PATHOLOGICAL OBSERVATION OF FUNGAL (*ASPERGILLUS FUMIGATUS*) OPHTHALMITIS IN CHICKS

Chitoshi ITAKURA and Masatomo GOTO

Department of Veterinary Pathology, Faculty of Agriculture,
Tottori University, Tottori

Mitsuo FUJIWARA

Okayama Diagnostic Service Center for Livestock, Prefecture of Okayama

(Received for Publication March 27, 1973)

The pulmonary form of *Aspergillus fumigatus* infection in chicks is of frequent occurrence. On the other hand, infection of the eyes with this fungus is very rare, judging from previous reports^{2,4,5}). Especially, the pathology of this infection is apparently less well known.

This article describes mainly the pathology of the chick eyes affected naturally with *Aspergillus fumigatus* and bears a review of literature on this disorder.

MATERIALS AND METHODS

The materials used for the investigation consisted of ten chicks (Table 1) which had been collected from two poultry farms, A (in Hyogo Prefecture) and B (in Okayama Prefecture), as mentioned below.

Three chicks were collected from poultry farm A. On this farm chicks were reared in windowless houses where chip was used as litter. Affected birds were detected by confirming swelling of the eyelid, although they were a few in number.

Seven chicks were collected from poultry farm B. On this farm the infection was observed after chip began to be used as litter. The incidence seemed to have been recognized for the first time at about 16 days of age, judging from the symptom of swelling of the eyelid. It reached 0.4 percent up to 31 days of age. No morbid birds were observed in other flocks at the same age, for which rice straw was used as litter.

Clinically, the infected chicks had usually unilateral eye lesions. The affected eye was closed and showed a marked swelling of both lower and upper eyelids. Suppurative lacrimation was occasionally observed. A yellow caseous mass was adherent to the cornea (Figs. 1 & 2). Therefore, the iris and lens were not visible. The affected birds were high-spirited and normal in gait.

A fungus was isolated in culture media using blood, corn-meal, and Sabouraud's agar from eye lesions found on poultry farm B. The isolated fungus was also examined microscopically by slide culture. From its cultural characters¹⁾ the fungus was identified as *Aspergillus fumigatus* (Fig. 14).

All the chicks were sacrificed for the pathological examination. After autopsy, tissue specimens were collected from the whole body, including the eyes, and fixed in 10 percent formalin solution. Paraffin sections were made by the routine method and stained with hematoxylin and eosin for general examination. Many sections from eyes, lungs, and air sacs were also treated with PAS, Gridley, and azan stains.

Table 1. Materials Subjected to Investigation

Poultry farm	Case No.	Age (days)	Sex	Side of affected eye	Main concurrent lesions
A	1	28	Male	Right	Fungal granulomas in air sacs and <i>E. acervulina</i> in small intestine
A	2	28	Male	Left	Fungal granulomas in lungs and <i>E. acervulina</i> in small intestine
A	3	22	Male	Left	
B	4	25	Female	Right	
B	5	25	Female	Right	Fungal granulomas in lungs
B	6	25	Female	Right	
B	7	25	Female	Right	Productive serositis in small intestine
B	8	25	Female	Right	
B	9	25	Female	Right	
B	10	25	Female	Right	

Remarks: All the chicks were White Cornish and White Rock hybrids and killed for examination.

RESULTS

1. Macroscopical findings

The macroscopical findings of the affected eyes were identical with changes observed clinically (Figs. 1 & 2). No lesions were found out in any visceral organ.

2. Histopathological findings

a. Eyes

In all the chicks examined, eye lesions were almost identical in character and high in severity. Usually, an unilateral eye was infected and the opposite eye showed no changes.

Lesions characteristic of this disease were confined to the anterior part of the eye; namely, cornea, bulbar conjunctiva, anterior chamber, iris, and ciliary body (Figs. 3 & 4). It was frequently difficult to distinguish one tissue from another, especially the cornea from the anterior chamber (Figs. 4~6), because every lesions were high in severity.

