STAPHYLOCOCCI AS AN INDICATOR FOR BACTERIAL EMISSIONS FROM A BROILER HOUSE

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Introduction

The air of animal houses contains bioaerosols like viruses bacteria, fungi, endotoxins and allergens that present a health risk for humans and animals [1]. Airborne pathogen bacteria, fungi that cause allergies and endotoxins were detected in poultry houses [2]. The spreading of bioaerosol emissions from poultry houses is less understood and therefore it is difficult to estimate the health risk on humans and animals caused by this emissions. A possibility to show the distribution of bioaerosols originating from the air of these livestock buildings is to use viable indicator bacteria. These bacteria need a sufficient survival time in the airborne state and should reach high emission rates because the detection of these organisms in longer distances downwind of the animal house depends on their source strength [3, 4]. Bacteria that possess these characteristics are staphylococci.
Staphylococci are commensales of the skin of poultry. Pathogenic and non pathogenic species were isolated from the skin and the nasal passages of the birds. Skin debris, broken feather barbules and particles from litter and faeces are dust compounds in poultry houses [2] to which the staphylococci can become attached. Staphylococci were the predominant airborne bacteria and the most present genus in airborne dust of broiler houses.
The objectives of this study were to quantify and to identify airborne staphylococci in the air of a Louisiana type broiler barn holding initially 40.000 broilers on litter and to estimate the travelling distance of these bacteria in the ambient air downwind the building.

Material and Methods

Sampling of bioaerosols

Bioaerosols were sampled by AGI-30 impingers in 50 ml 1:1 glycerol-phosphate buffer solutions placed at 1,5m height in the middle of the broiler house and at 1,5m, 4,0m and 10m heights at defined sampling places in the surroundings of the Louisiana barn. The sampling times were 30 min. in the barn and 90 min. in the field. The Impingers for the outdoor measurements were fixed in coloured [uv protection] white and insulated plastic holders at weather masts (Clark Masts Teksam NV, Belgium) to reflect sun radiation and prevent freezing in winter. The positions of the masts relative to the middle point of the barn were determined with a TC 110 Tachymeter (Leica Geosystems, Switzerland).

Meteorology

The wind speed and the wind direction were measured downwind (*lee* side) the barn with a UNIKLIMA 7 weather station and calculations of the weather data were made with the UK_TOSS-SOFTWARE (TOSS, Potsdam).

Cultivation of micro-organisms and identifying staphylococci to the genus level

Aliquots or diluted aliquots from the impinger solutions were plated on mannit-salt agar and on blood-agar basis (OXOID LTD, Basingstoke, Hampshire, England). After incubation the airborne colony forming units (cfu) per m^3 were calculated as described by Lin et al. [5].
Grown colonies of staphylococci on mannit-salt agar were identified to the genus level by morphology, motility, gram staining, catalase test, oxidase test and lysostaphin susceptibility.

PCR identification

A 16S-23S rDNA intergenic spacer PCR was used to identify *staphylococcus* species. A part of typical colony grown from staphylococci was transferred in a PCR reaction tube and incubated with 5 µl of a 100µg ml^{-1} lysostaphin solution for 15 min at 37°C. Then 5 µl of a GeneReleaser (BioVentures, Inc.) was added and a thermocycle programme followed by the manufacture's procedure was started.
A modified PCR amplification method based on a proposal of Mendoza et al. [6] was carried out.

Electrophoresis and imaging

PCR products were separated in 3 % agarose gels in TBE buffer. The gels were stained in TBE buffer with 0,5 µg ml^{-1} ethidium bromide and photographed with a BioDocAnalyze system (Biometra, Göttingen).

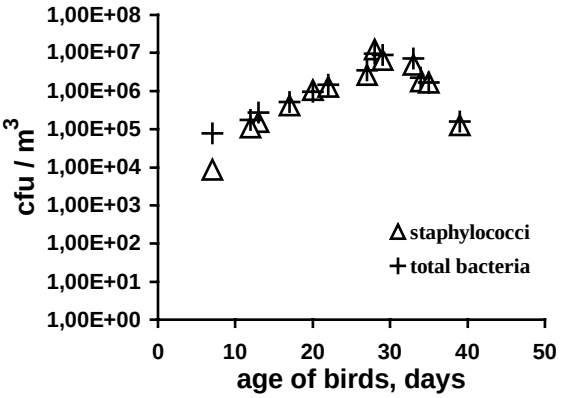
Identification of staphylococci species

Identification of the airborne staphylococci species was carried out as proposed by Mendoza et al. [6] and also with the ID 32 STAPH test (bioMérieux, Lyon).
Sometimes variations of the PCR fragments migration provide unclear results. In these cases we cleaned the PCR products with the MinElute PCR Purification Kit (QIAGEN, Hilden) and restricted the purified products with 10U DraI as described by the manufacturers protocol (Qbiogene, Heidelberg). For identification these patterns were compared to restricted PCR products of staphylococci strains from the DSZM (Deutsche Stammsammlung für Mikroorganismen und Zellkulturen, German type culture collection, Braunschweig).

