

Occurrence of *Listeria* spp. in mattress dust of farm children in BavariaMelanie Korthals^{a,*}, Markus Ege^b, Sonja Lick^c, Erika von Mutius^b, Johann Bauer^a^aInstitute of Animal Hygiene, Technische Universität München, Weihenstephaner Berg 3, 85354 Freising, Germany^bUniversity Children's Hospital, Ludwig-Maximilians-University of Munich, Lindwurmstr. 4, 80337 Munich, Germany^cFederal Institute for Risk Assessment, Thielallee 88-92, 14195 Berlin, Germany

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Abstract

Several epidemiological studies have shown that the farm environment impacts allergy protection mechanisms in children. These associations are not well understood, but it is thought that contact to microorganisms may mediate this effect. For example, heat-inactivated *Listeria* (*L.*) *monocytogenes* have been successfully used as an adjuvant in mouse immunotherapy to modulate airway hyperreactivity and inflammation. In investigating the link between farming lifestyle and prevention of childhood allergy, we examined the prevalence of *Listeria* spp. in dust specimens from the environment of rural children.

A total of 26 farms located in Bavaria (South Germany) were examined. Dust samples taken from mattresses ($n = 63$), cow-sheds ($n = 30$) and swine-sheds ($n = 10$) were qualitatively screened for the presence of viable *Listeria* spp. according to the ISO 11290-1 method and additionally by *L. monocytogenes* specific *iap*-based real-time PCR. Isolates were further characterized by biochemical techniques, serology and multiplex PCR.

Nineteen of 26 farms tested positive for *Listeria* spp. and seven were tested negative. The dominant species found by culturing methods were *L. innocua* ($n = 12$) and *L. monocytogenes* ($n = 8$). Viable *Listeria* spp. were detected in 8% of the mattress dust samples, whereas real-time PCR revealed 60% *L. monocytogenes* positive specimens. Regarding animal sheds, 28% of dust samples showed viable *Listeria* spp., while using real-time PCR found that 28% of specimens were *L. monocytogenes* positive. All strains of *L. monocytogenes* except one (4ab) belonged to the serotype 1/2a.

Our data demonstrate that a substantial number of farm children's beds contain *L. monocytogenes*. The importance of this result regarding the health of children must be evaluated by epidemiological investigations on both the risk of listeriosis and the effects on protection against allergies.

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1. Introduction

Several epidemiological studies have shown that growing up on a farm has a considerable impact on the development of the immune system, especially regarding protection from asthma and allergic sensitisation (Ege et al., 2006; Lauener et al., 2002; Braun-Fahrlander and Lauener, 2003; Kabesch and Lauener, 2004; Perkin and Strachan, 2006). Due to their complexity, these associations are not understood, but it is assumed that extensive exposure to microorganisms

may mediate this protective effect (Braun-Fahrlander et al., 2002).

Farming families usually live in close contact with animals or have intensive contact with soil through farming activities. They are therefore exposed to higher numbers and diversities of bacteria than non-farming families. Moreover, microorganisms are thought to play a crucial role during the maturation of a child's immune system (Riedler et al., 2001; Braun-Fahrlander et al., 2002). Exposure to different bacterial compounds such as lipopolysaccharides and *N*-acetyl-muramic acid have been associated with protection from childhood allergic diseases (Von Mutius et al., 2000; Braun-Fahrlander et al., 2002; Van Strien et al., 2004). Some bacterial species such as

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Acinetobacter lwoffii F78 and *Lactococcus lactis* G121, identified in farm cowsheds, are candidates for protection from allergies such as asthma, hay fever and atopic sensitization in children (Debarry et al., 2007; Ege et al., 2007).

Heat-killed *Listeria* (*L.*) *monocytogenes* were successfully used as an adjuvant in immunotherapy to modulate the murine immune responses of airway hyperreactivity and inflammation (Hansen et al., 2000). *Listeria* spp. are ubiquitous microorganisms which are widespread in nature that have been isolated from soil, vegetation, marine sediments and water. Six species are currently known: *L. monocytogenes*, *L. innocua*, *L. ivanovii*, *L. seeligeri*, *L. welshimeri* and *L. grayi*. Of these, the species *L. monocytogenes* is of special importance, because it is a pathogen to humans and animals (Ramaswamy et al., 2007).

