The Microbial Flora of Broiler-house Litter and Dust

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SUMMARY

A survey of litter samples from broiler houses at widespread localities in the U.K. revealed a significant difference between the fungal floras before and after the houses had been used for a single crop of birds. *Paecilomyces varioti*, *Trichoderma* spp. *Aureobasidium pullulans*, and *Hyalodendron lignicola* predominated in the fresh litter, but were replaced by *Scopulariopsis brevicaulis* and *Aspergillus* spp. in the final samples. The fungi present in the final litter samples were also found in the initial and final dust samples, indicating that the dust is a potential source of inoculum. These results are discussed in relation to the formation in broiler-house litter of 2,3,4,6-tetrachloroanisole (a compound associated with a musty taint in chickens), and to the potential formation of mycotoxins.

INTRODUCTION

Curtis *et al.* (1972), reported a musty taint in broiler chickens and showed that it was caused by 2,3,4,6-tetrachloroanisole. Evidence was obtained that this anisole is formed by the microbiological methylation of 2,3,4,6-tetrachlorophenol, and three fungal species isolated from litter associated with musty birds were shown to be capable of methylating this phenol. Published information about the microbial flora of broiler-house litter is restricted to the reports of Halbrook, Winter & Sutton (1951), Schefferle (1965) and Lovett, Messer & Read (1971). Only the last authors examined the fungal flora in any detail, and then carried identification only to the generic level. The present survey was undertaken to determine the fungal flora of broiler house litters and to establish whether the flora of litters containing 2,3,4,6-tetrachloroanisole and giving rise to musty birds is unusual.

METHODS

Samples of litter and dust

Samples of litter and dust were taken from 31 broiler houses at different localities in the United Kingdom (Cambridgeshire, Co. Durham, Essex, Hertfordshire, Lincolnshire, Norfolk, Sussex, Wiltshire, Yorkshire and Aberdeenshire) before and after they had been used for a single crop of birds. The average time for a crop of birds to be in a broiler house was 56 days. The huts sampled were heated in three ways: viz. with brooder heaters in a single longitudinal bank down the centre of the hut (type A); in two or more parallel longitudinal banks (type B); or by hot air blown into the hut from one end (type C) (Fig. 1). When the chicks are initially placed in either of hut types A or B they congregate beneath the heaters whereas in type C their movement is restricted by a movable curtain. In hut types A and B samples were taken from each square yard of a rectangular area of approximately 30 sq yards (i.e. 30 samples), covered by 500 to 600 birds. The width of these huts differed; the area sampled always extended from the centre of the hut to the side wall,

Fig. 1. The position of sampling areas in the three types of broiler houses.

midway along the length of the hut. In huts of type C, 30 samples were taken at approximately equal intervals along a central line from one end to the other. Each sample was taken through the whole depth of the litter. The combined sample from each hut weighed approximately 2.0 to 3.0 kg for fresh litter and approximately 4.0 to 5.0 kg for used litter. Where available, samples of dust of at least 1 g were taken from beams and ledges above the area used for sampling the litter. All samples were collected in unused, clean polythene bags and mixed by shaking thoroughly before mycological examination and moisture determinations. The average air temperature in the broiler house at the end of the rearing cycle was 19.5 °C. Litter temperatures were not recorded, but in a previous similar survey they were approximately 5 °C higher than the corresponding air temperatures.

Samples of litter associated with musty birds were those used in previous work (Curtis *et al.* 1972) and were examined at that time.

Isolation and identification of organisms

All samples of litter and dust were treated as follows. Each of three 0.5 g portions was added to 10 ml of a sterile 0.1 % (v/v) solution of Tween 80 in 1 oz Universal square-based masticator bottles and macerated for 1 min by a M.S.E. masticator (Measuring and Scientific Equipment, London). Samples (0.1 ml) of appropriate dilutions were spread over the whole surface of dried agar plates. The following media were used: (1) 2 % Difco Bacto malt extract (Difco Laboratories, West Molesey, Surrey), pH 4.0, solidified with 2 % Davis New Zealand Agar. The agar was autoclaved separately to avoid breakdown at acid pH. (2) Oxoid Sabouraud Dextrose agar (Oxoid Ltd, London), pH 5.6, containing 12 µg/ml of penicillin and 40 µg/ml of streptomycin sulphate. (3) 2.1 % Difco Bacto YM broth, pH 3.4, solidified with 2 % Davis New Zealand Agar (Davis Gelatine Ltd, Warwick). The agar was

autoclaved separately to avoid breakdown at acid pH. (4) Difco Bacto Heart Infusion Agar, pH 7.4.

