THE MYCOFLORA OF WILD PINK-FOOTED GEESE SAMPLED IN ICELAND AND SCOTLAND, 1953

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SUMMARY

An attempt was made, (i) to study the fungi isolated from the upper respiratory tracts of wild Pink-footed Geese at their breeding-grounds in Iceland and at their winter quarters in Scotland, in order to discover whether they harboured *Aspergillus fumigatus* in the wild, and (ii) to test suitable methods for collecting samples in the field under adverse conditions. Cultures were made by streaking throat-swabs on to Sabouraud's dextrose agar slopes and neither these nor the controls revealed *A. fumigatus* or any other potential pathogenic fungus, but the isolates obtained showed that the quality and quantity of the fungus content of the upper respiratory tracts of the geese may be dependent on their environment. Suggestions are made for improving technique. A species of Ascomycete grown from the throats of three geese in Iceland appears to be new to science.

INTRODUCTION

Avian Aspergillosis is a common and usually fatal disease especially of young poultry and of wild birds in captivity (Ainsworth and Rewell, 1949). It is caused almost exclusively by *A. fumigatus*, a cosmopolitan fungus which can be readily isolated from soil, plant remains, and generally from the environment of animals. Very little is known about the natural occurrence of the disease in wild birds, though Ainsworth and Austwick (1955) have recorded it in a Rook, a Jackdaw and four Woodpigeons. The disease causes much of the mortality among adults in the Wildfowl Trust collection, and during recent years has killed many valuable specimens. Nothing is known about the natural occurrence of the disease in wildfowl and the present investigation was undertaken to ascertain the presence of *A. fumigatus* in the upper respiratory tracts of wild Pink-footed Geese in their natural habitats.

Previous investigations (Sladen, 1952 and 1954) among penguins in the Antarctic, and among Kelp Geese and Steamer Duck brought back to the Wildfowl Trust did not reveal the presence of *A. fumigatus*, or other potentially pathogenic fungi before captivity. In addition, Sladen swabbed the throats of 23 wild geese in Scotland in November 1952, after they had been caught for ringing by rocket nets. A number of non-pathogenic fungi were grown from these swabs after streaking on to Sabouraud's dextrose agar, and one culture gave an abundant growth of *A. fumigatus*. Contamination due to the primitive field conditions may have been responsible for this positive result, because two out of five control, and apparently sterile, swabs also grew isolated colonies of the same species. The work however suggested further lines of approach. First, a much larger sample of swabs from the upper respiratory tracts of wild birds was indicated, and second, methods of collection and culturing needed testing in the field.

The Wildfowl Trust's expedition to Central Iceland in 1953 (see Scott, Boyd and Sladen, 1955) provided opportunities for following up this work. Two

hundred and fifty-four of the 9,005 adult and gosling Pink-footed Geese captured for ringing had their throats swabbed and at the same time 46 cultures were made from the feathers of the geese and the soil in the catching area and by exposing agar slopes to the air during streaking operations, (termed ' controls ' throughout this account). In November 1953, a further sample was taken from 40 of the same species caught by rocket-nets in Dumfriesshire and a further 10 controls collected. Sladen was responsible for the field work and Austwick for the examination of the isolates.

EQUIPMENT FOR FIELD WORK

Cultures were made on Sabouraud's dextrose agar slopes in Bijou bottles. Standard throat swabs were used which were sterilised twice, the second time after being packed into tin containers. These tins were kept sealed until required, thus reducing the danger of contamination to a minimum.

METHODS

In Iceland. Swabs and culture media were transported to the catching area by pack-pony. Each goose was held firmly round the wings and body by an assistant. The left hand was used for opening the bill and the swab, held in the right hand, inserted and rubbed against the fauces where the nares entered the hard palate and then rubbed across the epiglottis until the bird gave a little ' cough.' Care was taken not to rub the swab against the fingers used for keeping the mouth open. The ever present wind and dust were also potential sources of contamination, and there were further disturbances when birds were caught for ringing. Swabs were therefore collected on the windward side of the catching cage and, if inoculated on to the culture media immediately, the Bijou bottle heads were held pointing away from the wind. Speed in technique also helped to overcome danger of contamination. Some of the swabs were replaced in their sterile containers and inoculated within six inches of a primus stove on return to camp. A comprehensive set of controls was taken from the feathers of the geese, the ringers' hands, clothing and beards, and from the gravel and vegetation in the catching area. More controls were also taken to make sure that the swabs and culture media had remained sterile in spite of their travels under adverse conditions.

In Scotland. Results from the Iceland cultures and controls indicated that the technique of inoculating the swabs on to the culture media immediately after being collected from the geese was as satisfactory as bringing the swabs back to camp and inoculating them by a primus stove. All the Scottish swabs were therefore collected from the geese before they were ringed, and inoculated immediately on to the slopes. Controls were again collected from goose feathers, the ringers, from the surrounding vegetation, and also to test that the swabs and medium had remained sterile in transit.