Listeria spp., especially *L. monocytogenes*, are frequently found in the rural environment (animal sheds, silage and faeces) (Borucki et al., 2005; Nightingale et al., 2004; Skovgaard and Morgen, 1988), so we assumed that farm children might be exposed to these bacteria by playing in this environment. As a consequence, children can transfer dust particles and microorganisms into their homes through their hands, clothes or hair. In the domestic environment little is known on *Listeria* spp. as a part of the microbial flora (Wagner et al., 2007).

In the household environment, mattress dust was chosen as the sample material because of the intense contact of this matrix to one person, reflecting an individual long-term exposure. The prevalence of *Listeria* spp. was investigated using conventional qualitative culturing methods according ISO 11290-1 and *L. monocytogenes* specific LightCycler® real-time PCR targeting the *iap* (invasion associated protein) gene with fluorescence resonance energy transfer (FRET) probes (Schwaiger et al., 2005).

2. Materials and methods

2.1. Field samples

Dust samples of mattresses ($n = 63$), cow-sheds ($n = 30$) and swine-sheds ($n = 10$) were collected from 26 farms in Bavaria (South Germany) during Spring 2006 (Table 1). Mattress dusts were collected according to a standard protocol (Schram-Bijkerk et al., 2005). In brief, the head of a vacuum cleaner was cleaned with 70% ethanol and a nylon collection sock was connected to a standard vacuum (Miele S 711, Germany). Blankets and sheets were removed from the bed and the whole area of the mattress was

vacuumed at full power for 3 min. The collection socks were then removed, closed and sealed in a plastic bag. The socks were shipped to the laboratory within 24 h and stored at -20°C . In animal sheds, at least 200 mg (about 2 teaspoons) of sediment dust was taken from various surface areas 0.5–1.5 m above the floor (e.g. shelf, windowsill, etc.) using brushes previously treated with 70% ethanol.

The study was approved by the local ethics committee and written informed consent was obtained from the children's parents.

2.2. Isolation of *Listeria* spp.

The isolation of *Listeria* spp. was performed according to the ISO 11290-1 reference method for food microbiological quality control. Aliquots of 100 mg of dust samples were added to 1.9 ml of half-Fraser broth (HFB; Merck, Germany) and incubated at 30°C for 48 h. One milliliter was then transferred into 100 ml of Fraser broth (FB) and incubated again for 24 h at 37°C . A total of 100 μl aliquots were streaked directly onto Oxford and Palcam agar (Merck, Germany) and incubated for 48 h at 37°C . Typical *Listeria* spp. colonies—greenish-yellow glistening, iridescent and pointed colonies of about 0.5 mm diameter surrounded by a diffused black zone of aesculin hydrolysis—were chosen for further investigations. Three characteristic colonies of each plate were transferred onto tryptic soy agar plates (TSA) (Oxoid, Germany) containing 5% sheep blood (Fiebig Nährstofftechnik, Germany) and incubated for 24 h at 37°C . All isolates were identified on species level using the API-*Listeria* test kit (Biomérieux, Germany) and by species-specific PCR according to Bubert et al. (1999), Gallien et al. (2000). Serotypes of *L. monocytogenes* were determined using a slide agglutination assay according to Seeliger and Hone (1979). The antisera used (commercially prepared by Denka Seiken Co., Japan) were purchased from Innogenetics GmbH (Germany). Serotypes were verified in parallel with the multiplex PCR recently published by Doumith et al. (2004). Reference strains were: *L. monocytogenes* DSM20600^T serovar 1/2a, *L. monocytogenes* ATCC19112 serovar 1/2c, *L. monocytogenes* SLCC2755 serovar 1/2b, *L. monocytogenes* ATCC19114 serovar 4a, *L. monocytogenes* DSM15675 and ATCC19111 serovar 4b, *L. monocytogenes* NCTC10528 serovar 4ab, *L. innocua* DSM20649^T, *L. ivanovii* DSM20750^T, *L. seeligeri* ATCC35967 and *L. welshimeri* ATCC35897.

