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Antibiotic-Resistant Pathogens in Wastewater From a Pig Slaughterhouse and Their Dissemination into the Aquatic Environment
Antimicrobial Resistance in Hunted Wild Boars in Germany

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Abstract

Wild boars might be a suitable indicator species for the spread of resistant bacteria from animal houses and waste water treatment plants to the environment as they live in the environment and may potentially feed on contaminated material. Therefore, we investigated the prevalence of resistant bacteria among wild boars in Germany in 2016. In the framework of the national monitoring for zoonotic agents samples from hunted wild boars were collected in 2016 and tested for the presence of Salmonella spp., MRSA, E. coli and extended-spectrum beta-lactamases producing (i.e. cefotaxime-resistant) E. coli. Samples were distributed across the federal states of Germany based on the number of wild boars hunted in the respective federal state in 2014. Primary isolation was carried out by the accredited regional state laboratories using harmonized procedures. Results of the investigations were reported to the Federal Office of Consumer Protection and Food Safety (BVL). Isolates from positive samples were submitted to the National Reference Laboratories (NRLs) at the German Federal Institute for Risk Assessment (BfR) for further microbiological analyses. Testing for antimicrobial resistance (AMR) was performed as prescribed by Commission Implementing Decision 2013/652/EU.

Salmonella spp. were isolated from 13 of 551 tested samples. Among 11 Salmonella-isolates submitted to the NRL nine were susceptible to all tested substances. None of the samples yielded MRSA. Among the 219 isolates of E. coli tested for AMR, 210 were susceptible to all 14 tested substances. Selective isolation on MacConkey-Agar supplemented with cefotaxime, however, yielded 6.4 % of samples with cefotaxime-resistant E. coli. Among the 25 confirmed isolates that were resistant to cefotaxime, ceftazidime and ampicillin, 11 (44 %) showed no further resistance, while 14 showed up to five additional resistance traits. Results indicate that while overall the prevalence of AMR is low in bacteria from hunted wild boars in Germany, some may harbor bacteria with specific resistance traits including those to substances of highest priority such as 3rd generation cephalosporins, fluoroquinolones and colistin. The origin of these isolates is not known, however uptake of the resistant bacteria via feed or drinking water is a likely reason for carriage. Further analyses with respect to regional distribution and genetic traits need to be carried out to examine potential regional hot spots of AMR in wild boars in order to examine potential associations with livestock or human wastewater.

Keywords: Wild boar, antimicrobial resistance, monitoring

Introduction

The spread of resistant bacteria from animal houses and waste water treatment plants to the environment is well documented. Wild boars might be a suitable indicator for this spread as they live in the environment and may potentially feed on contaminated material. We therefore investigated the prevalence of resistant bacteria among wild boars in Germany in 2016.
Material and Methods

In the framework of a national monitoring program for zoonotic agents and antimicrobial resistance samples from hunted wild boars were collected and tested for the presence of *Salmonella*, MRSA, *E. coli* and extended-spectrum beta-lactamases producing (i.e. cefotaxime-resistant) *E. coli*. Allocation of samples to the federal states of Germany was proportional to the reported number of wild boars hunted in the respective federal state in 2014. Primary isolation was carried out by the accredited regional state laboratories using harmonized procedures [1]. Results of the microbiological analysis of samples were reported to the Federal Office of Consumer Protection and Food Safety (BVL) for analysis and reporting on the national level. Isolates from positive samples were submitted to the corresponding National Reference Laboratories (NRLs) at the German Federal Institute for Risk Assessment (BfR) for further characterization and resistance testing. Testing for antimicrobial resistance (AMR) was performed using broth microdilution and epidemiological cut-off values as prescribed by Commission Implementing Decision 2013/652/EU [2].