The lesions examined were divided into two main categories, fibrinous exudation with heterophils and proliferation of granulation tissue. Exudative changes were observed chiefly in the cornea and anterior chamber (Figs. 3~7). The exudative substance frequently showed regressive changes, especially in the superficial area of the cornea. The cornea lost its proper structure and disintegrated. A small or large number of proliferated hyphae were in the superficial area of the cornea and invaded the exudative substance within the anterior chamber through the cornea (Figs. 5 & 6). Hyphae were also occasionally demonstrated in some areas of the sclero-corneal junction where the epithelium was lost (Fig. 8). A large amount of exudative substance was seen in the anterior chamber, adhering to the cornea and the iris (Figs. 4~6 & 12).

On the other hand, the granulation tissue consisted mainly of fibroblasts almost always accompanied by heterophils, lymphocytes, plasma cells, and histocytes (Figs. 8~10 & 12). This tissue developed from the sclero-corneal junction (Fig. 9), the tunica propria of the bulbar conjunctiva (Figs. 4 & 8), and the iris (Fig. 12). Furthermore, foreign-body giant cells were scattered or formed nodules in the granulation tissue (Figs. 10 & 13). They frequently ingested and encircled degenerated hyphae (Fig. 10 & 13).

The iris and ciliary body showed hyperemia, hemorrhages, a marked increase in lymphocytes, a marked infiltration of heterophils and, moreover, a slight proliferation of

fibroblasts and plasma cells (Figs. 11 & 12). Other changes were scarcely observed in their pigmented epithelia.

In the bulbar conjunctiva, there were a proliferation and desquamation of epithelial cells, and an infiltration of heterophils into the epithelium. Furthermore, hyperemia, an increase in lymphocytes, and an infiltration of heterophils were in the conjunctival tunica propria.

In addition marked hyperemia in the choroid and atrophy of the lens were rarely pointed out. No alterations were noted in the retina or any other tissue.

b. Miscellaneous tissue (Table 1)

Granulomas with hyphae were sporadically formed in the bronchi of cases 2 and 5, and in the wall of the air sacs of case 1. A circumscribed increase in lymphocytes was often present in some visceral organs of many cases. Coccidial (*Eimeria acervulina*) infection was observed in the small intestine of cases 1 and 2, and productive serositis in that of case 7.

DISCUSSION

In the pathological changes characteristic of fungal ophthalmitis described in the present paper it should be emphasized that lesions were localized in the anterior part of the affected eye and consisted of exudative inflammation with fungal hyphae and reactive proliferation of granulation tissue. HUDSON²⁾, REIS⁴⁾, and SPERLING⁵⁾ pointed out the confinement of lesions. Lesions mentioned in their reports were formation of a yellow cheesy pellet beneath the nictitating membrane²⁾, formation of white patches on the cornea⁴⁾, and keratitis^{2,4,5)}, though no minute description of any lesion was made.

On the other hand, REIS⁴⁾ reported that in some of the affected chicks practically all parts of the eye had been involved. It was also revealed that main pathological changes were found in the posterior part of the eye in poult, the lungs of which were also involved³⁾.

All the chicks examined in this study had unilateral eye lesions. The same result was obtained by SPERLING⁵⁾. HUDSON²⁾ reported that only one of fifteen chicks examined had shown infection of both eyes. The reason why only unilateral eye was affected predominantly is unknown.

As for lesions related to the fungal infection in other organs, SPERLING⁵⁾ and REIS⁴⁾ observed no respiratory lesions or symptoms. HUDSON²⁾ found out visible pulmonary involvement in two chicks of the fifteen affected. The lungs and air sacs were involved in two birds of the ten examined in this study. It is presumed, however, that there may be no direct relationship on pathogenesis between the respiratory and eye infections.

The following findings suggest that the causative agent may have invaded by way of the cornea or conjunctiva from the exterior: localization of the lesions, unilateral eye infection, lack of connection with lesions of visceral organs, and histological pictures indicating that hyphae invaded from the surface of the cornea into the anterior chamber of the eye.

REIS⁴⁾ reproduced the experimental infection of this disorder by inoculating a pure culture of the fungus into the anterior chamber of the eye and by dropping a suspension of spores on the deeply scarified cornea. He thought that sawdust litter might be the source of infection. HUDSON²⁾ reported the appearance of affected chicks in houses where bagasse was used as litter. In the present investigation, chip litter may be responsible for the infection, judging from the aspects of the occurrence mentioned above.