2.3. Polymerase chain reaction (PCR)

2.3.1. Sample preparation

A total of 2.85 ml phosphate buffered saline (PBS) ($\text{Na}_2\text{HPO}_4 \times 2\text{H}_2\text{O}$ 41.57 mM, NaCl 0.62 M, KH_2PO_4) was added to 150 mg of dust and shaken at 350 rpm for 30 min. Dust particles heavier than microorganisms were separated by a centrifugation step ($200 \times g$, 3 min). The supernatant was transferred into a 1.5 ml tube and centrifuged at $10,000 \times g$ for 10 min to obtain a microorganism pellet, which was subjected to DNA extraction.

2.3.2. DNA extraction

The DNA extraction was performed according to the protocol of Somerville et al. (2005) with minor modifications. The pellet was resuspended in 500 μl Tris-EDTA buffer (Tris 0.010 mM, EDTA 0.001 mM, pH 8.0) and heated for 20 min at 80°C . Lysozyme was added

Table 1
Origin of the field samples taken from different farm types

Group	Cow	Swine	Crop farming	Number of farms sampled	Number of dust samples		
					Mattress	Cow-shed	Swine-shed
1	+	+	+	8	24	8	8
2	+	–	+	11	24	15	–
3	+	+	–	7	15	7	2
Total				26	63	30	10

(final concentration, 1 mg/ml), followed by incubation at 37 °C for 2 h. Ten percent sodium dodecyl sulfate (SDS; final concentration, 1.1%) and proteinase K (final concentration, 0.2 mg/ml) were added, the tubes were vortexed gently and incubated for 20 min at 65 °C. A mixture of *N*-acetyl-*N,N,N*-trimethyl ammonium bromide (CTAB, final concentration, 40 mM) and NaCl (final concentration, 0.1 M) was added, followed immediately by another addition of NaCl (final concentration, 0.6 M). The tubes were gently shaken until the suspension turned milky and were incubated again for 10 min at 65 °C. One volume of chloroform–isoamyl alcohol (24:1) was added and the tubes were gently shaken and then centrifuged in a microcentrifuge at $13,000 \times g$ for 5 min at room temperature. The aqueous phase was transferred into a 1.5 ml tube, and 2 μ l glycogen (Invitrogen, Germany) and 600 μ l ice-cold isopropanol (100%) were added to the tube and gently shaken, followed by centrifugation in a microcentrifuge (VWR, Germany) at $16,000 \times g$ for 10 min at room temperature. The pellet was washed in 600 μ l of 70% ethanol and centrifuged at $16,000 \times g$ for 2 min. The ethanol was removed and the resulting genomic DNA pellet was resuspended in 30 μ l nuclease-free water.

2.3.3. Preparation of a quantitative positive control for real-time PCR: spiking of dust samples with *L. monocytogenes* ATTC 7644

Twenty-five mattress dust specimens of 150 mg each were γ -radiated (26 kGy for 22 h) in order to destroy naturally occurring DNA. A 10-fold dilution series of a McFarland 1 *L. monocytogenes* ATTC 7644 suspension in PBS was prepared and 100 μ l of each dilution was added to the 150 mg mattress dust specimens. In parallel, colony forming units (cfu) were determined on TSA agar, which ranged from 2×10^4 to 2×10^0 per mg dust (Table 2). The artificially contaminated samples were shaken vigorously and then lyophilized under aseptic conditions.

DNA was extracted as described above. The DNA concentration of the standards was measured using the Nanodrop ND-100 spectrometer (Peqlab, Germany). All quantifications of DNA were based on the calculation that 1 ng of bacterial DNA equals about 3×10^5 copies of the

genome (*L. monocytogenes*, genome size 2.9 Mbp) and contained only one copy of the target *iap*-gene (Hein et al., 2001).

2.3.4. LightCycler® real-time PCR for *L. monocytogenes*

The oligonucleotide primers and hybridization probes for PCR targeted the *iap*-gene. Although it is not very well conserved in the genus *Listeria*, *L. monocytogenes* strains contain conserved portions at the 5' and the 3' end, whereas its central regions are highly variable. The primers were used as described by Hein et al. (2001) with minor modifications according to Schwaiger et al. (2005) generating a 175 bp amplification product and using probes (TIB Molbiol, Germany) for the LightCycler® system based on the sequence of the *iap*-gene (Fig. 1).

