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EXPOSURE TO NON-INFECTIOUS MICROORGANISMS AND ENDOTOXINS IN AGRICULTURE*

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Abstract: Farmers and farm workers can be exposed to large concentrations of airborne fungi and bacteria including actinomycetes, and microbial constituents such as endotoxins. Measurement methods for microorganisms may give different results and need to be further developed to allow personal sampling and species characterization of viable and non-viable microorganisms. Farm work includes many differently exposed tasks and processes. A large number of measurements is therefore needed to estimate long-term exposure in epidemiological studies of farming populations. A more efficient strategy is probably exposure modeling using different determinants of exposure. The literature was therefore reviewed for studies on determinants of exposure to microorganisms in agriculture. In most studies tasks, process and/or production had been studied. Little information is available on other determinants. It was found that exposure to fungi was high during handling grain, hay and bedding material, especially when mouldy, and when tending cattle; exposure to bacteria was high during handling of grain, hay and bedding material and in animal houses; and exposure to endotoxins was high during chopping of bedding material and in animal houses except probably in cowsheds. Exposure showed wide variability between measurements of the same task even within studies. Further studies of other determinants of exposure are recommended in order to improve the accuracy of exposure assessment in epidemiological studies and in future compliance testing.

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BIOAEROSOLS IN AGRICULTURE

Farmers and farm workers are often exposed to airborne dust, especially when working with plant and animal material. Plant fragments and skin scales form a large part of this dust [18]. Microorganisms can be important components as well since they occur naturally in such materials as manure, silage, and compost and can colonize other farm materials when conditions are favourable for growth, e.g., during storage of moist grain, hay and straw.

Infectious microorganisms must be viable to cause infections, but infectious as well as non-infectious microorganisms may pose other health hazards even if they are dead and disintegrated. Inhalation of non-infectious microorganisms and their constituents can cause inflammation of the respiratory system while antigens and allergens may activate the immune system and cause allergic and immunotoxic effects [43, 44, 57].

Structural constituents of microorganisms are referred to as primary metabolites while compounds excreted by microorganisms into the environment, e.g., mycotoxins

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and proteolytic enzymes, are referred to as secondary metabolites. Examples of primary metabolites that have been associated with health effects are endotoxins present in the cell walls of Gram-negative bacteria, and (1→3) and (1→6)-β-glucans in the cell walls of many fungi. Secondary metabolites may be found in particles from colonized materials as well as in the microorganisms and their spores. Mycotoxins can be highly toxic and carcinogenic. They can cause intoxication in animals and in man if their food is infested with toxigenic fungi. The contribution from airborne mycotoxins to occupational disease among farmers needs further clarification, however, as exposure by inhalation is much lower than oral exposure although the lungs may be more susceptible to toxic effects than is indicated by oral dosing [37].

The need for specific identification of microorganisms is dependent on whether exposure-response relationships differ between species. A few animal studies suggest that such differences do exist [4, 23, 60]. However, much more data on exposure-response relationships from human studies is needed before health based occupational exposure limits for specific organisms or broader taxonomic groups can be established. The same applies to substances of microbial origin such as glucans and allergens. The situation is different for endotoxins as experimental and epidemiological studies have provided human data that have been regarded as a sufficient basis to propose health based occupational exposure criteria [30].

Most microorganisms have small cells with bacterial cells typically 0.5-5 μm diameter and fungal spores 2-10 μm. Spores from fungi and actinomycetes are often liberated as single spores or small aggregates when mouldy hay and grain is handled and many inhaled spores may reach the alveoli. However, microbial particles may also show larger sizes as microorganisms can form big aggregates and/or be attached to other particles.

MEASUREMENT METHODS

Culture methods have been used widely for measurements of airborne microorganisms in the work environment [12, 13, 21]. Microorganisms that are able to grow in culture with the media and incubation temperatures used can be measured, and species identification is most readily done by these methods. However, non-culturable microorganisms which can also cause health effects cannot be measured. The total number of microorganisms is generally substantially underestimated by culture-based-methods since often only 1 in 10 microbial cells, sometimes only 1 in 1000 are culturable. The proportion of culturable microorganisms within the same work environment may even vary widely over time [8, 28]. A further disadvantage of many culture methods is that most sampling instruments cannot be used as personal samplers and the sampling time is short, typically less than 1 h and less than 1 min in highly contaminated work environments. However, more hardy microorganisms as spores from fungi and actinomycetes can be collected on filters using personal sampling instruments and prolonged sampling periods.