Viable mould counts were made on media 1 and 2 and viable yeast counts on medium 3, after 5 days of incubation at 25 °C. Total viable bacterial counts were made on medium 4 after 2 and 4 days of incubation at 37 °C and 25 °C respectively. The presence of one colony on the lowest dilution plate corresponded to a count of 200 viable units/g of litter or dust.

The moulds were identified as far as possible, usually to the genus, on the isolation plates. Representative colonies including those which could not be identified on the isolation plates, were subcultured, purified and stored on Difco Bacto Malt Extract agar slopes at 1 °C before further identification. All isolates were grown on malt extract agar for identification. *Aspergillus* spp. and *Penicillium* spp. were also grown on Czapek Dox agar, containing either 3 % or 20 % sucrose.

Determination of moisture contents of litter

The moisture content of samples of fresh shavings and used litter was determined by drying 5 g and 20 g samples respectively to constant weight at 120 °C.

RESULTS

The micro-organisms found in the initial and final samples of litter and dust are shown in Tables 1 and 2. The mean total fungal count (viable units/g) of all the initial litters was slightly lower than that of the final samples of litter. By contrast there was little difference between the initial and final dust samples, but the counts for the dust were consistently higher than those for the litters. More bacteria were present in the dust than in the litter. The bacterial counts were similar at 25 °C and 37 °C except that with four samples of dust no bacteria grew on plates incubated at 37 °C although the count at 25 °C exceeded 10⁶/g. The total bacterial counts of the initial and final samples of dust were similar, but the count for the final litter samples was consistently higher than that of the initial litter samples (cf. Schefferle, 1965). The total yeast count was similar in both the initial and final samples of litter and dust.

The majority of dust samples had a different fungal flora from that of the initial litter. *Aspergillus* spp. especially *A. sydowi*, *A. repens* and *A. candidus*, and *Scopulariopsis brevicaulis* predominated in the dust, while *Paecilomyces varioti*, *Trichoderma* spp., *Aureobasidium pullulans* and *Hyalodendron lignicola* were most abundant in the fresh litter. *Penicillium* spp. were present in over 90 % of initial litter and dust samples. Where *Aspergillus* spp. and *S. brevicaulis* were detected in the fresh litter the counts were much lower than in the dust.

The mould flora of the final litter, collected after the birds had been on the litter for between 52 and 77 days, was very different from that of the initial shavings. *Aureobasidium pullulans*, *Trichoderma* spp. and *Hyalodendron lignicola* were not found in the final litter, and there was a marked decrease in the incidence of *Paecilomyces varioti*. Although the shavings may be diluted up to ten-fold by the faeces, between initial and final sampling (L. J. Parr, M. G. Gee, D. G. Land and D. Robinson, unpublished), dilution alone does not account for the above observations. *Aspergillus* spp. and *Scopulariopsis brevicaulis* predominated in the final litter samples. Of the former, *A. sydowi*, *A. repens* and *A. candidus* occurred most frequently, while *A. ruber* and *A. chevalieri* were found in approximately 50 % of the samples and *A. versicolor*, *A. amstelodami*, and *A. flavus* in 25 % of the samples. The combined count for *Penicillium* spp. was less in the final litter than initially but *P. crustosum* still remained the predominant member of the genus. *Absidia cylindrospora* and

Table 1. *Microbial flora of poultry litter and dust: initial samples*

The *Penicillium* species isolated from the shavings included *P. crustosum*, *P. brevicompactum*, *P. notatum*, *P. roqueforti*, *P. frequentans*, *P. waksmani*, *P. janthinellum*, *P. nigricans*, *P. corylophilum*, *P. citrinum*, *P. carneo-lutescens*, *P. viridicatum*, *P. lanosum*, and *P. funiculosum*, while *P. crustosum*, *P. brevicompactum*, *P. chrysogenum*, *P. notatum*, *P. frequentans*, and *P. waksmani* were isolated from the dust samples.