The reaction volume of 20 μ l contained a mixture of 2 μ l of LC-FastStart Reaction Mix (Roche, Germany), primers 0.4 μ M each, FRET probes 0.2 μ M each, MgCl₂ 3 mM and 4 μ l target DNA. A DNA extract of γ -radiated dust (26 kGy for 22 h) was used as a negative control. A 10-fold dilution series of the spiked dust samples was used as (quantitative) positive control standard (see above).

The standard LightCycler® protocol was as follows: an initial activation phase at 95 °C for 10 min; 50 cycles of amplification consisting of a 95 °C denaturation for 10 s, 60 °C annealing for 10 s, and 72 °C extension for 5 s; the quantity of amplified product was monitored in the F2 mode by detection of fluorescence emitted at 640 nm. Melting curve analysis was undertaken from 45 °C to 72 °C with a temperature transition rate of 0.1 °C/s and a rapid cooling phase.

A selection of PCR products was sequenced by Sequiserve (Germany) in order to confirm the detected species *L. monocytogenes*.

2.3.5. Statistical analysis

Concordance between the occurrence of *Listeria* spp. in sheds and mattresses was calculated as kappa coefficient (κ [95%-confidence interval]) of presence of *Listeria* spp. in animal shed dust and presence of *Listeria* spp. in any mattress dust per farm.

Table 2

Detection of *Listeria* spp. in mattress- and shed-dusts by a culture-based method and real-time PCR

Group	Source	Sample	Culture			<i>L. monocytogenes</i> real-time PCR calculated genome copies per 150 mg dust
			<i>L. monocytogenes</i> (serotype)	<i>L. innocua</i>	<i>L. seeligeri</i>	
1	Swine-shed	220 SSt	–	+	–	n.d.
	Mattress	448 K4	–	+	–	n.d.
	Mattress	448 K5	–	+	–	938
	Swine-shed	448 SSt	–	+	–	210
	Cow-shed	484 KS	–	+	–	n.d.
	Swine-shed	776 SSt	–	+	–	n.d.
	Mattress	795 K2	–	+	–	n.d.
2	Cow-shed	133 KS	+(1/2a)	–	+	n.d.
	Mattress	413 K1	+(1/2a)	+	–	435
	Cow-shed	413 KS	+(1/2a)	+	–	218
	Cow-shed	510 KS	+(1/2a)	–	–	450
	Cow-shed	710 KS	–	+	–	n.d.
	Cow-shed	863 KS1	+(1/2a) and +(4ab)	–	–	n.d.
	Cow-shed	863 KS2	+(1/2a)	–	–	n.d.
3	Mattress	187 K3	+(1/2a)	+	–	248
	Cow-shed	969 KS	+(1/2a)	+	–	n.d.

n.d., not detected.

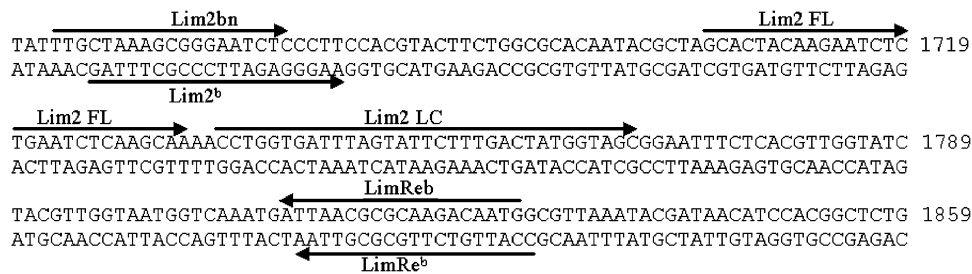


Fig. 1. Sequence of primers (Lim2bn and LimReb) and probes (Lim2 FL and Lim2 LC) of the *iap*-gene designed from Schwaiger et al. (2005) compared to primers (Lim2^b and LimRe^b) according to Hein et al. (2001).

3. Results and discussion

3.1. Culture methods for detection of *Listeria* spp.

Since direct quantitative culturing according to the ISO11290-2 method failed to demonstrate any growth of *Listeria* spp., qualitative culturing could be performed only as is described in ISO 11290-1.

