Zoonoses Monitoring 2016

Summary of Findings and Conclusions
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Salmonella spp.

Salmonella was detected in 2.4% of wild boar faecal samples. This included the serovars S. Enteritidis and S. Typhimurium, which are relevant for poultry. Wild boar were thus clearly less often Salmonella positive than fattening pigs, of which 6% to 9% were found to carry Salmonella in Zoonoses Monitoring programmes of previous years.

Fresh wild boar meat examined in 2011, on the other hand, was more frequently contaminated with Salmonella than fresh pork, which had 3.4% positive samples (fresh pork in 2011 and 2015: 0.4% positive samples). This indicates a lack of hygiene in the handling of game meat. This is probably due to the special conditions of game meat production, which bring about an increased risk of contamination with germs (e.g., by shot-related injuries to the digestive tract, less bleeding compared to slaughter animals, and delayed evisceration of the game). In order to prevent transmission of zoonotic agents to game meat, hygiene must be particularly strictly followed when obtaining, handling, processing and marketing game meat.

The 2016 Zoonosis Monitoring results show that the decline in Salmonella detection rates observed over the past few years in the broilers and fattening turkeys food chains did not continue in 2016. Salmonella findings in neck skin samples of broiler carcases (6.7% positive samples) and fresh broiler meat samples (4.7% positive) were roughly the same level as in 2014. The rate of positive samples in caecal content of broilers (2.3%) was even slightly increased in 2016 over 2013 (1.0% positives).

In the fattening turkeys food chain, turkey carcases were found with a higher Salmonella contamination degree than in 2014, with 11.9% of neck skin samples positive (compared to 7.1% positive neck skin samples in 2014). Fresh turkey meat, too, showed with 2.6% of positive samples a slightly higher degree of contamination than in the previous year of investigation (1.7% of positive samples). Fewer of the birds, however, actually carried Salmonella in 2016 (1.0% positive samples of caecal content), compared to findings of the 2012 Zoonosis Monitoring, when 1.7% of the caecal samples were positive. Striking differences were noted among the various slaughterhouses in the frequency of Salmonella contamination findings in carcases, both as regards findings in broilers and turkeys.

Both in broilers and in fattening turkeys, Salmonella were found significantly more frequently on the carcases than in intestinal content, which indicates a considerable degree of cross-contamination during the slaughter process. This makes clear that, apart from relevant measures in poultry farms, it is necessary to improve hygiene practices in the slaughtering of broiler chickens and turkeys, in order to prevent Salmonella contamination of the meat. The striking variance in the percentage of findings
in individual slaughterhouses should be a reason to oblige slaughterhouses to strictly obey the processing hygiene criteria for *Salmonella* on bird carcasses, and to take appropriate measures if the criteria are not met.

Tomatoes, which were sampled at retail level, did not contain *Salmonella* spp. in any of the samples. So in Germany, tomatoes do not seem to play a role as a possible source of infection of humans with *Salmonella*.

Sprouts, which were also tested at retail level, had 0.8% of positive samples, which makes them a potential source of infection of humans with *Salmonella*, in particular because sprouts are often eaten raw, so that germ contamination is not reduced before consumption. Legal regulations provide that ready-to-eat sprouts which are found positive with *Salmonella* are harmful to health and must be removed from the market. Susceptible consumer groups, such as small children, elderly, and immune-suppressed people, as well as pregnant women, are advised to abstain from sprouts.

Depending on the origin of *Salmonella* isolates, the resistance situation was extremely heterogenic, as it was also found in previous years. As a whole, only 20.2% of the isolates were susceptible to all of the tested antibiotics. *Salmonella* isolates obtained from the broilers food chain displayed higher resistance rates than in the 2014 monitoring programme, which is connected to the fact that the share of *S. Indiana* – which has very low resistance rates – in the total number of isolates was notably smaller in 2016 than in 2014. The predominant *Salmonella* isolates in the 2016 monitoring, namely, *S. Infantis* and *S. Paratyphi* B dT+, in contrast, have a long record of high resistance rates. The reason why the serovar spectrum of *Salmonella* isolates has changed in this way is not known. The *Salmonella* isolates obtained from the fattening turkeys food chain also showed an increase in resistance rates, compared to the rates found in the 2014 programme. A favourable finding of the 2016 Zoonosis Monitoring is that none of the *Salmonella* isolates tested was resistant towards third-generation cephalosporins, nor towards carbapenems. On the other hand, isolates were very often resistant towards the (fluoro-)quinolones ciprofloxacin (71.8% resistant isolates) and nalidixic acid (66.7% resistant isolates). These substances are particularly important in antibiotic treatments of humans.