As to the morbidity of this disease, REIS⁴⁾ exhibited that 10 percent of 1,000 chicks two to five weeks of age had been affected with an eye involvement. HUDSON²⁾ found

that 3.2 percent of 6,250 chicks ten to eleven days of age had been involved in this disease. The morbidity may be dependent largely on the housing condition of birds. Generally speaking, young chicks seem to be involved in this disorder as they are suffering from the respiratory infection.

SUMMARY

Ten chicks with eye lesions caused by *Aspergillus fumigatus* were studied pathologically. All of them had unilateral eye infection. The histological lesions were confined to the anterior part of the eye, and consisted of exudative inflammation with hyphae and reactive proliferation of granulation tissue. They had no connection with those in other organs. Direct invasion of an etiological agent from the exterior was suspected to be the mode of infection of this eye disorder.

REFERENCES

- 1) CHUTE, H. L. (1965): Diseases of Poultry, edited by BIESTER, H. E. and SCHWARTE, L. H., 5th Ed., p. 495, Ames, Iowa, Iowa State University Press.
- 2) HUDSON, C. B. (1947): *Poult. Sci.*, **26**, 192~193.
- 3) MOORE, E. N. (1953): *Poult. Sci.*, **32**, 796~799.
- 4) REIS, J. (1940): *Arg. Inst. biol. Artigo*, **11**, 437~450. Cited from HUDSON, C. B. (1947) and MOORE, E. N. (1953).
- 5) SPERLING, F. G. (1953): Proc. 25th Ann. Meet. N.E. Conf. Lab. Wkrs Pullorum Dis. Contr., June 16 & 17, p. 2. Cited from *Vet. Bull. (Weybridge)*, **24**, 266~267.

鶏雛のカビ (*Aspergillus fumigatus*) 性眼炎に 関する病理学的観察

板倉智敏・五藤精知

鳥取大学農学部家畜病理学教室

藤原三男

岡山県家畜病性鑑定所

(昭和48年3月27日受付)

Aspergillus fumigatus の眼への感染は、REIS (1940), HUDSON (1946), SPERLING (1953) が鶏において、MOORE (1953) が七面鳥において、認めているに過ぎず、本症の病理像はいまだ明らかでない。著者らは、鶏雛において、本菌の眼への自然感染例に遭遇したので、それらを病理学的に観察した。

検索材料は、10羽の雛（白色コーニッシュと白色ロックとの交雑種、22～28日齢、雄3例、雌7例、すべて殺例）から成る。これらは、兵庫県（A群）、岡山県（B群）の2養鶏場において発生した。

罹病眼は、すべての例において一側性（右眼8例、左眼2例）であった。それらは、肉眼的には、上・下両眼瞼の著明な腫脹と、角膜表面に黄色乾酪化物の付着を示した。なお、B群例の罹病眼からは、*Aspergillus fumigatus* が分離された。

組織病変は、角膜、前眼房、眼球結膜、虹彩、毛様体に限局していた。その病変は、偽好酸球を混じた線維素を主成分とする滲出性変化と、これに対する異物巨細胞を含む幼若な肉芽組織の増殖から成っていた。前者の滲出性変化は、角膜および前眼房に主座し、後者の肉芽性病変は、それを囲繞する周囲組織から発達していた。滲出物の表層および異物巨細胞内には、菌糸が認められた。水晶体を含め、眼深部には、特記すべき変化は認められなかった。

本菌に起因する内臓病変としては、2例の肺と1例の気嚢に、肉芽腫の形成が所見されたのみであった。

本菌の眼への感染機序としては、外部からの直接感染が示唆された。感染源としては、A・B両群とも、敷料として用いられたチップに疑いがもたれた。

EXPLANATION OF PLATES

Figs. 1 and 2 are macroscopical photographs and Figs. 3~13 microphotographs taken from affected eyes, and Fig. 14 is a microphotograph from the fungal culture.