	Incidence		Maximum count/g	
	Shavings	Dust	Shavings	Dust
Total no. of samples	31	24	—	—
Fungal species				
<i>Aspergillus sydowi</i>	6	24	8.0×10^2	7.3×10^6
<i>A. versicolor</i>	3	8	7.2×10^2	7.0×10^5
<i>A. repens</i>	4	20	4.0×10^2	6.0×10^6
<i>A. amstelodami</i>	1	4	6.0×10^1	2.5×10^6
<i>A. ruber</i>	0	9	—	7.0×10^6
<i>A. chevalieri</i>	1	11	6.0×10^3	7.0×10^5
<i>A. flavus</i>	0	4	—	3.3×10^5
<i>A. flavus</i> var. <i>columnaris</i>	0	1	—	9.0×10^3
<i>A. niger</i>	2	1	1.3×10^2	1.3×10^2
<i>A. petrakii</i>	0	3	—	3.0×10^3
<i>A. wentii</i>	1	0	6.6×10^1	—
<i>A. candidus</i>	3	18	6.0×10^2	2.5×10^6
<i>Scopulariopsis brevicaulis</i>	10	17	2.7×10^3	7.8×10^7
<i>Penicillium</i> spp.	30	21	2.2×10^6	2.9×10^6
<i>Absidia cylindrospora</i>	9	12	4.0×10^3	1.2×10^4
<i>Mucor racemosus</i>	2	6	6.0×10^2	8.0×10^3
<i>Rhizopus oryzae</i>	3	3	6.0×10^2	4.0×10^4
<i>Paecilomyces varioti</i>	20	2	8.6×10^4	2.0×10^3
<i>Trichoderma viride</i>	7	0	6.0×10^3	—
<i>T. hamatum</i>	11	1	1.3×10^4	1.0×10^5
<i>T. harzianum</i>	2	0	1.3×10^3	—
<i>T. koningii</i>	3	0	6.0×10^3	—
<i>Hyalodendron lignicola</i>	16	0	1.7×10^6	—
<i>Acremonium</i> spp.	6	2	8.6×10^4	1.0×10^3
<i>Cladosporium herbarum</i>	7	0	2.0×10^4	—
<i>Phialophora fastigiata</i>	2	0	6.6×10^5	—
<i>Graphium penicillioides</i>	4	0	6.0×10^4	—
<i>Alternaria</i> spp.	1	0	2.6×10^3	—
<i>Stemphylium botryosum</i>	1	0	6.6×10^1	—
<i>Fusarium</i> sp.	1	0	6.6×10^3	—
<i>Sepedonium</i> sp.	0	1	—	7.0×10^4
<i>Mycelia sterilia</i>	1	0	6.6×10^1	—
<i>Basidiomycete</i>	1	0	4.6×10^4	—
<i>Sphaeropsidales</i>	2	0	2.1×10^5	—
<i>Aureobasidium pullulans</i>	30	0	1.4×10^6	—
<i>Yeast</i> spp.	29	19	3.5×10^7	1.9×10^7
Total mould count (mean)	—	—	4.0×10^5	1.7×10^7
Bacteria (37 °C)	30	20	1.1×10^7	9.5×10^9
Bacteria (25 °C)	31	24	3.8×10^7	1.3×10^{10}

Incidence denotes the number of samples from which each species was isolated.

Mucor racemosus occurred at similar frequencies in the initial and the final litter. The fungal species present in the final litter were thus closely similar to the species present in the dust at this time. The incidence of *Aspergillus chevalieri*, *A. amstelodami*, *A. ruber*, *A. flavus*, *Absidia cylindrospora*, *Mucor racemosus*, *Circinella simplex*, *Botrytis cinerea*, *Cladosporium herbarum*, *Aureobasidium pullulans* and yeast species increased in the dust during the growth of the birds.

Table 2. *Microbial flora of poultry litter and dust: final samples*

The *Penicillium* species isolated from the litter included *P. crustosum*, *P. chrysogenum*, *P. brevicompactum*, *P. roqueforti*, *P. diversum*, *P. corylophilum*, *P. frequentans*, *P. cyaneum*, *P. waksmani*, *P. biforme*, *P. lilacinum*, *P. variable*, while *P. crustosum*, *P. chrysogenum*, *P. brevicompactum*, *P. roqueforti*, *P. corylophilum*, *P. corymbiferum*, and *P. steckii* were isolated from the dust samples.