The *Salmonella* isolate obtained from sprouts was susceptible to all substances tested.

*Campylobacter* spp.

Detection rates of *Campylobacter* spp. continue to be at high level in the food chains based on broilers and fattening turkeys. It was found in 76.9% of the neck skin samples gathered from broilers at slaughter, which is yet more frequent than in previous years, when about 50% of the carcasses were found contaminated with *Campylobacter* spp. The birds, too, were again found to be frequent
carriers of *Campylobacter* spp., with 43.5% positive samples of caecal content. Near to a quarter (24%) of the broiler carcases had a *Campylobacter* count higher than 1000 cfu/g. This limit came into force as a process hygiene criterion in 2018. This means the percentage of neck skin samples with high *Campylobacter* count has still increased since 2013, when about 20% of the neck skin samples had counts at this level. Fresh chicken meat had a *Campylobacter* contamination rate of 47.2%. Compared to the findings of the 2014 monitoring programme, where 54.0% of fresh chicken legs were positive with *Campylobacter*, the contamination rate of chicken meat was thus somewhat lower in the past year. This may, however, be owed to the fact that last year, chicken meat was analysed without skin, while in 2014, the analysed meat samples contained skin.

Fattening turkeys were still much more frequently colonised with *Campylobacter* spp. than broiler chickens, with near to 74% positive samples of caecal content. Fresh turkey meat, in contrast, had a clearly lower contamination rate than chicken meat, with only near to 16% of positive samples. This relation was the same in previous monitoring programmes. The percentage of fresh turkey meat samples found positive with *Campylobacter* spp. in the 2016 monitoring programme was significantly lower than in 2014 (26.5% positives). Here again, this might be attributable to the composition of the sample material, as in 2016, meat was analysed without skin, while in 2014, skin was part of the meat samples analysed.

The findings show that efforts to reduce the presence of *Campylobacter* in the poultry meat food chain must be further intensified. The process hygiene criterion of 1000 cfu/g for *Campylobacter* in broiler carcases which took effect in 2018 will be one step in this direction, as it requires that action must be taken to ensure processing hygiene if this limit value is exceeded. At the same time, the findings highlight the need to consistently inform consumers about the risks associated with fresh poultry meat, because *Campylobacter* will remain a relatively frequent finding in raw chicken and turkey meat.

Like in previous years, *Campylobacter coli* isolates displayed higher resistance rates throughout than *Campylobacter jejuni*. Both *Campylobacter* species were most resistant to the (fluoro-)quinolones ciprofloxacin and nalidixic acid, the rates of resistant isolates being 92.7% in *C. coli* and 75.0% in *C. jejuni*. The rates had even enhanced compared to the findings of the 2014 programme, when 87.4% and 50.2% of the respective isolates were found resistant. A positive fact is that resistance of *Campylobacter* isolates towards the substance erythromycin had declined, compared to the findings of 2014. This is important in so far as this antibiotic plays a role in the treatment of campylobacteriosis in humans. Only some single *C. jejuni* and up to 13% of the *C. coli* isolates displayed resistance towards erythromycin.
**Listeria monocytogenes**

None of the tomato samples taken at retail level were found positive with *Listeria monocytogenes*. So, tomatoes do not seem to play a role as a source of infection of humans with *L. monocytogenes* in Germany.

Fresh sprouts were found positive with *Listeria monocytogenes* in 1.8% of samples. Quantitative examinations, however, did not produce any *L. m.* bacterial counts higher than the detection limit of 10 cfu/g. The findings show that while one has to reckon with the presence of *Listeria monocytogenes* in fresh sprouts, the actual amounts do not pose a potential danger to human health. Still, the humid environment provided by fresh, ready-packed sprouts, has the potential to encourage growth of present Listeria, which is why this food is not suited for susceptible consumer groups, namely, small children, elderly and immune-suppressed people, or pregnant women. These consumer groups should eat sprouts only thoroughly cooked.

**Verotoxin-forming Escherichia coli (VTEC)**

VTEC was found in 6.9% of wild boar faecal samples. This shows that wild boar, too, can be a reservoir of VTEC. VTEC isolates included *O* groups known as a frequent cause EHEC infections and the haemolytic-uraemic syndrome in humans.