INCUBATION

The two sets of cultures received different treatment. Those from Iceland were inoculated and kept with the caps loose at field temperatures for up to seven days before they were screwed down for despatch to England by air. Three days later the caps were loosened again and the tubes incubated at 25 °C. for seven days before examination. The caps of the Scottish culture bottles

were screwed up immediately after inoculation and sent by post, the tubes remaining closed until they were similarly incubated four days later.

In each case examination consisted of recording those fungi which could be identified immediately, and subculturing the remainder on to 2% malt agar. The subcultures were further incubated and recorded when characteristic growth had appeared. Some of the Iceland cultures may have been 12 days older than the corresponding Scottish ones, a fact which no doubt favoured the appearance of slow-growing fungi from the Iceland inocula.

RESULTS

A. fumigatus was not detected in any of the 350 cultures, either from swabs or controls. Other fungi belonging to 17 different genera were isolated, and eight species were identified. Some of these, for example *Truncatella truncata* and *Pseudogymnoascus roseus*, appear to be new records (regional records, for almost all would count as new records from the ' substrate '), and at least one of the unidentified species, an Ascomycete (P. 71), is probably new. A large number of isolates produced sterile white or dematiaceous mycelium and hence have not been determined further.

None of the species recorded are known to be pathogenic. There is little doubt that the majority grew from spores present on the mucosæ of the geese. The clue to the source of these spores is given in the isolations from the swabs and controls of the Scottish sample, where the fungi recorded from the throats and those from the stubble were in many cases identical. Table I gives details of the fungi isolated, and their frequency in the various samples, together with the total numbers of cultures examined.

From swabs. Fungi were recorded from 23% of the Icelandic cultures and 97% of the Scottish, whilst bacteria occurred in 75% and 100% of the samples respectively. 15% of the first sample gave sterile cultures.

It is the occurrence of large numbers of fungi in the Scottish cultures that is of interest, for most of the species encountered are characteristic of decaying vegetation and more particularly decaying cereals. Thus species of *Fusarium*, *Cephalosporium*, *Trichoderma* and dematiaceous fungi were dominant and were isolated in similar quantities from the controls made by inoculating slopes direct from the stubble. By comparison, the Iceland cultures did not show such an abundance of these species and it is clear that by the time the birds had been in Scotland for a short while they had picked up much of the new fungus content of their throats from their surroundings, either by inhalation or during feeding.

From controls. A comparison of the fungi in the various controls with those from the swabs suggested that little contamination of the cultures had occurred in spite of the adverse conditions. The controls made from the gravel or stubble present in the catching areas gave fungi typically isolated from these substrates, and the extent of the controls seems to have been quite adequate to deal with any suspected sources of contaminants. (Table II).

DISCUSSION

Although *A. fumigatus* was not isolated, the data have proved interesting from several points of view, and suggest ways in which the sampling and culturing technique could be further improved.

The negative finding of this investigation does not indicate that *A. fumigatus* is necessarily absent from the throats of wild Pink-footed Geese, for it seems fairly certain that its normal ubiquitous occurrence would provide a source of spores, even if in small numbers, in almost every environment.

The sampling technique used, although no doubt providing a fairly satisfactory sample of the throat mucus, could not be expected to indicate the spore content of lower portions of the respiratory tract. Moreover, the conditions following the inoculation of the slopes were not ideal for the growth of *A. fumigatus* and the development of other saprophytic fungi was probably favoured at the expense of this species, which requires an unrestricted air supply and relatively high temperature (35–37 °C.) for optimum growth.

A more intensive sampling technique, covering the larynx and tracheæ of the birds, would appear advisable, with a complete examination, both clinical and cultural, of a representative number of respiratory tracts (*i.e.* by killing the birds). During the present investigation however, swabs taken after dissection from the lower parts of the tracheæ of three goslings and one adult bird which had died at the Arnarfellsalda catch (29 July 1953), produced only one positive culture, giving a yeast and bacteria. Moreover in the course of the Agricultural Research Council Survey of Animal Mycoses extensive cultural examination

TABLE I

The fungi isolated

Species and Totals	Swabs		Controls		Totals	
	Site*				Swabs	Controls
	I	S	I	S		
Acremoniella atra (Corda) Sacc.	—	3	—	—	3	—
Aspergillus glaucus series	—	—	3	—	—	3
Cephalosporium spp.	4	13	—	2	17	2
Cladosporium herbarum Link	—	—	1	1	—	2
Fusarium spp.	2	21	2	3	23	5
Mucor spp.	—	—	2	3	—	5
Penicillium spp.	5	—	6	—	5	6
Phoma spp.	2	—	1	—	2	1
Pseudogymnoascus roseus Raillo	1	—	—	—	1	—
Stemphylium sp.	—	—	1	—	1	—
Trichoderma viride Fr.	—	4	—	1	4	1
Truncatella truncata (Lév) Steyeart	2	—	3	—	2	3
Indet. ascomycete (P. 71)	3	—	—	—	3	—
Indet. yeasts	1	2	4	—	3	4
Indet. mycelial fungi (white)	25	31	12	5	37	36
Indet. mycelial fungi (dematiaceous)	4	14	3	6	18	9
Bacteria	192	40	12	3	204	43
Actinomycetes (aerobic)	4	—	2	—	4	2
Sterile slopes	38	—	24	2	38	26
Total fungi isolated	55	58	31	21	112	51
No. of species (or genera if not identified further)	11	6	12	6	13	14
Total no. of cultures	254	40	46	10	294	56