Table 1. Sources of microorganisms on the farm (summarised from Lacey and Dutkiewicz [38]).

Taxonomic group	Source
Gram-negative bacteria	Cereal grain, plant surfaces, animal breeding
Gram-positive cocci	Animal breeding
Corynebacteria	Animal breeding, plant dust
Actinomycetes	Stored hay and grain, compost, soil
Fungi	Plant surfaces, stored hay and grain, compost

Filter samples can also be analysed by non-culture-based methods [21]. The total number of microorganisms can be measured by microscopical methods such as light, fluorescence and scanning electron microscopy. Their potential for identification of species is limited although classification by morphological features is feasible in some work environments, e.g. fungal spores in sawmills [22]. Metabolites from microorganisms can be measured with bioassays and immunoassays, and by chemical and molecular biological methods.

Endotoxins are usually measured by biological assays based on the reaction of *Limulus* Amoebocyte Lysate (LAL) with lipopolysaccharide [29, 47, 55]. Endotoxins and (1→6)-β-glucans can also be measured as specific markers for Gram-negative bacteria and fungi, respectively. Immunoassays and molecular biological methods usually have high specificity and allow detection of specific organisms in complex environments. However, the microflora on the farm is complex [19, 20, 38] and many different assays are needed for characterisation of the work environment unless assays for broader taxonomic groups of microorganisms are used.

EXPOSURE ASSESSMENT

The assessment of exposure to non-infectious microorganisms in epidemiological studies and compliance testing, once occupational exposure limits have been established, should be based on methods that allow personal sampling. In epidemiological studies both short term and long term exposure estimates are interesting as little is known about exposure-response relationships.

Farm work includes many different tasks and processes each differing in the nature of exposure to microorganisms. Since concentrations of microorganisms in the farm environment are often changeable, a large number of measurements is needed if long-term exposure of a single farmer or farm worker is to be estimated by task specific measurements. The measurement effort may be reduced by exposure modelling [7] which has been described in a

Table 2. Exposure to fungi measured in farm environments by culture methods.

Task	Reference	Fungi, cfu/m^3
		10^3 10^4 10^5 10^6 10^7 10^8
Grain		
harvest	Batel [5]	⟷
drying	Lappalainen *et al.* [39]	⟷
handling	Kotimaa *et al.* [32]	⟷
	Kotimaa [33]	⟷
crushing	Wardrop *et al.* [63]	⟷
	Lappalainen *et al.* [39]	⟷
Hay handling		
unbaling	Wardrop *et al.* [63]	⟷
loose hay	Kotimaa *et al.* [32]	x
baled hay		x
?	Kotimaa [33]	⟷
Bedding material		
handling straw	Kotimaa [33]	⟷
chopping[a]	Pratt *et al.* [52]	⟷
chopping, dry	Jones *et al.* [31]	⟷
chopping, wet		⟷
Dairy and cattle		
cowshed air	Batel [5]	x
	Hanhela *et al.* [25]	⟷
	Dutkiewicz *et al.* [20]	⟷
tending	Pasanen *et al.* [51]	⟷
	Lappalainen *et al.* [39]	⟷
Horse		
horse stable air	Dutkiewicz *et al.* [20]	⟷
Swine		
pig house air	Batel [5]	x
	Clark *et al.* [10]	< >
	Travers *et al.* [61]	⟷
	Bækbo [2]	? ⟷
	Cormier *et al.* [11]	→
	Dutkiewicz *et al.* [20]	⟷
	Crook *et al.* [14]	⟷
tending	Haglind *et al.* [24]	x
Poultry		
poultry house air	Batel [5]	x
	Clark *et al.* [10]	⟷
turkey house air	Mulhausen *et al.* [49]	⟷

⟷ = range; x = single value or narrow range; [a] = only *Aspergillus fumigatus*

study of pig farmers using relationships between measured exposure to endotoxins, farm characteristics and duration of tasks [53]. Relationships between exposure to microorganisms and determinants of exposure, e.g. materials, processes, tasks and modifying factors therefore are interesting for exposure modelling in epidemiological studies. Such relationships may also be useful in future compliance testing.