Results and Discussion

*Salmonella* were isolated from 13 of 551 (2.4 %) tested faecal samples from wild boars. This figure is lower compared to the prevalence of *Salmonella* observed in caecal samples of fattening pigs at slaughter within the same monitoring framework of the years 2015 and 2017 (6.1 %). Among the 551 samples 391 originated from adult animals while 139 originated from animals reported as younger than one year of age. For 21 isolates no information on the age of the animal was available. All isolates of *Salmonella* spp. originated from adult animals older than one year. Of the 11 isolates submitted to the NRL 81 % were susceptible to all test substances (Table 1).

The isolation of commensal *E. coli* from faecal samples was performed to investigate resistance patterns of the bacterial population submitted to the NRL, not to estimate the prevalence. The vast majority of 219 *E. coli*-isolates tested for AMR (95.9 %) were susceptible to all 14 test substances (Table 1). However, among the nine resistant isolates, resistance to colistin was most frequent (n=4, 1.8 %). Likewise, resistance to the fluoroquinolone ciprofloxacin was observed in two isolates. Resistance to cefotaxime was not observed among commensal *E. coli* isolates. These results indicate that within the *E. coli* population harbored by wild boars in Germany, overall resistance is fairly rare. However, wild boars may also harbor *E. coli* with antimicrobial resistance traits that are considered a public health concern such as resistance to fluoroquinolones or to colistin, two substances that are considered highest priority critically important antimicrobials by the WHO [3].

In line with that, selective isolation yielded presumptive ESBL/AmpC-producing *E. coli* in 6.4 % of the samples. This confirms previous results from other groups in Europe [4]. Among the 25 confirmed isolates that were resistant to cefotaxime, ceftazidime and ampicillin, 11 (44 %) showed no other resistance, while 14 showed up to five further resistances (table 1). In contrast to the non-selectively isolated *E. coli*, resistance to colistin was not observed in these isolates. However nine isolates (36 %) were resistant to tetracycline and eight isolates (32 %) to ciprofloxacin. Apart from colistin, tigecycline and meropenem resistance was observed to all substances in at least one isolate. The differences in AMR between the selectively isolated ESBL/AmpC-producing *E. coli* and the randomly chosen commensal *E. coli* confirm earlier results comparing these populations in poultry in Germany[5].
Table 1: Microbiological resistance of isolates of *Salmonella*, randomly picked indicator *E. coli* and selectively isolated ESBL/AmpC-producing *E. coli* from wild boars in 2016 to 14 antimicrobials according to the susceptibility testing as described in Commission Implementing Decision 2013/652/EU [2].

<table>
<thead>
<tr>
<th>Program</th>
<th>Salmonella spp.</th>
<th></th>
<th>Commensal <em>E. coli</em></th>
<th></th>
<th>Presumptive ESBL/AmpC forming <em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Number of isolates tested</td>
<td>11</td>
<td>219</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>5</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>1</td>
<td>9.1</td>
<td>2</td>
<td>0.9</td>
<td>4</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>25</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>24</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>1</td>
<td>9.1</td>
<td>2</td>
<td>0.9</td>
<td>4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1</td>
<td>9.1</td>
<td>2</td>
<td>0.9</td>
<td>8</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1</td>
<td>9.1</td>
<td>1</td>
<td>0.5</td>
<td>25</td>
</tr>
<tr>
<td>Colistin</td>
<td>1</td>
<td>9.1</td>
<td>4</td>
<td>1.8</td>
<td>0</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>0</td>
<td>0.0</td>
<td>2</td>
<td>0.9</td>
<td>7</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0</td>
<td>0.0</td>
<td>2</td>
<td>0.9</td>
<td>6</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1</td>
<td>9.1</td>
<td>1</td>
<td>0.5</td>
<td>9</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>2</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>Susceptible</td>
<td>9</td>
<td>81.8</td>
<td>210</td>
<td>95.9</td>
<td>0</td>
</tr>
<tr>
<td>1x resistant</td>
<td>0</td>
<td>0.0</td>
<td>7</td>
<td>3.2</td>
<td>0</td>
</tr>
<tr>
<td>2x resistant</td>
<td>1</td>
<td>9.1</td>
<td>1</td>
<td>0.5</td>
<td>11</td>
</tr>
<tr>
<td>3x resistant</td>
<td>1</td>
<td>9.1</td>
<td>0</td>
<td>0.0</td>
<td>5</td>
</tr>
<tr>
<td>4x resistant</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>&gt;4x resistant</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>8</td>
</tr>
</tbody>
</table>