No VTEC were found in tomatoes, which means that tomatoes do not seem to play a role as a source of infection with VTEC in humans.

Nor were any of the retail-level fresh sprouts samples found positive with VTEC, either. Still, there were VTEC infections of humans in the past which were definitely attributed to consumption of fresh sprouts. Therefore, sensitive consumer groups, such as small children, elderly and immune-suppressed people and pregnant women, should abstain from consuming fresh sprouts, or consume sprouts only after thorough cooking.

The VTEC isolates from wild boar faecal samples were nearly all susceptible to the antimicrobial substances tested.

**Methicillin-resistant Staphylococcus aureus (MRSA)**

As in previous years, MRSA was only very rarely found in broiler flocks. Only 0.6% of the dust samples and 1.9% of the skin swabs taken in conventional farms were positive with MRSA. Broiler carcases, in contrast, were repeatedly found with high MRSA contamination rates of up to 50% in the framework
of the Zoonosis Monitoring. These findings indicate that colonisation or contamination of broilers with MRSA takes place only during transport to the slaughter establishment or in the slaughterhouse. Fresh chicken meat had - with 13.0% of positive samples - a significantly lower contamination rate than in the 2013 Zoonosis Monitoring (24.2% positives). The reason for this is not known. Further testing in the framework of the Zoonosis Monitoring will show whether this turns into a trend.

The results of analyses of fresh turkey meat are in about the same range as in previous years’ programmes and confirm that MRSA occurs very frequently in the fattening turkeys food chain. The contamination rate of fresh turkey meat with MRSA was 44.5% and thus still markedly higher than in fresh chicken meat. The current state of knowledge is that the consumption or handling of food contaminated with MRSA is not connected with a higher risk of being colonised or infected with these bacteria (EFSA 2009b).

Nasal swabs of wild boar were not detected with MRSA. In the fattening pigs food chain, in contrast, MRSA was found frequently, with 26.3% positive samples from breeding sows’ farrowing areas and 41.3% positive samples from young pigs’ raising areas.

As it was expected, all isolates were resistant towards beta-lactam antibiotics. In addition, nearly all isolates displayed resistance towards tetracycline, which is typical for farm animal-associated MRSA. The resistance rate in MRSA isolates from turkey meat towards ciprofloxacin, which is an important substance in human medicine, increased further (2016: 65.1% resistant isolates, 2014: 55.4%). This trend was already noted in the monitoring programmes of previous years. Good news is that compared to 2014, fewer MRSA isolates from turkey meat were resistant to the antibiotic linezolid, which is applied as a treatment of MRSA in humans (2016:1.2%; 2014: 5.1%).

**Presumptive Bacillus cereus**

Presumptive Bacillus cereus were detected in 28.4% of tomato samples and 8.3% of fresh sprouts samples. The isolates obtained from tomatoes were nearly all Bacillus thuringiensis, which has so far rarely been connected to human diseases. Presumptive Bacillus cereus isolates obtained from sprouts, on the other hand, did not include Bacillus thuringiensis.

The findings have confirmed that bacteria of the Bacillus cereus group may be present in tomatoes and sprouts. In order to be able to better assess potential health risks emanating from food contaminated with this bacterial species, future monitoring programmes should also make a bacterial count of presumptive Bacillus cereus.
Commensal *Escherichia coli*

Commensal *E. coli* were analysed using a quantitative method in 4.8% of samples of fresh sprouts. In three samples (0.8%), counts were higher than the limit of 1,000 cfu/g set in Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs for pre-cut, ready-to-eat vegetables. Which indicates hygienic deficiencies in the manufacturing process. The highest bacterial count detected was 1.7x10E4 cfu/g. *E. coli* are indicators of possible faecal contamination of a product. Findings – though rare – of high bacterial counts in fact indicate that fresh sprouts are in part of dissatisfying hygienic quality. The findings show how important it is to thoroughly rinse sprouts under running water before consumption, in order to reduce bacterial contamination.

The percentage of resistant *E. coli* isolates obtained from conventional broiler flocks decreased slightly, as a whole, with most of the tested antibiotics. Still, the number of isolates resistant to colistin, which is important in human medicine, was higher than in previous years. This requires further observation, all the more as there was a declining tendency in resistance towards this antibiotic in the years before, which was also in line with the declining sales of this substance to veterinarians. What is striking is that *E. coli* isolates from conventional broiler farms had a much higher resistance rate of 86.7% than isolates obtained from ecological chicken farms, of which only 29% were resistant towards at least one of the tested antibiotics. In addition to that, the proportion of *E. coli* isolates resistant to ciprofloxacin, which is important in human medicine, was obviously higher in isolates stemming from conventional farms (44.5% and 59.9% resistant isolates in faecal and caecal samples, respectively) than in isolates stemming from ecological chicken farms (9.7% resistant isolates in faecal samples).

