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Treatment of Experimental Candidiasis in Broilers with Griseofulvin

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Abstract

Candidiasis is a mycotic disease of avian hosts. Some species of the genus candida are the etiological agents of candidiasis. Griseofulvin is an antifungal metabolic byproduct of some fungi especially those belong to the phylum Ascomycota. Due to the shortage of research regarding this drug use in birds, this study was conducted. A number of 60 broiler chicks of 10 days old was divided in to 3 groups, 20 chicks each. Group one (G1), control group, did not receive infection nor treatment. Group two (G2), infection group, was challenged with candida albicans. Group three (G3), treatment group was infected similar to G2 then treated with griseofulvin. At 20 days of age, a sample of 5 birds from each group were necropsied to document gross lesions and collect tissue samples for histopathology. Results show that crops from infected birds in G2 have thickened wall and the lumen were lined with diphtheritic material while crops from birds that received treatment with griseofulvin showed significantly milder lesions compared to G2. Histopathological sections from infected birds of G2 were showing inflammatory cell infiltration and sloughing of the superficial epithelial layer and fungal hyphae penetrating the crop mucosa. On the other hand, histopathological sections of crops from G3 were less affected by the infection. There was very few tissues reaction and mycelial growth was only superficially penetrating the epithelium. From the finding of the present study, it can be concluded that griseofulvin is effective for the treatment of candidiasis.

Keywords: Thrush, moniliasis, *Candida albicans*, Crop mycosis.

Introduction

Poultry industry is an important economic sector of the Iraqi national economy and employs a large number of the work force [1–3]. Economic losses due to diseases is one of the important challenges that face this sector in Iraq [4]. Candidiasis is a mycotic disease of avian hosts. Some species of the genus candida mainly *C. albicans* are the etiological agents of candidiasis [5, 6]. These yeasts are part of the natural flora in the animal's upper alimentary tract, and they cause sickness when the bird has a deteriorating condition [7]. Additionally, candidiasis develops when the number of microflora reduces as a result of long-term antibiotic usage [8]. Griseofulvin is an antifungal metabolic byproduct of some fungi especially those belong to the phylum Ascomycota despite the fact that it was initially derived from *Penicillium griseofulvum* in 1939 [9]. The medication is currently applied to people who have dermatophyte infections. [10]. There are multiple research examining the use of griseofulvin in various animals [11–13]. There were few published experiments examining the use of this drug in avian species [14–17]. Due to the insufficient research regarding the use of this drug use in birds, this study was conducted to examine the therapeutic effect of griseofulvin against candidiasis which is experimentally induced in broiler chicks.

Material and Methods

Location and period of the study: The research was carried out at the University of Fallujah's College of Veterinary Medicine between 11 June and 27 August 2023.

Source of isolate

Candida albicans was isolated from a field infection in a local rooster submitted by the owner for diagnosis and treatment purpose. The mycological

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identification of the pathogen was carried out and the isolate was used in previous studies by the authors [18].

Experiment design:

A number of 60 broiler chicks of 10 days old were divided in to 3 groups (Table 1). Each group included 20 chicks. Group one (G1) was designated as control and did not receive candida infection nor treatment. Group two (G2) was named as an infection group and was challenged orally with 3 billion colony forming unit of *candida albicans* at the age of 10 days old. Group three (G3) was named as a treatment group and was infected similar to G2 then treated with 50 milligrams of griseofulvin per kilogram of body weight [19]. The treatment was administered orally 3 days after infection at 13 days of age and continued for 7 days till the end of the experiment at 20 days of age.

TABLE 1. Chick groups used in the study

Group	Challenge	Treatment
G1 (Control)	-	-
G2 (Infection)	+	-
G3 (Treatment)	+	+

Gross examination

At 20 days of age, a sample of 5 birds from each group were euthanized humanly and necropsy techniques were performed. Lesions in the crop were documented by photographs. Small pieces of the crop tissue were cut and placed in a fixative solution for histopathological examination.

Histopathological examination:

Small pieces of tissues were placed in a neutrally buffered formalin for fixation for at least 72 hours. Tissue samples were then processed for staining with hematoxylin and eosin stain (HE) and Periodic acid–Schiff stain (PAS) according to Luna [20].

Results and Discussion

Clinical signs: Birds were monitored closely throughout the study period to document any clinical signs associated with the disease. There were no significant clinical signs related to the diseases other than slight unthriftiness. This finding agrees with other researchers who also mentioned that the disease causes no particular clinical signs (5). On the other hand, some references reported general non-specific signs such as stunted development, listlessness, harshness of feathers, and low growth [21].