None of the 575 nasal swab samples tested for MRSA yielded *Staphylococcus aureus* isolates that were confirmed as MRSA by the NRL at the BfR. This is in line with previous studies on MRSA in wild boars in Germany [6]. In contrast, in other European countries, MRSA have sporadically been detected in wild boars [7, 8]. Results indicate that while overall the prevalence of AMR is low in bacteria from hunted wild boars in Germany, some may harbor bacteria with specific resistance traits including those to substances of highest priority such as 3rd generation cephalosporins, fluoroquinolones or colistin. The origin of these isolates is not known, however due to the lifestyle of wild boars selective pressure in their intestinal tract can be considered minimal, thus, the uptake of resistant bacteria via feed or drinking water can be considered a likely reason for carriage. Further analyses with respect to regional distribution and genetic traits need to be carried out to examine regional differences in the spread of AMR among the wild boar population in order to explore potential associations with livestock or human wastewater.

References

Antibiotikaresistenzen bei zoonotischen und kommensalen Bakterien, 2013/652/EU. In: Amtsblatt der Europäischen Union, p 26-39


Abstract

The study aimed at contributing to the surveillance of food safety and antimicrobial resistance by providing information on Salmonella strains circulating in animals of the bushmeat chain in Côte d’Ivoire. Swabs and feces samples were collected among reared grasscutters in farms and dead wild animals sold in a bushmeat market. Sixty-five Salmonella spp were isolated from 425 samples. Serogroups and antimicrobial susceptibility of Salmonella isolates were determined. We found overall Salmonella positive proportion of 14.6% with a significant higher frequency in wild animals (22.4%) compared to the reared grasscutters (7%) (OR = 3.8, CI 95% 2 – 7.6). The isolated strains belonged to the serogroups B (39.4%), E or G (29.6%), D (16.9%) and C (14.1%). The highest rates of resistance were observed for quinolone (53.9%), aminoglycoside (44.6%) and cephalosporin (16.9%) antibiotic classes. Multi-resistance to three or more classes of antibiotics was observed for 9.2% of strains. Antimicrobial resistances to quinolone and cephalosporin classes observed underlined a potential exposure of people handling animals in the bushmeat chain, as well as of consumers. Also, animals used in this sector could be reservoir of resistant-Salmonella to quinolones and aminoglycosides, but more research is needed to understand resistance pathways.

Key words

Salmonella, Wild animals, Grasscutter, Antimicrobial Resistance, bushmeat

1. Introduction

Hunting of wild animals for consumption is a widespread practice in Côte d’Ivoire as in many sub-Saharan Africa countries. In a context of conservation of the biodiversity, breeding of wild animal species was introduced as an alternative for provision of well appreciated bushmeat that serves also as an animal source food. Thryonomis swinderianus (Grasscutter) is one of the most consumed species and one of the most suitable for rearing (Fantodji and Soro, 2004). Thus, both sources of bushmeat, wild and farmed, are exploited for food. Nevertheless, the bushmeat sector is informal in Côte d’Ivoire; also no sanitary control is carried out along the marketing chain before the meat reaches the consumer. In this context, microorganisms responsible for foodborne diseases (FBD) and infectious diseases or transmission of multidrug resistant bacteria strains spread uncontrolled (Founou, et al., 2016).