Resistance rates of *E. coli* isolates obtained from the fattening turkeys food chain were found slightly decreased in the 2016 monitoring programme, compared to results of previous years’ programmes. Also, they were less frequently resistant to the substance colistin, compared to 2014. In this, they differed from the isolates obtained from the broiler food chain.

*E. coli* isolates obtained from tomatoes and sprouts were all sensitive to the substances tested. Isolates obtained from mussels and from wild board were also in the majority sensitive to the tested substances (82.4% and 95.9% of the isolates, respectively). However, there were also some single isolates of these two origins that were resistant to ciprofloxacin and colistin.

**ESBL/AmpC-forming Escherichia coli**

ESBL/AmpC-forming *E. coli* were detected by selective methods in about half of the faecal samples from conventional broiler chicken farms and of fresh chicken meat samples (50.2% and 49.8%
positive samples, respectively). Faecal samples from ecological chicken farms were positive with ESBL/AmpC-forming \textit{E. coli} to 25.7\%, and thus significantly less frequently positive than in conventional farms. The findings show that ESBL/AmpC-forming \textit{E. coli} are frequently present in the broiler food chain, and that there is a considerable degree of cross-contamination during slaughtering and processing, as it was also noted with regard to other pathogens. Compared to the findings of the 2013 Zoonoses Monitoring, however, when more than 60\% of the faecal samples gathered in broiler chicken farms and of fresh chicken meat were positive with ESBL/AmpC-forming \textit{E. coli}, the detection rate declined in 2016.

With 36.5\% positive samples, caecal content of fattening turkeys sampled in the slaughterhouse had noticeably less frequent findings of ESBL/AmpC-forming \textit{E. coli} than faecal samples of broiler chickens (50.2\% positives). Fresh turkey meat, too, had a significantly lower contamination rate with ESBL/AmpC-forming \textit{E. coli} (38.8\%) than fresh chicken meat (49.8\%).

In faeces of wild boar, ESBL/AmpC-forming \textit{E. coli} was found in 6.4\% of samples. This shows that ESBL/AmpC-forming \textit{E. coli} is also present in the environment apart from animal farm holdings.

No ESBL/AmpC-forming \textit{E. coli} were found in tomato samples.

The contamination rate of fresh sprouts with ESBL/AmpC-forming \textit{E. coli} was 2.2\%. The detection of ESBL/AmpC-forming \textit{E. coli} in samples of fresh sprouts is in so far important under a point of view of consumer health protection, as sprouts are often eaten raw and contamination is thus not reduced by a treatment before consumption.

The importance of the various ways of transmission of ESBL/AmpC-forming \textit{E. coli} are subject to intensive research. The current state of knowledge is that ESBL/AmpC-forming \textit{E. coli} may be transmitted to humans also by foodstuffs (BfR 2011c). It is not known, however, to what extent this fact contributes to the problems with ESBL/AmpC-forming \textit{E. coli} resistance in human medicine.

\textbf{Carbapenemase-forming Escherichia coli}

The results of testing for carbapenem-resistant \textit{E. coli} indicate a need for improving the specificity of testing methods because, while considerable amounts of \textit{E. coli} with suspected carbapenem resistance were identified in both the broiler and fattening turkey food chains, none of the isolates sent in could finally be phenotypically confirmed as carbapenem resistant.
Enterococcus faecalis and Enterococcus faecium

The isolates of *Enterococcus faecalis* and *Enterococcus faecium* stemming from the broiler and the fattening turkey food chains were resistant to at least one of the antibiotic substances tested in 68% and 80%, respectively. Both species displayed high rates of resistance towards tetracycline and erythromycin.

Round-up

The Zoonoses Monitoring serves to raise relevant and comparable data on the occurrence of the most important zoonotic agents at any level of the food chain that allow drawing conclusions on the infection risk of consumers through consumption of foodstuffs. Continuous testing in the framework of the Zoonoses Monitoring allows monitoring trends in the prevalence of zoonotic agents in animals and in foods. Resistance testing contributes to essential improvement of the data basis in this field and helps to better be able to analyse links between use of antibiotics and development of resistance.