Gross lesions: A sample of 5 birds were necropsied to document any gross lesions. Figure 1 shows a crop's typical gross look within control birds in G1. On the contrary, crops from infected birds in G2 have thickened wall and the lumen were lined with diphtheritic material (Figure 2). These are typical lesions of candidiasis and usually referred to as curd-like pseudomembrane [21]. In natural infections, the lesions vary from bird to another and lesions of predisposing factors maybe seen during necropsy overlap with lesions of candidiasis (22). Crops from birds that received treatment with griseofulvin showed significantly milder lesions (Figure 3). There were small white spots of exudate significantly smaller than the lesions in G2. The mucosal lining was slightly hyperemic when compared to G1.

Histopathology:

Figure 4 and Figure 5 show normal histological appearance of the crop mucosae taken from G1. Sections from infected birds of G2 were showing inflammatory cell infiltration and sloughing of the superficial epithelial layer (Figure 6). Staining with PAS stain aid in visualization of the fungal hyphae that penetrate the crop mucosa (Figure 7). This stain is preferred for fungal hyphae staining (5). The direction of hyphal growth is opposite to the epithelium's surface and it penetrates the outer layers of the stratified squamous epithelium involving the stratum corneum layer and the superficial portion of the stratum spinosum (23, 24). The fungal growth causes tissue reaction comprising inflammatory cells infiltration. Macrophages are seen in the section along with heterophils, lymphocytes and plasma cells [25].

Histopathological sections of crops from G3 that were treated with griseofulvin were less affected by the infection. There was very few tissue reactions and the stratified squamous epithelium was intact (Figure 8). Special staining showed that in this group, mycelial growth was only superficially penetrating the epithelium (Figure 9). Since this is the first study that examine the effectiveness of griseofulvin as a treatment for candidiasis in chicks, we could not find previous research to compare with our findings. Griseofulvin have been tested in-vitro against candida and other fungal species (26). The drug was authorized in 1959 by the Food and Drug Administration (FDA) to be used as a human medicine. It is usually used in human for the treatment of ringworm infections and dermatophytes (27).

Conclusions

From the findings of the present study, it can be concluded that griseofulvin is effective for the treatment of candidiasis. Further studies are required to examine the side effects of the drug and its effects on physiological parameters.

Fig. 1. Photograph of normal crop from control birds in G1 showing normal gross appearance.

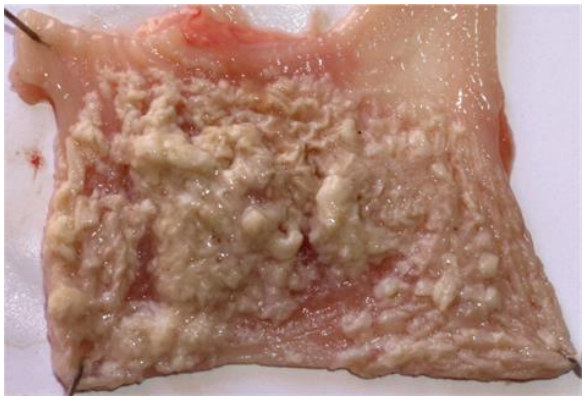

Fig. 2. Photograph of a crop from infected birds in G2 showing thickened wall with a diphtheritic material.

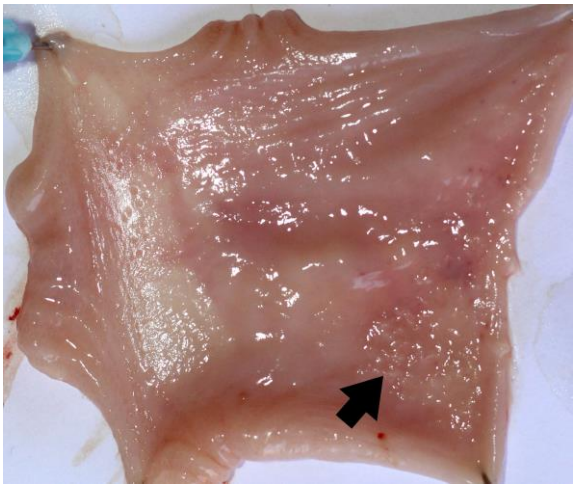

Fig. 3. Photograph of a crop from treated birds in G3 showing normal gross appearance except small pieces of diphtheritic exudate (arrow).