	Incidence		Maximum count/g	
	Litter	Dust	Litter	Dust
Total no. of samples	31 (19)	30	—	—
Fungal species				
<i>Aspergillus sydowi</i>	23 (10)	30	1.0×10^7 (1.1×10^7)	1.7×10^7
<i>A. versicolor</i>	8 (5)	10	2.1×10^6 (2.4×10^5)	1.0×10^6
<i>A. repens</i>	26 (5)	29	6.0×10^5 (5.0×10^5)	1.7×10^7
<i>A. amstelodami</i>	7	8	6.0×10^4	3.0×10^6
<i>A. ruber</i>	16 (6)	16	1.2×10^6 (9.0×10^5)	3.1×10^6
<i>A. chevalieri</i>	15	24	2.7×10^5	3.0×10^6
<i>A. chevalieri</i> var. <i>intermedius</i>	1	1	2.0×10^4	3.0×10^5
<i>A. flavus</i>	7	12	1.5×10^4	5.0×10^5
<i>A. flavus</i> var. <i>columnaris</i>	0	3	—	2.0×10^6
<i>A. restrictus</i>	1	1	8.7×10^5	6.0×10^6
<i>A. petrakii</i>	1	0	6.0×10^3	—
<i>A. candidus</i>	26 (15)	25	2.4×10^6 (1.0×10^7)	9.0×10^6
<i>A. wentii</i>	0	1	—	1.0×10^5
<i>A. niger</i>	0	1	—	1.0×10^4
<i>Scopulariopsis brevicaulis</i>	30 (18)	21	1.4×10^6 (1.2×10^7)	4.0×10^7
<i>Paecilomyces varioti</i>	3	5	6.0×10^3	1.0×10^4
<i>P. parvus</i>	1	0	6.0×10^4	—
<i>Penicillium</i> spp.	21 (12)	29	6.6×10^5 (1.0×10^6)	6.0×10^6
<i>Absidia cylindrospora</i>	7	19	6.0×10^3	3.0×10^5
<i>Mucor racemosus</i>	1	11	6.0×10^2	1.2×10^6
<i>Circinella simplex</i>	0	4	—	1.0×10^5
<i>Rhizopus oryzae</i>	0	5	—	2.0×10^4
<i>Botrytis cinerea</i>	1	5	6.0×10^2	1.0×10^5
<i>Cladosporium herbarum</i>	3	6	4.0×10^4	3.0×10^5
<i>Acremonium</i> spp.	2	0	4.6×10^4	—
<i>Chrysosporium keratinophilum</i>	0	1	—	1.0×10^3
<i>Monascus ruber</i>	2	0	1.0×10^2	—
<i>Alternaria</i> spp.	2	2	2.0×10^4	4.0×10^4
<i>Aureobasidium pullulans</i>	0	3	—	7.0×10^4
Yeast spp.	24 (13)	30	3.5×10^7 (4.0×10^8)	6.0×10^7
Total mould count (mean)	—	—	1.9×10^6	2.2×10^7
Bacteria (37 °C)	31	30	1.5×10^{10}	8.2×10^9
Bacteria (25 °C)	31	30	1.1×10^{10}	9.6×10^9

Incidence denotes the number of samples from which each species was isolated. Figures in parentheses show the incidence and maximum counts for the predominant fungi isolated from 19 samples of litter, associated with musty birds, examined previously.

Although the maximum mould count in the initial and final samples of litter ranged between 10^5 and 10^7 /g, many samples had a much lower count for most of the species. This is in contrast with the counts for the dust samples, which showed less variability.

Samples of the final litter associated with musty birds (Curtis *et al.* 1972) had a similar fungal flora to the present final litter samples in that *Scopulariopsis brevicaulis*, *Penicillium* spp. and *Aspergillus* spp. were the predominant fungi (Table 2). Although fewer *Aspergillus* spp. were isolated and a lower incidence of *A. repens* and *A. ruber* recorded, the maximum counts of the species present were of the same order as those in the present survey.