*Salmonella* control measures in poultry flocks led to a decrease in detection rates in the broiler and fattening turkey food chains in the past few years, accompanied by a decrease in the number of cases of salmonellosis in humans. However, the contamination of poultry carcases and meat was not found further reduced in the 2016 Zoonosis Monitoring, compared to previous programmes. The unchanged percentage of *Salmonella*-positive carcases, differences between individual slaughterhouses, and a rising percentage of positive caecal samples of broiler chickens should be reason to make further efforts in this field in order to achieve a uniform, high level of hygiene.

Findings of the 2016 Zoonosis Monitoring showed no progress in the reduction of *Campylobacter* spp. in the poultry meat food chain. Given the importance of *Campylobacter* as a pathogen in humans, there is need for action to reduce contamination of foodstuffs with this bacterium. To this end, the EU has established a process hygiene criterion for *Campylobacter* on broiler carcases of 1000 cfu/g, which became valid in 2018. The results of the monitoring programme show that a considerable portion of the carcases sampled do not meet this criterion. Continued testing in the framework of the Zoonosis Monitoring will allow assessing the effects of this hygiene criterion on the presence of *Campylobacter* in the poultry meat food chain.

The findings of the 2016 Zoonosis Monitoring also confirmed that wild boar constitute a reservoir of various zoonotic pathogens. In addition, the particular circumstances of producing game meat by hunting facilitate contamination of the meat with germs. Game meat should therefore only be consumed thoroughly cooked.
In sprouts, there were some single cases of contamination with potential pathogens. The manufacturing process of sprouts encourages growth of germs which are partially already present in the seed. Thus, sprouts are connected with a high microbiological risk. Sensitive consumer groups such as small children, elderly and immune-suppressed people, and pregnant women should therefore eat sprouts not raw, but only thoroughly cooked.

MRSA and ESBL/AmpC-forming E. coli occur frequently in poultry. However, the rate of detection of these multi-resistant bacteria in the broilers food chain has declined in the past few years. Whether this is going to develop into a trend will be shown by ongoing and future monitoring programmes.

In the transmission of MRSA to humans, consumption of food seems to play a negligible role. As regards ESBL/AmpC-forming E. coli, in contrast, the current state of knowledge is that these multi-resistant germs may well be transmitted to humans by foodstuffs, without being able to quantitate the degree to which transmission is attributable to foodstuffs.

The results of the antimicrobial susceptibility testing show that the portion of resistant isolates continues to be high in isolates obtained from fattening poultry. Partially increasing resistance towards fluoroquinolones gives rise to concern and makes clear that the use of antibiotics, and in particular of fluoroquinolones, in poultry must be further reduced. It was conspicuous that isolates from broiler chickens in ecological farms had clearly lower resistance rates than isolates obtained from conventional farms. There may be a connection between this observed fact and the less frequent use of antibiotic therapy in ecological farms. More studies to this end are needed in order to identify possible differences between ecological and conventional poultry farming with regard to the contamination of birds and foodstuffs with antibiotic-resistant germs. In this context it is necessary to raise reliable data on the use of antibiotics in conventional and ecological livestock keeping.

The low rates of resistance in isolates stemming from wild boar reflect the low anti-microbial selection pressure on intestinal bacteria in game.

The results of the Zoonoses Monitoring programmes give hints to where the foci of veterinary surveillance should be placed. They deliver important information helping government authorities to take suitable measures to reduce the occurrence of zoonotic pathogens.

So, with the overriding goal of reducing consumers’ exposure to zoonotic agents, the Zoonoses Monitoring significantly contributes to health protection of consumers.

Consumers can protect themselves from food-borne infections by thoroughly cooking meat and following strict kitchen hygiene, preventing pathogens from being transmitted from raw meat to other, ready-to-eat food (such as, salads) while preparing the food. In order to counteract microbial
growth in meat or certain ready-to-eat food, particular attention should be paid to keeping up cold chains and defining appropriate, short durability or consumption dates. Raw minced meat and raw meat or milk products as well as certain ready-to-eat foodstuffs pose a potential health risk and should therefore not be consumed by sensitive consumer groups, such as infants, elderly and immune-suppressed people, or pregnant women. Particular advice on how to minimise the risks of infection with *Campylobacter*, VTEC, or *Listeria*, and on how to protect from food-borne infections in the private household, have been published by the Federal Institute of Risk Assessment BfR (BfR 2007b, 2009b, 2011b, and 2014b).