Culturable *Listeria* spp. could be detected in 5 of 63 (8%) mattress samples. Species identification performed with API tests and multiplex-PCR showed the presence of *L. innocua* in 5 mattresses (8%) and of *L. monocytogenes* in 2 (3%) mattresses (Table 2). These findings correspond to data recently published by Wagner et al. (2007), who detected *Listeria* spp. in 8.7% of kitchen dust samples; *L. monocytogenes* was determined in 1.1% of all samples. As expected, shed dust samples showed a significantly higher prevalence of *Listeria* spp. than the domestic area. Eleven of 40 specimens (28%) were *Listeria* spp. positive, *L. monocytogenes* could be identified in 6 shed specimens (15%), *L. innocua* in 7 shed samples (18%) and *L. seeligeri* in one sample only. Determination of the serotypes of *L. monocytogenes* showed in all but one case serotype 1/2a (Table 2). Among 13 described serovars of *L. monocytogenes*, 3 serovars (1/2a, 1/2b and 4b) account for 95% of most findings (Kathariou, 2002). Serovar 1/2a is already known to usually be over-represented when recovered from foods and food processing plants. In one case the very rare serotype 4ab could be isolated from a cow-shed.

3.2. *Iap*-based real-time PCR for *L. monocytogenes*

Real-time PCR was performed since this technique is a highly sensitive and specific method that can quantify the presence of *Listeria* spp. in environmental samples (Gasanov et al., 2005).

Based on the work of Hein et al. (2001), Schwaiger et al. (2005) developed an *iap*-gene based PCR with additionally designed FRET hybridization probes for use in a Light-Cycler[®] real-time detection system which was used in this study. Identification criteria for the amplification of the *iap*-gene were the increase of fluorescence and the accordance of the melting curves with reference melting

Table 3

Traceability of *L. monocytogenes* in spiked dust samples by real-time PCR

Cfu/mg	Cfu per 150 mg dust	Mean value of genome copies per PCR (SD) ^a	Calculated genome copies per 150 mg dust
2×10^4	3×10^6	836,000 (297,855)	6,270,000
2×10^3	3×10^5	59,732 (19,859)	447,990
2×10^2	3×10^4	4193 (1909)	31,448
2×10^1	3×10^3	508 (237)	3810
2×10^0	3×10^2	41 (19)	308

SD, standard deviation.

^aTriplicate measurement.

curves (*L. monocytogenes* ATTC 7644). Due to occasional sequence polymorphisms of single basepairs in the stretch of the hybridization probes, slight shifts in the melting temperature could be observed. Additional sequencing of those samples confirmed the detected species as *L. monocytogenes*.

In order to determine the sensitivity of the real-time PCR, the DNA amount extracted from dust samples spiked with a 10-fold dilution series of *L. monocytogenes* was measured and used as the quantification standard. Real-time PCR experiments were carried out in triplicate (Table 3). The validation showed that this method was able to detect down to 41 copies per PCR reaction on average of the *iap*-gene of *L. monocytogenes*.

An overview of the results obtained from different locations carried out with culturing and real-time PCR is presented in Tables 2, 4 and 5.

Concerning the mattress dust, 38 of 63 (60%) samples tested positive for *L. monocytogenes* (Table 4) using real-time PCR. The genome copy numbers obtained ranged between 15 and 23,420 copies per PCR reaction, corresponding to 113 to 175,650 genome copies per 150 mg dust. Real-time PCR results obtained from shed samples revealed that 12 of 43 (28%) specimens tested positive for *L. monocytogenes*, with copy numbers varying between 22 and 973 per PCR reaction, corresponding to 165 to 7298 genome equivalents per 150 mg dust (Table 4). The negative control dust samples were consistently negative in real-time PCR.