Table 3. Exposure to bacteria including actinomycetes measured in farm environments by culture methods.

Task	Reference	Bacteria incl. actinomycetes, cfu/m^3
		10^3 10^4 10^5 10^6 10^7 10^8 10^9
Grain		
harvest	Batel [5]	⟷
handling	Kotimaa *et al.* [32]	⟷
handling	Kotimaa [33]	⟷
crushing	Wardrop *et al.* [63]	⟷
Hay handling		
unbaling	Wardrop *et al.* [63]	⟷
loose hay	Kotimaa *et al.* [32]	⟷
baled hay		⟷
Bedding material		
handling straw	Kotimaa [33]	⟷
Dairy and cattle		
tending	Kotimaa *et al.* [32]	⟷
cowshed air	Batel [5]	x
	Dutkiewicz *et al.* [20]	⟷
Horse		
horse stable air	Dutkiewicz *et al.* [20]	⟷
Swine		
pig house air	Curtis *et al.* [15]	⟷
	Batel [5]	x
	Clark *et al.* [10]	⟷
	Travers *et al.* [61]	⟷
	Bækbo [2]	? ⟷
	Cormier *et al.* [11]	⟷
	Crook *et al.* [14]	⟷
	Heederik *et al.* [27]	⟷
	Dutkiewicz *et al.* [20]	⟷
tending	Haglind *et al.* [24]	x
	Attwood *et al.* [1]	⟷
Poultry		
poultry house air	Batel [5]	x
	Clark *et al.* [10]	⟷
tending	Reynolds *et al.* [56]	⟷

⟷ = range; x = single value or narrow range

DETERMINANTS OF EXPOSURE

Microorganisms are common constituents of materials that are handled on the farm such as manure, silage and compost, and can also be found on plant surfaces, skin scales and in soil [38]. Many different species of Gram-negative bacteria, of Gram-positive bacteria including actinomycetes, and of fungi have been identified in the farm environment [19, 20, 38]. The most important sources for main groups of bacteria and fungi as recognised by Lacey and Dutkiewicz [38] have been summarised in Table 1.

Very high exposure may occur if mouldy grain and hay are handled. During storage, plant materials can be colonised by microorganisms if the water content is too high, typically >14%. As water content increases, increasing metabolic activity leads to spontaneous heating. With water contents greater than 35%, temperatures of 65°C may be attained. There is also a fire risk as combustible compounds are produced during

Table 4. Exposure to microorganisms measured in farm environments by culture methods.

Task	Reference	Microorganisms cfu/m^3
		10^3 10^4 10^5 10^6 10^7 10^8 10^9
Grain		
silo unloading	May *et al.* [45]	⟷
Dairy and cattle		
cowshed air	Dutkiewicz [19]	x
Poultry		
poultry house air	Dutkiewicz [19]	⟷
Vegetables		
tomato greenhouse	Davies *et al.* [17]	⟷
greenhouse	Blomquist *et al.* [6]	⟷

⟷ = range; x = single value or narrow range

Table 5. Exposure to microorganisms in farm environments measured by non-culture methods.

Task	Reference	Microorganisms cells/m^3
		10^3 10^4 10^5 10^6 10^7 10^8 10^9 10^9
Grain		
harvest	Darke *et al.* [16]	⟷
drying	Lappalainen *et al.* [39]	⟷
barley unloading	Lacey [35]	⟷
crushing	Lappalainen *et al.* [39]	⟷
Hay		
harvest, baling	Lacey [35]	⟷
shaking mouldy hay	Lacey & Lacey [34]	
- fungi		⟷
- actinomycetes		⟷
Dairy and cattle		
cowshed air	Baruah [3]	⟷
	Hanhela *et al.* [25]	⟷
tending	Larsson *et al.* [40]	⟷
	Rask-Andersen *et al.* [54]	⟷
	Pasanen *et al.* [51]	⟷
	Lappalainen *et al.* [39]	⟷
milking	Lacey & Lacey [34]	
- actinomycetes		⟷
- fungi		⟷
Mushrooms		
compost tipping and spawning	Lacey [35]	
- actinomycetes		⟷
growing	Sastre *et al.* [58]	
- Shiitake spores packing		x
- Shiitake spores		⟷

⟷ = range; x = single value or narrow range

Table 6. Exposure to endotoxins in farm environments.