PLATE I

- Fig. 1. The eyelid is swollen and a large amount of yellow caseous substance attached to the cornea. Case 1.
- Fig. 2. The eyelid is swollen. A knob-shaped yellow caseous mass has been formed at the lateral angle. Case 3.
- Fig. 3. Cornea, anterior chamber, iris, ciliary body, and lens. All of them, except the lens, are hardly identified from one another because of severe lesions. The lesions consist of fibrinous exudation with heterophils, which is observed mainly in the cornea and anterior chamber, and of a proliferation of granulation tissue which has developed from the sclero-corneal junction and iris. No significant alterations are seen in the lens. Case 2. Hematoxylin and eosin (H-E) staining. $\times 16.5$.
- Fig. 4. Cornea, anterior chamber, and iris. The cornea and anterior chamber (leftward) have been replaced by fibrinous exudation with heterophils. Therefore, it is difficult to distinguish one tissue of the eye from the other. The superficial area of this lesion shows regressive changes and is associated with hyphae. There is a fibroblastic proliferation with heterophils, sometimes accompanied by foreign-body giant cells, in the sclero-corneal junction and tunica propria of the bulbar conjunctiva. The corneal epithelium in the sclero-corneal junction is swollen and desquamating. The iris has been markedly thickened due to an increase in lymphocytes and fibroblasts, and an infiltration of heterophils. Case 1. H-E staining. $\times 46$.
- Fig. 5. Cornea and anterior chamber. The cornea (about upper half) has disintegrated, accompanied by heterophils and hyphae. The anterior chamber (about lower half) contains a large amount of exudative substance consisting of fibrin and degenerated heterophils. Case 10. PAS staining. $\times 228$.

PLATE II

- Fig. 6. Cornea and anterior chamber. The cornea (central part) has disintegrated. Beneath it there is the anterior chamber (lowerward) into which fibrinous substance has been exuded with heterophils. Hyphae have been proliferated on the cornea with heterophil exudation (upperward) and invaded into the anterior chamber through the cornea. Case 3. Gridley staining. $\times 228$.
- Fig. 7. Exudative substance with many hyphae on the cornea and in the anterior chamber. The substance consists of fibrin and degenerated heterophils. Case 9. PAS staining. $\times 455$.
- Fig. 8. Sclero-corneal junction. A large amount of fibrin accompanied by a few heterophils has been exuded in the limbus of the cornea, from which the epithelium has been lost. In addition to the exudative substance, fibroblasts have been proliferated in the cornea, accompanied by heterophils, lymphocytes, and plasma cells originated from the surrounding tissues. Hyphae are seen in the lesion where the corneal epithelium is desquamating (arrow). Case 1. H-E staining. $\times 115$.
- Fig. 9. Sclero-corneal junction. A marked proliferation of fibroblasts, the appearance of a few heterophils, hyperemia, and hemorrhage are observed in the limbus of the cornea. The sclera is seen in the lower part. Most of the corneal epithelial cells have been lost and fibrin has been exuded in the lesion. Case 2. H-E staining. $\times 115$.

PLATE III

- Fig. 10. Granulation tissue in the limbus of the cornea. The tissue consists of fibroblasts, heterophils, lymphocytes, plasma cells, and histiocytes. Foreign-body giant cells are formed, within which degenerative hyphae are demonstrated by PAS and Gridley staining. Case 2. H-E stain. $\times 228$.
- Fig. 11. Iris. A marked increase in lymphocytes, an infiltration of heterophils, and a slight proliferation of fibroblasts are seen in the stroma iridis. No significant changes are observed in the pigmented epithelium. Case 1. H-E staining. $\times 228$.
- Fig. 12. Iris. A large amount of fibrin accompanied by a few heterophils is adherent to the anterior part (upperward) of the iris. Fibroblasts proliferated in the iris have invaded the exudative substance. An infiltration of heterophils and an increase of lymphoid cells are seen in the stroma iridis. Some of the pigmented epithelial cells are degenerative. Case 9. PAS staining. $\times 115$.
- Fig. 13. An aggregation of foreign-body giant cells with degenerative hyphae in the granulation tissue which has been formed in the limbus of the cornea. Case 2. Gridley staining. $\times 455$.
- Fig. 14. *Aspergillus fumigatus* from slide culture on cornmeal agar. Lactophenol cotton blue staining. $\times 860$.