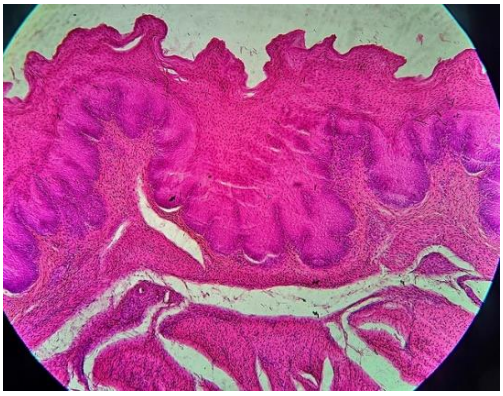

Fig. 4. Histological section of crop from G1 showing normal histological appearance. H&E, 40X.

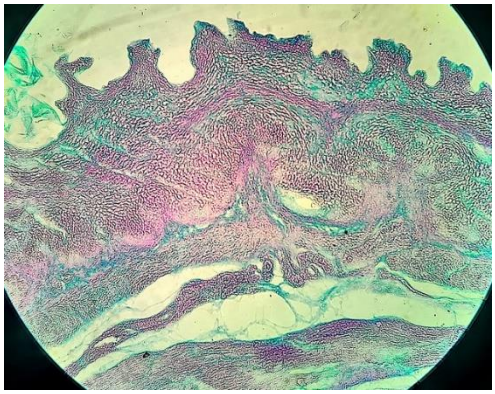

Fig. 5. Histological section of crop from G1 showing normal histological appearance. PAS, 40X.

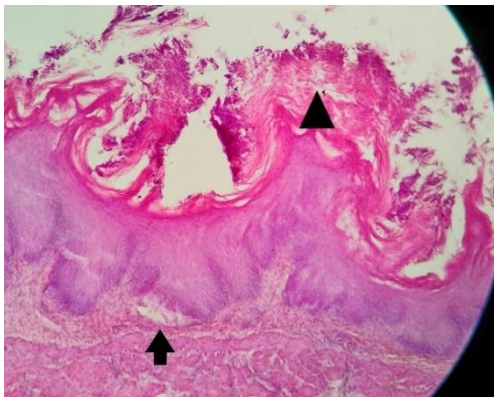

Fig.6. Histological section of crop from G2 showing inflammatory cell infiltration (arrow) and sloughing of the superficial epithelial layer (arrowhead). H&E, 40X.

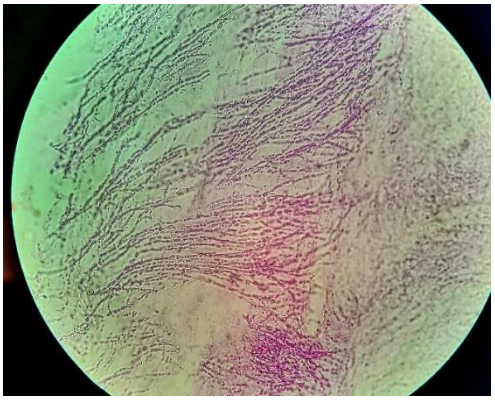

Fig. 7. Histopathological section of crop from G2 showing fungal hyphae. PAS, 100X.

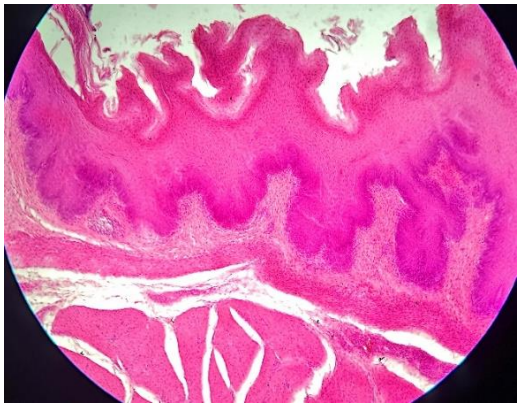

Fig. 8. Histopathological section of crop from G3 Show normal histological architecture. H&E, 40X.

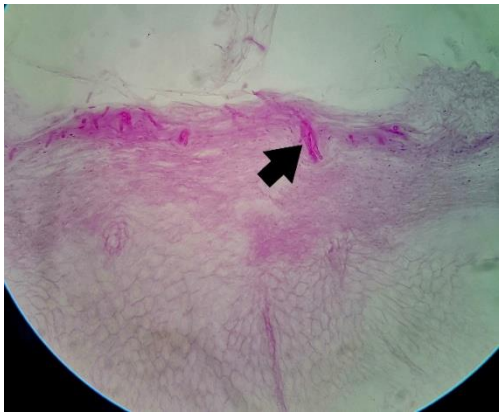

Fig. 9. Histopathological section of crop from G3 showing superficial mycelial infestations. PAS, 100X.