Table 4

Detection of the *L. monocytogenes* specific *iap*-gene in mattress- and shed-dusts by real-time PCR

Group	Number of samples	Positive samples	PCR range copies per 150 mg dust		
			Mattress	Cow-shed	Swine-shed
<i>Mattresses</i>					
1	24	13	35–5233		
2	24	20	26–23,420		
3	15	5	15–121		
Total	63	38	15–23,420		
<i>Sheds</i>					
1	16	4		55–60	28–973
2	15	6		29–680	
3	9	2		–	22–92
Total	43	12		55–680	22–973

Table 5

Occurrence of *L. monocytogenes* in mattress and shed dusts: comparison of the results of enrichment culturing and real-time PCR

Farm type	Real-time PCR/culture			
	+/+	-/-	+/-	-/+
Mattresses	2	25	36	0
Sheds	2	24	10	4
Total	4	49	46	4

+, *L. monocytogenes* positive samples.

–, *L. monocytogenes* negative samples.

3.3. Comparison of results derived from culturing and real-time PCR

From 103 samples (mattresses and sheds), 53 samples gave concordant results with both culture and real-time methods: four samples (2 mattress specimens, 2 shed specimens) tested positive and 49 (25 mattress specimens and 24 sheds specimens) tested negative (Table 5).

A total of 50 samples showed discordant results comparing both techniques. Most of them tested positive with the *iap*-based real-time PCR (36 mattress specimens, 10 shed specimens), but no viable *Listeria* spp. was detected. This is not entirely unexpected. It might be due either to DNA stably bound to dust particles remaining detectable or to an often-discussed dormant state of microorganisms described as viable but non-culturable (VNBC). This metabolic state might be favoured under suboptimal bacterial growth conditions (Besnard et al., 2002).

In contrast to this, only a few samples (4 shed samples) tested negative for *Listeria* spp. according to real-time PCR, but positive for *Listeria* spp. based on our culture method. This might be explained by, for example, a non-

homogenous distribution of *Listeria* spp. throughout the samples or by PCR inhibitory substances like humic acids in the DNA extract coming from soil and particles in the dusts (Von Wintzingerode et al., 1997). In addition, *L. innocua* were isolated in some of the dust samples, but no *L. monocytogenes* (Table 2) were found, although real-time PCR reacted positively to *L. monocytogenes*. Hein et al. (2001) and Bubert et al. (1999) reported that *L. innocua* is frequently found associated with *L. monocytogenes* and therefore considered *L. innocua* as a potential indicator bacterium for the presence of *L. monocytogenes*. Since usually only a few colonies—in our study three colonies—are taken from each enrichment culture plate for further species identification, one should be aware that not all *Listeria* spp. existent in the sample might have been detected.

4. Conclusions

In conclusion, of 26 farms investigated, 14 farms tested positive (culture method and real-time PCR) and seven farms tested negative in both locations (sheds and mattresses) for the occurrence of *L. monocytogenes*. Discordant results were found for five farms, which were either only *Listeria* spp. positive for animal sheds ($n = 3$) or for mattresses ($n = 2$). The concordance of *Listeria* spp. between animal sheds and mattresses was substantial ($\kappa = 0.59$ [0.26–0.9]), thereby rendering the transfer of *Listeria* spp. from the farm environment to the mattresses rather likely. The transfer might involve skin, hair or respiratory tract, but it has to be considered that *Listeria* spp. in home environments may also originate from contaminated food (Wagner et al., 2007). Transmission of certain strains from one location to another still needs to be proved by subtyping of isolates.

Our data demonstrate that mattress dust samples of farm children contained viable *L. monocytogenes*, *L. innocua* and *L. seeligeri* and possibly *L. monocytogenes* at a non-culturable state (dead or VBNC) at a substantial prevalence. Hence, the children appear to be continuously exposed to potentially antigenic epitopes of *Listeria* species. Therefore, the immunomodulative properties of the farm environment mentioned at the beginning might occur through *L. monocytogenes* exposure, amongst others. However, the importance of these results regarding the health status of children must be evaluated by further epidemiological investigations on both the risk of listeriosis and allergy prevention effects. Furthermore, high-level epidemiologic studies will help to trace the sources of *Listeria* spp. with respect to distinct farm-related exposures.

