177.
13. Tadros, G. and Iskander, A. R. (1975). *Hadjelia truncate* (Crepl., 1825) Gendre, 1921 (Spiruroidea): A new parasite of pigeons in Egypt and its pathogenicity. *Egypt J. Vet. Med. Assoc.*, 35:283-301.
14. Burg, R.W.; Miller, B. M.; Baker, E. E.; Birnbaum, J.; Curries, J. A.; Harman, R.; Kong, V. L.; Turner, M. J.; Schaeffer, J. M. and Campbell, W. C. (1989). In Ivermectin and Abamectin. New York: Springer-Verlag. Mode of action of ivermectin: Pp: 73-78.
15. Shoop, W. L.; Mrozic, K. H. and Fisher, M. H. (1995). Structure and activity of avermectins and milbemycins in animal health. *Vet. Parasitol.*, 59(2):139-156.
16. Gyatth, de Silva N.; Bundy, D. (1997). Anthelmintics: a comparative review of their clinical pharmacology. *Drugs*, 53(5):769-788.
17. Julie, M. K.; Gabriel, S. C.; Bruce, R. C.; Richard, W. G.; Tiffany, J. S. and Mark, C. (2013). Treatment, Diagnostic trials, and construction of species- specific PCR primers of *Hadjelia truncate* in pigeons (*Columba livia*). *Parasitol Res.*, 112(1):327-333.
18. Basit, M. T.; Pervez, K.; Avais M. and Rabbani, I. (2006). Prevalence and chemotherapy of nematodes infestation in wild and domestic pigeons and its effects on various blood components. *J. Anim. Pl. Sci.*, 16(1-2):24-27.
19. Sharma, R. L.; Bhat, T. K. and Hemaprasanth, I. (1990). Anthelmintic activity of ivermectin against experimental *Ascaridia galli* infection in pigeons. *Vet. Parasitol.*, 37(3-4):307-314.
20. Okaem, A. N. (1988). Ivermectin in the control of helminthiasis in pigeon. *Vet. Q.*, 10(1):70-71.
21. Howard, L. L. (2002). Fenbendazole and Albendazole toxic in Pigeons and Doves. *J. Avia. Med. Surg.*, 16(3):203 -210.
22. Okuda, T. and Fujiwara, A. (1982). Calvatic acid and product by the *Lycoperdecea 2*. Distribution among the *Gastromycetes*. *Trans. Mycol. Soc. Jpn.*, 23:235-239.
23. Takaishi, Y.; Murakami, M.; Uda, T.; Ohashi, M.; Hamamura K. and Kadota S. (1998). Hydroxyphenylazoformamide derivatives From *Calvatia craniiformis*. *Phytochemistry*. 45(5):997-1101.
24. Reynolds, L. D. and Wilson, N. G. (1991) *Scribes and Scholars 3rd Ed* Oxford. Pp: 193-194.
25. Wanamaker, B. P. and Massey K. L. (2004). Topical antiseborrheics. *Applied pharmacology for Veterinary Technician*. 3rd ed. Saunders. Pp: 206-207.

26. Hagerman, A. E. and Butler, L. G. (1991). Tannins and lignins. In Herbivores. Their interaction with secondary plant metabolites (ed. GA Rosental and TH Janzen), Pp: 355–376. Academic Press, San Diego, USA.
27. Rittner, U. and Reed, J. D. (1992). Phenolics and in vitro degradability of protein and fibre in west African Browse. J. Sci. Food Agricul., 58:21–28.
28. Reed, J. D. (1995). Nutritional toxicology of tannins and related polyphenols in forage legumes. J. Anim. Sci., 73: 1516–1528.
29. Dawson, J. M.; Buttery, P. J.; Jenkins, D.; Wood, C. D. and Gill, M. (1999). Effects of dietary Quebracho tannin on nutrient utilisation and tissue metabolism in sheep and rats. J. Sci. Food Agricul., 79:1423–1430.
30. Waller, P. J. and Thransborg, S. M. (2004). Nematode control in green ruminant production systems. Trends in Parasitology. 20(10):493-497.
31. Assefa, F. (2015). The role of plant phenolic compounds in controlling parasitic nematodes of small ruminants. Global Sci. Res. J., 3(1):134-139.
32. Boa, E. (2004). Wild Edible Fungi: A Global Overview of Their Use and Importance to People. Non-Wood Forest Products 17. Food and Agriculture Organization of the UN. Pp: 132. ISBN 978-92-5-105-157-3.
33. Miller, H. R. and Miller, O. K. (2006). North American Mushroom: A field Guide to Edible and Inedible Fungi. Guilford, Connecticut: Falcon Guide. 459. ISBN 0-7627-3109-

علاج الحمام (*Columba livia domestica*) المصاب بطفيلي *Hadjelia truncata* بالعالق الكحولي للقطر *Calvatia craniiform* بالمقارنة مع الأيفرمكتين

غسان حمدان جميل¹ و عامر مرجم عبد² و ميساء غني ظاهر³ و زاهد اسماعيل محمد⁴
¹ فرع الأحياء المجهرية، ² فرع الطفيليات، ³ فرع الصحة العامة، كلية الطب البيطري ⁴ جامعة ديالى، ² جامعة بغداد، ³ فرع الامراض،
 كلية الطب، جامعة ديالى، العراق.

E-mail: M.murhum@yahoo.com

الخلاصة

شملت الدراسة ثلاثين زوجاً من الحمام كانت تعاني من فقدان الوزن الشديد وضعف عام والموت المفاجئ لبعض من هذه الطيور. ولوحظ وجود بعض الديدان الخيطية تتمركز تحت طبقة الكويلين المتقرنة للقانصة بعد تشريح ثلاثة طيور هالكة. أظهر التشريح العياني للطيور الهزيلة وجود ديدان خيطية للنوع *Hadjelia truncata* وهي من الديدان الخيطية التي تصيب الجهاز الهضمي للدواجن. حضرت ثلاثة تراكيز مختلفة من معلق القطر وهي (0.25 و 0.5 و 1 مليغرام/ملييلتر) واستعملت بالمقارنة مع الأيفرمكتين الشائع استعماله في علاج الطفيليات. كانت جميع التراكيز المحضرة من القطر فعالة ضد الطفيليات وأعطت نتائج جيدة لكنها أقل فعالية من الأيفرمكتين.

الكلمات المفتاحية: هاتجيبيا ترانكاتا، الأيفرمكتين، كالفاتيا كرانيفورمس، الحمام.