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The authors declare that the present study has no financial issues to disclose.

Conflict of interest: None

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References

1.Al-Wasity, R. T., Mahmood, Z. H. and Al-Sammarraie, M. H. Measuring efficiency of broiler breeding projects using stochastic cost limit in iraq (baghdad governorate). *Iraqi J. Agric. Sci.,* **54** (2), 535–541 (2023). https://doi.org/10.36103/ijas.v54i2.1729.

2. Barbaz, D. S. and Al-Hiyali, A. D. K. Economic evaluation of some agricultural initiative projects in Iraq. *Iraqi J. Agric. Sci.,* **51** (3), 797–804 (2020). https://doi.org/10.36103/ijas.v51i3.1035.

3. Al-Abd Alaali, H. J. and Mahmood, Z. H. An economic research of broiler projects for some provinces in the middle of Iraq in 2019. *Iraqi J. Agric. Sci.,* **52** (4), 1019–1030(2021). https://doi.org/10.36103/ijas.v52i4.1413.

4. Al-Ani, G. A. A. and Al-Badri, A. A. N. Reasons related to the deterioration of poultry industry for broiers in the middle and southern region of Iraq. *Iraqi J. Agric. Sci.,* **52** (2), 429–436(2021). https://doi.org/10.36103/ijas.v52i2.1304.

5. Ibrahim, Z. Y., Ali, B. H., Ali, R. K., Jarad, A. S., Farhan, W. H. and Hasan, M. S. Avian Candidiasis: A Review. *Int. J. Pharm. Res.,* **12** (1), 1088–1091(2020). https://doi.org/10.31838/ijpr/2020.12.01.199.

6. AL-Shimmery, F. A. Investigation On The Occurrence of Yeast Species in the Digestive Tracts of Broiler. *Euphrates J. Agric. Sci.,* **3** (9), 73–42(2011).

7. Kaab, H. T. Genotyping of Candida albicans Isolated from broilers by 25S rDNA Analysis. *Kufa J. Vet. Med. Sci.,* **4** (1), 105–110 (2013).

8. Kemoi, E. K., Okemo, P. and Bii, C. C. Isolation of Candida species in domestic chicken (Gallus gallus) droppings in Kabigeriet Village, Nakuru County Kenya. *Eur. Sci. J.,* **9** (36), 309–318(2013).

9. Ribeiro, A. I., Costa, E. S., Thomasi, S. S., Brandão, D. F. R., Vieira, P. C., Fernandes, J. B., Forim, M. R., Ferreira, A. G., Pascholati, S. F. and Gusmão, L. F. P. Biological and chemical control of Sclerotinia sclerotiorum using Stachybotrys levispora and its secondary metabolite griseofulvin. *J. Agric. Food Chem.,* **66** (29), 7627–7632 (2018). https://doi.org/10.1021/acs.jafc.7b04197.

10. Lee, H. B., Mun, H. Y., Nguyen, T. T. T., Kim, J.-C. and Stone, J. K. Abieticola koreana gen. et sp. nov., a griseofulvin-producing endophytic xylariaceous ascomycete from Korea. *Mycotaxon.,* **131** (4), 749–764(2016). http://dx.doi.org/10.5248/131.749.

11. Mohamed, A., Hassan, A., Amer, M. and Abdel-Aziz, E.S. The effects of oral ketoconazole and griseofulvin on the fertility of male rabbits. *Mansoura Vet. Med. J.,* **21** (2), 32–38(2020). https://doi.org/10.21608/mvmj.2020.21.2.0203.

12. López-Gómez, N., Piñeiro, I. R. and Fernández, Y. Successful treatment but delayed complete recovery of griseofulvin-induced bone marrow hypoplasia in an FIV-seronegative cat. *Vet. Rec. Case Rep.,* **10** (4), 480–487. (2022). https://doi.org/10.1002/vrc2.480.

13. Chandrahas Sannat, M. O. K., Hirpurkar, S. D., Rawat, N., Jolhe, D., Gupta, A. K., Gumasta, P. and Kamble, M. V. Isolation of Microsporum canis from dog and its therapeutic management. *J. Entomol. Zool. Stud.,* **7** (2), 516–518(2019).

14. Marx, K. L. Therapeutic agents. In Clinical avian medicine, Vol. Vol. 1, pp. 214–342(2006). Spix Publishing Inc Palm Beach, FL.