Task	Reference	Endotoxin, ng/m^3
		10^1 10^2 10^3 10^4 10^5 10^6
Bedding material		
chopping	Olenchock *et al.* [50]	⟷
chopping dry	Jones *et al.* [31]	⟷
chopping, wet		⟶
Dairy and cattle		
tending	Rask-Andersen *et al.* [54]	⟶
cowshed air	Dutkiewicz *et al.* [20]	⟷
Horse		
horse stable air	Dutkiewicz *et al.* [20]	⟷
Swine		
pig house air	Crook *et al.* [14]	⟷
	Attwood *et al.* [1]	⟷
	Heederik *et al.* [26]	⟷
	Vinzents *et al.* [62]	⟷
	Preller *et al.* [53]	⟷
	Dutkiewicz *et al.* [20]	⟷
weighing pigs	Larsson *et al.* [41]	⟷
Poultry		
tending	Reynolds *et al.* [56]	⟷
collection, loading, unloading birds	Thelin [59]	⟶

⟷ = range; x = single value or narrow range

microbial growth. Thermotolerant and thermophilic fungi and actinomycetes usually dominate the microflora after 'spontaneous' heating [36] and these microorganisms may produce large numbers of spores which are easily liberated into the air. In a Finnish study it was found that exposure during handling of stored hay, grain and straw increased with storage time of the hay [33].

The water content of farm materials influences exposure also in a different way as dust is much more easily released from dry than from damp materials, e.g., during bedding chopping [31].

Many other factors can be expected to influence exposure, and factors may also be interrelated. E.g. mechanical operations may liberate more dust into the air than manual work but the worker may be located further from the source during mechanical operation and may even be enclosed in a ventilated cab. The exposure time is also likely to be shorter. Type of equipment, housing and ventilation were related to dust concentrations in poultry houses [42] and farm characteristics and activities were related to endotoxin exposure of pig farmers [53]. Only few studies have been found of other determinants of exposure to microorganisms than task, production and process.

EXPOSURE TO BIOAEROSOLS ON THE FARM

Studies of task, production and process specific exposure have been stratified and are summarised in Tables 2 to 6. The results should be compared with care, as different sampling and analytical methods, sampling times and strategies may have been used.

The results indicate that different tasks and forms of production may lead to different exposure of workers. Exposure to fungi and bacteria (mainly actinomycetes) from handling grain, hay and bedding material is all in the same range and may be very high, >10^8 cfu/m^3, e.g., when mouldy material is handled. Similar concentrations of airborne fungi have been found when tending cattle but smaller concentrations when tending swine and poultry, typically <10^5 cfu/m^3. Concentrations of bacteria are generally large in animal houses, up to 10^8 cfu culturable microorganisms/m^3 and 10^9 total cells/m^3. Concentrations of culturable microorganisms were about an order of magnitude less than concentrations determined with non-viable methods.

Large endotoxin concentrations have been found in animal houses, see Table 6. Very large concentrations

were found in a Swedish study of dairy farmers [54] whereas concentrations were much lower in a Polish study [20]. The authors of the former study suggested that their results could be due to a positive reaction to non-endotoxin components in the LAL test [54]. This has been demonstrated for glucans and other polysaccharides [46, 48]. The LAL test has therefore been improved by several authors [29, 47, 55]. In conclusion, it is more likely that endotoxin concentrations in cow barns are low.

Few studies have measured airborne fungal antigens [9, 52] and mycotoxins [39, 45]. The specificity of immunochemical methods may limits its use in the farm environment which has a complex microflora unless common antigens of broader groups of microorganisms can be measured. Airborne mycotoxins concentrations during grain handling were low.

CONCLUSIONS

Exposure of farmers and farm workers to airborne non-infectious microorganisms and endotoxins depends on task and production and can be highly variable. Thus, estimates of long-term exposure are likely to be inaccurate if based on a limited number of measurements. Development of more efficient strategies, e.g., by exposure modelling using task and other determinants of exposure are therefore needed. Further study of determinants of exposure to microorganisms and endotoxin in agriculture is therefore warranted in order to improve the accuracy of exposure assessment in epidemiological studies of farming populations. There is also a need for further development of methods for the measurement of exposure to viable and non-viable microorganisms allowing personal sampling and species characterisation.

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