*I = Iceland
S = Scotland

TABLE II

Control from	No. of Tubes							
	Fungi		Bacteria		Sterile		Total No. of Tubes	
	Site*							
	I	S	I	S	I	S	I	S
Gosling feathers	1	—	—	—	—	—	1	—
Adult feathers	5	2	3	2	—	—	6	2
Ringers' hands, clothing, etc. ..	5	—	3	—	1	—	7	—
Gravel or stubble	4	5	—	—	—	—	4	5
Caps left off for varying periods ..	7	—	6	1	8	—	16	1
Swab exposed to air	1	—	1	—	2	—	4	—
Unused swab streaked	—	—	—	—	14	2	14	2

*I = Iceland
S = Scotland

of swabs taken from the larynx and trachea of an adult wild White-fronted Goose from the New Grounds, Slimbridge, by Mr J. A. J. Venn, Veterinary Investigation Centre, Langford, Bristol, gave only two colonies of *A. fumigatus*, in spite of the presence of vast quantities of spores of this fungus on chronic bronchial lesions.

The incorporation of an antibacterial antibiotic into the media, and the replacement of Sabouraud's dextrose by either 2% malt or Czapek-Dox agar would probably enhance the possibility of isolating *A. fumigatus*, whilst the supplementary use of a dilution technique for a number of samples would check the efficiency of the swab-streak method for obtaining a true picture of the viable fungi present. Further, incubation as soon as possible after inoculation at a temperature of 35–37° C. would minimise the chances of other fungi suppressing the *A. fumigatus*, but this might not be easy in the field.

One point of considerable interest in these samples has been the number of isolations and the species of fungi found in the samples from the Scottish grounds. Here it is evident that the fungus content of the throat was derived directly from the stubble on which the geese were feeding and that in this way the fungus spores present on the mucosæ change in number and species with the environment of the birds. Thus *Truncatella truncata* cultured from two swabs in Iceland is chiefly known from plant material. It was also obtained from the feathers of one bird and appeared in a tube exposed to the air for an hour at the collecting site. Similarly, *Trichoderma viride* is a common soil fungus and was obtained in Scotland from four throat swabs and from the stubble of the catching area. Of other fungi common to both collecting grounds, the species of *Fusarium* were most abundant. These fungi are characteristic of decaying plant remains, especially cereals, and appeared in half the Scottish swab cultures and all three controls from the stubble, but only in two swab cultures and two controls from Iceland. It is expected therefore that *A. fumigatus* will be picked up from the surrounding plant life and soil as readily as those species of fungi found in this investigation.

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ASPERGILLOSIS IN WILD GEESE: A NEW TECHNIQUE FOR ITS DETECTION

A three-year study of the disease is going forward at the New Grounds. Mr J. V. Beer contributes the following note :

At the end of 1954 a new swabbing technique was used to detect *Aspergillus fumigatus* in Pink-footed Geese caught in rocket-nets in Britain. The technique aims at taking a fuller sample from the throat of the goose than the stick and cotton-wool method.

Briefly the methods of isolation are as follows. A rubber-tube swab is used to obtain material from the throat of the goose and is then washed in a sterile malt extract solution contained in a small screw cap bottle. This solution contains an antibiotic to keep down the growth of bacteria. The sample bottles are returned to the laboratory and kept in a refrigerator until the end of the expedition when a start is made to isolate the moulds. In isolating the mould the methods used were such that bacteria were completely inhibited from growing and large numbers of other moulds were inhibited by the relatively high incubation temperature. The moulds are grown out on a solid medium, of similar composition to that above, in a petri dish. Incubation is carried out at 40°C for one week. Suspected *A. fumigatus* colonies are purified by several successive transfers onto Czapek-Dox agar in petri dishes. Incubation is again carried out at 40°C and for 3–4 days. The purified culture is grown on a Czapek-Dox agar slope in a test-tube and kept at a low temperature. This culture is used to produce more growth for diagnostic studies and for experimental work.

A. fumigatus was found on 7 of 235 swabs. Just over 350 geese were swabbed and the remaining material is being worked up.

The full significance of these isolations is difficult to assess as the mould is a fairly common soil inhabitant.