Salmonellosis is the major contributor to global FBD and given its heavy burden of disease, is considered as one of the prioritized diseases by the European Union epidemiological authorities and the World Organization for Animal Health (Ciszewski, et al., 2015, Knight-Jones, et al., 2010). Outbreaks of Salmonella spp. occur frequently, causing more than 90 million of illnesses episodes and more than one hundred and half deaths per year worldwide (Oh and Park, 2017). In addition, the number of Salmonella strains developing multidrug resistance is increasing.

In this study, we address the food safety issue by exploring the antimicrobial susceptibility of Salmonella spp in wild animal species used for consumption. We aim to determine the distribution of Salmonella spp and antimicrobial resistances among wild animals and reared grasscutters from the bushmeat sector in Côte d’Ivoire.
During nine weeks, between January and March 2018, swabs and feces sampling were taken in two grasscutter farms and one bushmeat market in Abidjan, located in the South of Côte d’Ivoire. Samples from farmed live grasscutters were rectal swabs and feces and samples from market were from various dead wild animal species including rodents, antelopes, birds, small carnivorous and apes. Overall, we collected 215 samples in farms and 210 at the market. \textit{Salmonella} spp were investigated according the protocol ISO 6579. We used buffered peptoned water for pre-enrichment of the swabs and feces; for enrichment we used Selenite Faecal broth and Rappaport-Vassiliadis (RV) broth; and for isolation CHROMagar \textit{Salmonella} Plus and \textit{Salmonella Shigella} agar. We performed tests of oxidase, triple sugar iron, citrate and lysine decarboxylase as biochemical tests for genus identification. Furthermore, we did the \textit{Salmonella} latex agglutination test using the commercially available Wellcolex Colour \textit{Salmonella} identification kit, which provided the serogroup of the strain if belonging to the groups: A, B, C, D, E or G and the Vi antigen. Two isolates from a same sample were considered different if they belonged to different serogroups.

Antimicrobial susceptibility of \textit{Salmonella} isolates were realized on Mueller-Hinton agar using the disc diffusion method according the Kirby-Bauer protocol. Thirteen antimicrobial agents (or antibiotics) representing nine classes of antibiotics have been tested for their susceptibility. Susceptibility was interpreted according the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

We used logistic regression to describe relationships between variables and calculated odds ratios (OR) with 95% confidence intervals (95% CI). We denoted a multidrug resistant strain as an isolate resistant to three classes of antibiotics or more.

3. Results and Discussion

3.1. \textit{Salmonella} frequency and serogroup

Out of all samples cultivated for \textit{Salmonella} spp isolation, 62 were positive giving a sample proportion of 14.6% (95%CI 11.4 - 18.3). The frequency of \textit{Salmonella} spp isolated from wild animal samples (22.4%) was significantly higher than from farmed animals (7%) (OR = 3.8, 95%CI 2.1 – 7.6). In three samples we have found isolates of two different serogroups. The 65 isolated \textit{Salmonella} strains belonged to the serogroups: B (43.1%), E or G (32.3%), D (13.8%) and C (10.8%). Dadié, et al. (2017) found a proportion of 11.4\% \textit{Salmonella} spp. with also serogroup B as most prevalent when isolating and serotyping \textit{Salmonella} from frogs in Côte d’Ivoire. The serovar \textit{S. typhimurium} is the most common in the serogroup B and also the most frequently isolated from humans in Côte d’Ivoire (Boni-Cissé, et al., 2012, Coulibaly, et al., 2010). Regarding wildlife, there are only few studies on wildlife related to \textit{Salmonella} in Côte d’Ivoire; this study is one the first to report on this topic. \textit{S. typhimurium} responsible for non-typhoidal \textit{Salmonella} (NTS) infections, might be the most circulating in the bushmeat chain in Abidjan, suggesting that people handling bushmeat may be infected with NTS. Indeed, Smith, et al. (2014) found genotypic similarities between \textit{Salmonella} strains from captive wild animals and humans in South Africa.