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References

- Besnard, V., Federighi, M., Declercq, E., Jugiau, F., Cappellet, J.M., 2002. Environmental and physico-chemical factors induce VBNC state in *Listeria monocytogenes*. Vet. Res. 33, 359–370.
- Borucki, M.K., Gay, C.C., Reynolds, J., McElwain, K.L., Kim, S.H., Call, D.R., Knowles, D.P., 2005. Genetic diversity of *Listeria monocytogenes* strains from a high-prevalence dairy farm. Appl. Environ. Microbiol. 71, 5893–5899.
- Braun-Fahrlander, C., Lauener, R., 2003. Farming and protective agents against allergy and asthma. Clin. Exp. Allergy 33, 409–411.
- Braun-Fahrlander, C., Riedler, J., Herz, U., Eder, W., Waser, M., Grize, L., Maisch, S., Carr, D., Gerlach, F., Bufer, A., Lauener, R.P., Schierl, R., Renz, H., Nowak, D., von Mutius, E., 2002. Environmental exposure to endotoxin and its relation to asthma in school-age children. N. Engl. J. Med. 347, 869–877.
- Bubert, A., Hein, I., Rauch, M., Lehner, A., Yoon, B., Goebel, W., Wagner, M., 1999. Detection and differentiation of *Listeria* spp. by a single reaction based on multiplex PCR. Appl. Environ. Microbiol. 65, 4688–4692.
- Debarry, J., Garn, H., Hanuszkiewicz, A., Dickgreber, N., Blümer, N., von Mutius, E., Bufer, E., Gattermann, S., Renz, H., Holst, O., Heine, H., 2007. *Acinetobacter lwoffii* and *Lactococcus lactis* strains, isolated from farm cowsheds possess strong allergy-protective properties. J. Allergy Clin. Immunol. 119, 1514–1521.
- Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C., Martin, P., 2004. Differentiation of the major serovars by multiplex PCR. J. Clin. Microbiol. 42, 3819–3822.
- Ege, M.J., Bieli, C., Frei, R., van Strien, R.T., Riedler, J., Ublagger, E., Schram-Bijkerk, D., Brunekreef, B., van Hage, M., Scheynius, A., Pershagen, G., Benz, M.R., Lauener, R., von Mutius, E., Braun-Fahrlander, C., Parsifal Study Team, 2006. Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. J. Allergy Clin. Immunol. 117, 817–823.
- Ege, M.J., Frei, R., Bieli, C., Schram-Bijkerk, D., Waser, M., Benz, M.R., Weiss, G., Nyberg, F., van Hage, M., Pershagen, G., Brunekreef, B., Riedler, J., Lauener, R., Braun-Fahrlander, C., von Mutius, E., Parsifal Study Team, 2007. Not all farming environments protect against the development of asthma and wheeze in children. J. Allergy Clin. Immunol. 119, 1140–1147.
- Gallien, P., Schönberg, A., Perlberg, K.-B., Protz, D., 2000. *Listeria*. In: Sachse, K., Gallien, P. (Eds.), Molekularbiologische Nachweismethoden ausgewählter Zoonoseerreger. BgVV-Heft 02/2000, pp. 113–122.
- Gasanov, U., Hughes, D., Hansbro, P.M., 2005. Methods for the isolation and identification of *Listeria* spp. and *Listeria monocytogenes*: a review. FEMS Microbiol. Rev. 29, 851–875.
- Hansen, G., Yeung, V.P., Berry, G., Umetsu, D.T., DeKruyff, R.H., 2000. Vaccination with heat-killed *Listeria* as adjuvant reverses established allergen-induced airway hyperreactivity and inflammation: role of CD8+ T cells and IL-18. J. Immunol. 164, 223–230.
- Hein, I., Klein, D., Lehner, A., Bubert, A., Brandl, E., Wagner, M., 2001. Detection and quantification of the *iap* gene of *Listeria monocytogenes* and *Listeria innocua* by new real-time quantitative PCR assay. Res. Microbiol. 152, 37–46.
- ISO 11290-1, 1996. Microbiology of food and animal feeding stuffs—horizontal method for the detection and enumeration of *Listeria monocytogenes*—part 1: detection method. International Organization for Standardization, Geneva.
- ISO 11290-2, 1998. Microbiology of food and animal feeding stuffs—horizontal method for the detection and enumeration of *Listeria monocytogenes*—part 2: enumeration method. International Organization for Standardization, Geneva.