15. Onderka, D. K. and Doornenbal, E. C. Mycotic dermatitis in ostriches. *Can. Vet. J.,* **33** (8), 547–554(1992).

16. Samour, J. Pharmaceutics commonly used in avian medicine. *Avian Med.,* 388–418(2000).

17. Jensen, J. M. Current ratite therapy. *Vet. Clin. North Am. Food Anim. Pract.,* **14** (3), 485–502(1998).

18. Ibrahim, Z. Y., Hameed, N. A. and Jarad, A. S. Prevention of Avian Crop Candidiasis by dietary Supplementation of Saccharomyces Cerevisiae. *Rev. Electron. Vet.,* 435–444 (2022).

19. Harrison, G. J., Lightfoot, T. L. and Harrison, L. R. (2006). Clinical avian medicine. Spix publishing Palm Beach, FL.

20. Luna, L. G. (1968). Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3rd ed. McGraw-Hill. Inc., United States of America.

21. Arné, P. and Lee, M. D. Fungal Infections. In Diseases of Poultry, pp. 1109–1133(2020). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781119371199.ch25.

22. Shivaprasad, H. L. Fungal diseases. In Avian disease manual, pp. 140–152(2012). American Association of Avian Pathologists.

23. Dykstra, M. J., Charlton, B. R., Chin, R. P. and Barnes, H. J. Fungal Infections. In Diseases of Poultry, pp. 1075–1096. (2013). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781119421481.ch25.

24. Fletcher, O. J. and Abdul-Aziz, T. Alimentary System. In Avian Histopathology, pp. 271–354(2016). The American Association of Avian Pathologists.

25. Asfaw, M. and Dawit D. Review on Major Fungal Disease of Poultry. *Br. J. Poult. Sci.*, **6** (1), 16–25(2017). https://doi.org/10.5829/idosi.bjps.2017.16.25.

26. Mahmoudabadi, A. Z., Farrahei, F. and Zamin, M. In vitro synergism between miconazole and griseofulvin against Candida species. *Pak. J. Med. Sci.*, **22** (4), 454–456(2006).

27. Aris, P., Wei, Y., Mohamadzadeh, M. and Xia, X. Griseofulvin: An updated overview of old and current knowledge. *Molecules*, **27** (20),1–14(2022). https://doi.org/10.3390/molecules27207034.

علاج داء المُبَيِّضَات التجريبي في الفروج اللاحم باستخدام الغريزوفولفين

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داء المبيضات هو مرض فطري يسببه أعضاء من جنس المبيضات. الغرزيوفلفين هو ناتج ايضي مضاد للفطريات ينتج بشكل أساسي عن طريق الفطريات من شعبة الفطريات الزقية. أجريت هذه الدراسة بسبب قلة الأبحاث حول استخدام هذا الدواء في الطيور. تم تقسيم عدد 60 فروج تسمين عمرها 10 أيام إلى 3 مجموعات ، 20 طير لكل مجموعة. المجموعة الأولى (G1) ، مجموعة السيطرة ، لم تتلق العدوى ولا العلاج. المجموعة الثانية (G2) ، مجموعة العدوى ، اصيبت بفطر المبيضات. المجموعة الثالثة (G3) ، المجموعة العلاجية كانت مصابة على غرار G2 ثم عولجت بالغريزوفولفين. في عمر 20 يومًا ، تم تشريح عينة من 5 طيور من كل مجموعة لتوثيق الآفات العيانية وجمع عينات الأنسجة من أجل التقطيع النسجي. أظهرت النتائج أن الحواصل من الطيور المصابة في G2 لها جدار سميك وأن التجويف مبطّن بمواد خناقية بينما أظهرت حواصل الطيور التي تلقت العلاج باستخدام الغريزوفولفين آفات اقل. المقاطع النسجية المرضية لطيور G2 المصابة أظهرت ارتشاح للخلايا الالتهابية و انسلاخ الطبقة الظهارية السطحية والخيوط الفطرية تخترق الغشاء المخاطي للحوصلة. من ناحية أخرى ، كانت المقاطع النسجية المرضية لحواصل G3 التي تم علاجها باستخدام الغريزوفولفين أقل تأثراً بالعدوى. كان هناك عدد قليل جدًا من تفاعلات الأنسجة وكان النمو الفطري يخترق الظهارة بشكل سطحي فقط. من النتائج التي توصلت إليها الدراسة الحالية ، يمكن الاستنتاج أن الغريزوفولفين فعال في علاج داء المبيضات.

الكلمات الدالة: السلاق ، فطريات الحوصلة ، كانديدا البيكانز ، كانديدياسز.