Results

Figure 1 shows the results of the bacteria counts (total bacteria and staphylococci).

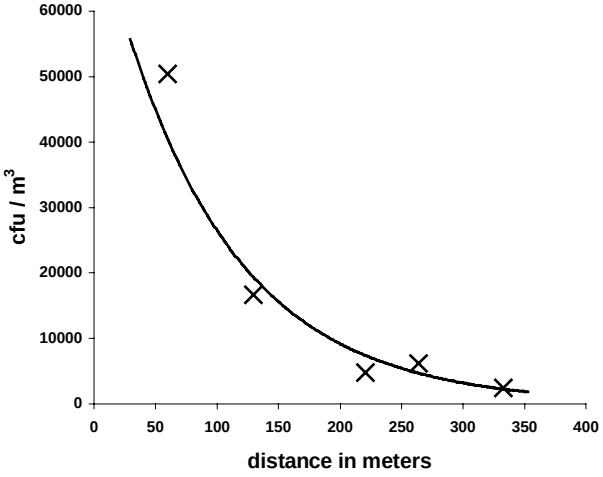
Figure 1 Numbers of airborne bacteria in a Louisiana stable holding initially 40.000 broilers on litter.



After one week the bacteria concentration is between 10,000 (staphylococci) and 100,000 (total bacteria). The counts rise to about 10 million after 30 days and drop to 100,000 again at day 40. This decrease is mainly due to the fact that at day 35 about 9000 of the biggest birds were taken to slaughter. The sometimes higher concentrations of staphylococci compared to the so called total bacteria count is caused by the different culture media which were used. It can happen that on blood agar base the colony counts vary considerably because of competitive growth or motile bacteria.

The staphylococci concentrations on the *lee* side showed a strong (r = 0,97) exponential decrease (Fig. 2). No staphylococci were found on the *luv* (upwind) side in the samples taken in parallel (detection limit 300 cfu of staphylococci per m^3).

Figure 2 Staphylococci on the *lee* side of a broiler house. Results from field measurements (May until September) at ages of birds older than 20 days. The sample points were positioned in the main wind direction and the wind speed was between 1.1 m/s and 4.1 m/s.



In addition to the overall quantification of staphylococci in the environment, the species of the genus *Staphylococcus* were also determined (Table 1).

Table 1 Predominant airborne *staphylococcus* species from samples in the house and from the parallel taken samples in the environment. At six different days 20 colonies from an outdoor sample and 20 colonies from an indoor sample were analysed.

Species found outdoor	Species found indoor
S. saprophyticus, S. cohnii, S. arlettae and *S. lentus*.	*S. saprophyticus, S. cohnii, S. arlettae* and *S. lentus*.

S. saprophyticus, S. cohnii and S. arlettae belong to the *S. saprophyticus* group whereas *S. lentus* is attributed to the *S. sciuri* group. *S. xylosus* was found in low concentrations and other species appeared occasionally only.

Discussion

The present study supports previous investigations in which the staphylococci were the predominant species in the air of broiler houses. The isolated and identified species in the indoor and the outdoor air were found on poultry skin and also in the litter. This indicates that skin particles and particles from the litter were the main source of airborne staphylococci. In this study the predominant species were coagulase negative. *Staphylococcus saprophyticus* is pathogen for humans and the other species may act as opportunistic pathogens in humans and animals. Additionally, these species can harbour a variety of resistant genes which could be transferred to pathogen bacteria like *Staphylococcus aureus*. A potential health risk from the identified species cannot be excluded for humans and animals.

The measured staphylococci concentration on the downwind side in relation to the distance of the animal house were higher as the concentration of indicator bacteria found in previous investigations. One important factor for detecting the dispersion of airborne bacteria from an emission source is the source strength [3, 4]. The source strength of the explored Louisiana barn was calculated based on the CO_2 balance method and it was about 1 x 10^9 staphylococci per second when the broilers were older than 20 days. This source strength for indicator bacteria was clearly higher as in studies, e.g. of Müller et al. [4]. Other factors like the sampling method, the meteorological conditions, the type of animal house and the topographic features have an effect on the dispersion of airborne bacteria [4].

The identification of indicator bacteria in more than 300 m distance downwind of the animal house suggests that other airborne bacteria including pathogens and dust bound viruses from the animal house can travel the same distances by air. More studies are necessary to verify the presented results.

Conclusion

Staphylococci seem to be a useful indicator bacteria to estimate the travelling distance of airborne bacteria from broiler houses. The results of this study can help to define safe distances between animal houses and between animal houses and residential areas.

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More specific literature with the authors on request.