- Kabesch, M., Lauener, R.P., 2004. Why Old McDonald had a farm but no allergies: genes, environments, and the hygiene hypothesis. J. Leukocyte Biol. 75, 383–387.
- Kathariou, S., 2002. *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. J. Food Prot. 65, 1811–1829.
- Lauener, R.P., Birchler, T., Adamsky, J., Braun-Fahrlander, C., Bufer, A., Herz, U., von Mutius, E., Nowak, D., Riedler, J., Waser, M., Sennhauser, F.H., ALEX Study Group, 2002. Expression of CD14 and Toll-like receptor 2 in farmers' and non-farmers' children. Lancet 360, 465–466.
- Nightingale, K.K., Schukken, Y.H., Nightingale, C.R., Fortes, E.D., Ho, A.J., Her, Z., Grohn, Y.T., McDonough, P.L., Wiedmann, M., 2004. Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. Appl. Environ. Microbiol. 70, 4458–4467.
- Perkin, M.R., Strachan, D.P., 2006. Which aspects of the farming lifestyle explain the inverse association with childhood allergy? J. Allergy Clin. Immunol. 117, 1374–1380.
- Ramaswamy, V., Cresence, V.M., Rejitha, J.S., Lekshmi, M.U., Dharsana, K.S., Prasad, S.P., Vijila, H.M., 2007. *Listeria*—review of epidemiology and pathogenesis. J. Microbiol. Immunol. Infect. 40, 4–13.
- Riedler, J., Braun-Fahrlander, C., Eder, W., Schreuer, M., Waser, M., Maisch, S., Carr, D., Schierl, R., Nowak, D., von Mutius, E., 2001. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. Lancet 358, 1129–1133.
- Schram-Bijkerk, D., Doekes, G., Douwes, J., Boeve, M., Riedler, J., Ublagger, E., von Mutius, E., Benz, M.R., Pershagen, G., van Hage, M., Scheynius, A., Braun-Fahrlander, C., Waser, M., Brunekreef, B., 2005. Bacterial and fungal agents in house dust and wheeze in children. Clin. Exp. Allergy 35, 1272–1278.
- Schwaiger, K., Stiersdorfer, B., Schmah, W., Lehmann, S., Gallien, P., Bauer, J., 2005. Zum Vorkommen bakterieller ZNS-Infektionen beim Rehwild (*Capreolus capreolus*), Rotwild (*Cervus elaphus*) und Gamswild (*Rupicapra rupicapra*) in Bayern. Berl. Münch. Tierärztl. Wochenschr. 118, 45–51.
- Seeliger, H.P.R., Hone, K., 1979. Serotyping of *Listeria monocytogenes* and related species. In: Bergan, T., Norris, J.R. (Eds.), Methods in Microbiology, vol. 13. Academic Press, New York, pp. 31–49.
- Skovgaard, N., Morgen, C.A., 1988. Detection of *Listeria* spp. in faeces from animals, in feeds, and in raw foods of animal origin. Int. J. Food Microbiol. 6, 229–242.
- Somerville, W., Thibert, L., Schwartzman, K., Behr, M.A., 2005. Extraction of *Mycobacterium tuberculosis* DNA: a question of containment. J. Clin. Microbiol. 43, 2996–2997.
- Van Strien, R.T., Engel, E., Holst, O., Bufer, A., Eder, W., Waser, M., Braun-Fahrlander, C., Riedler, J., Nowak, D., von Mutius, E., The ALEX Study Team, 2004. Microbial exposure of rural school children, as assessed by levels of *N*-acetyl-muramic acid in mattress dust, and its association with respiratory health. J. Allergy Clin. Immunol. 113, 860–867.
- Von Mutius, E., Braun-Fahrlander, C., Schierl, R., Riedler, J., Ehlermann, S., Maisch, S., Waser, M., Nowak, D., 2000. Exposure to endotoxin or other bacterial components might protect against the development of atopy. Clin. Exp. Allergy 30, 1230–1234.
- Von Wintzingerode, F., Göbel, U.B., Stackebrandt, E., 1997. Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS Microbiol. Rev. 21, 213–229.
- Wagner, M., Auer, B., Trittmann, C., Hein, I., Schoder, D., 2007. Survey on the *Listeria* contamination of ready-to-eat food products and household environments in Vienna, Austria. Zoonoses Public Health 54, 16–22.