The moisture levels of the fresh shavings ranged from 9.0 % to 26.0 %, although the majority of samples varied between 11.0 % and 18.0 %. The used litter samples generally had a higher moisture content ranging from 19.0 % to 38.0 %, with the majority between 25.0 % and 32.0 %.

DISCUSSION

The fungal floras of the present samples of litter were remarkably uniform, and consistently showed a complete change after the growth of the birds on the litter. Lovett *et al.* (1971) did not observe a change in composition of the fungal population after the growth of the birds, and, although they report a high incidence of *Scopulariopsis*, *Aspergillus* spp. appear to be predominant in the present litter samples. Detailed comparison with their results cannot be made because they did not distinguish the various species of fungi. The number of bacteria in the used litters was of the same order as that reported by Lovett *et al.* (1971), Halbrook *et al.* (1951), and Schefferle (1965).

Most samples of shavings derived from imported timber contain chlorophenols (Parr *et al.* unpublished). The microbiological methylation to the corresponding chloroanisoles has been shown to occur in poultry litter and in pure cultures of *Aspergillus sydowi*, *Penicillium crustosum* and *Scopulariopsis brevicaulis* isolated from poultry litter (Curtis *et al.* 1972). Other fungi and bacteria in the litter may be capable of methylating chlorophenols, but their role has not yet been investigated.

Analysis of litter samples from the present survey showed that there was a marked decrease in the concentrations of both 2,3,4,6-tetra- and pentachlorophenol in the litter during the growth of the birds (Parr *et al.* unpublished). Some of the final litter samples in the present survey had a distinctly musty odour, and a slight musty odour could be detected in at least 50 % of the samples. In 83 % of the samples 2,3,4,6-tetrachloroanisole was detected (Parr *et al.* unpublished); the concentrations varied considerably and the greatest was 3.6 μ g/g whereas the experience in this laboratory is that tainted birds are associated only with concentrations of 5 μ g/g or above. We have found no major difference between the fungal flora of litter giving rise to musty birds, and of litter containing concentrations of tetrachloroanisole lower than 5 μ g/g. Mustiness in broiler chickens may occur only when there is a relatively high concentration of chloroanisoles in the litter immediately prior to harvesting of the birds. Such concentrations may be localized in a hut and be transient owing to the volatility of the chloroanisoles. It is unlikely that chloroanisoles are derived other than from the corresponding chlorophenols in the litter.

Some of the more numerous fungi found in the litters, notably certain species of *Aspergillus* and *Penicillium*, are potential mycotoxin producers. Some strains of *Scopulariopsis* have also been shown to produce toxic metabolites (Christensen, Nelson, Mirocha & Bates, 1968; Martin, Gilman & Keen, 1970; Lovett, 1972). Martin *et al.* (1970) reported a strain of *S. brevicaulis* to be toxic to ducklings. Although production of such toxins in the litter has not been proved, Lovett (1972) drew attention to the importance of this potential in the light

of the proposed use of poultry-house litter as a nitrogen source in livestock feeds (Walters, 1972).

Wood shavings initially have a low moisture content (9.0 % to 26.0 %). It is therefore significant that they should contain a number of species of the *Aspergillus glaucus* group (*A. repens*, *A. ruber*, *A. amstelodami* and *A. chevalieri*) which are characteristic of low moisture environments, and have been shown to initiate growth at low water activities (Snow, 1949; Scott, 1957; Ayerst, 1969). Semeniuk, Nagel & Gilman (1947), in their report on the development and deterioration of stored yellow dent shelled corn, state that the real importance of the *A. glaucus* group, along with the *A. restrictus* group, lies in their ability to initiate growth at minimum moisture levels, thus facilitating the invasion of slightly less xerophilic moulds, such as *A. candidus*, *A. versicolor*, *A. flavus*, and certain species of *Penicillium*, as additional water is released by their metabolic activities.

The fungal floras of both initial and final dust samples were similar to that of the final litter. Environmental conditions in the poultry houses appear to favour the development of organisms present in the dust rather than those present on the shavings initially. We have examined samples of feed for the presence of fungi and, contrary to the findings of Lovett *et al.* (1971), found no significant mould contamination. It would be impractical to remove all the dust from a broiler house during the routine cleaning between rearing cycles; observations from the present survey suggest that this dust is the major source of inoculum for each batch of fresh shavings.

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