3.2. Antimicrobial resistance concern

Regarding resistances, 63.1\% of strains were resistant to at least one antibiotic with a higher proportion in reread animals (73.3\%) than in wild animals (60\%). According to antibiotic classes, resistances were in the following order: quinolone (53.9\%), aminoglycoside (44.6\%), cephalosporin (16.9\%), monobactame (9.2\%), tetracycline (3.1\%), penicillin (1.5\%) and no resistance in the other classes (Phenicol, Sulfonamide, Beta lactam) (Table 1).
Full sensitivity to the traditional first-line antibiotics chloramphenicol, trimethoprim + sulfamethoxazole and 95% for ampicillin (belong to the classes, phenicol, sulfonamide and penicillin respectively) was observed. According to Harbarth, et al. (2015), when a new antibiotic is introduced to the market, resistances emerge generally five years after its commercial release. For instance in a Nepal hospital, Adhikari, et al. (2017) observed a decrease of Salmonella resistances to chloramphenicol, trimethoprim + sulfamethoxazole and ampicillin among patients. They suggested that this gain in sensibility was because the older antibiotics were replaced by new ones in the human therapy. Full sensitivity to amoxicillin + clavulanic acid observed may possibly be related to the presence of beta-lactamase inhibitor in this drug.

A low animoglycoside resistance of Salmonella has been reported in human clinical strains (Samadi, et al., 2015) while other studies recorded a higher resistance in wild animal species, particularly to streptomycin (Rubini, et al., 2016, Staji and Zandiar, 2017). Gentamicin, tobramycin and streptomycin are the most common antibiotics from the aminoglycoside class. Due to their toxicity, they are rarely used in human and veterinary medicine, since antibiotics of this class were replaced by fluoroquinolones or 3rd generation cephalosporin for treatments against gram-negative bacteria (Hanberger, et al., 2013).

The synthetic characteristic of quinolones implies that the resistance is more likely to occur by vertical gene transfer (Hernández, et al., 2011), in addition to the fact that wild animals species are not exposed to the antibiotic therapy suggest that the high quinolone-resistance observed in our study could be related to an intrinsic resistance of Salmonella strains harbored by these animals. Mechanisms of resistance, intrinsic or acquired, developed by the microbiota are related to plasmids or chromosomes encoding some genes such as beta-lactamase genes (Baguy, et al., 2014). A deeper investigation including screening of genes responsible would shed more light on the origins of Salmonella resistance to quinolone (and aminoglycoside) agents among wild animal species. The quinolone agents tested here were Ciproflaxin, Naxiladic acid and Norfoflaxin. The two former are commonly used in human therapy against severe infections due to enterobacteria and against Gram-positive bacteria (Hernández, et al., 2011). However, because of the general adverse effects of quinolone especially in children, cephalosporins became more suitable for treatments.

Among cephalosporin agents, ceftriaxone (3rd generation antibiotic of this class) is very commonly used in salmonellosis therapy (Stahlmann and Lode, 2013). Hence, its high prevalence of resistance observed is a concern in the bushmeat sector especially for those who are butchering and dissembling animals without any biosecurity measures, but also for farmers and consumers.

### Table 1: Antimicrobial susceptibility profile of Salmonella strains isolated from both samples wild and farmed animals

<table>
<thead>
<tr>
<th>Antimicrobial classes</th>
<th>Antimicrobial agents</th>
<th>Proportion of isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sensitive</td>
</tr>
<tr>
<td>Aminoglycoside</td>
<td>Gentamicin</td>
<td>15.4</td>
</tr>
<tr>
<td></td>
<td>Tobramycin</td>
<td>4.6</td>
</tr>
<tr>
<td>Beta lactam and</td>
<td>Amoxicillin + Clavulanic acid</td>
<td>0</td>
</tr>
<tr>
<td>combination with</td>
<td></td>
<td></td>
</tr>
<tr>
<td>inhibitors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalosporin</td>
<td>Cefepim</td>
<td>27.7</td>
</tr>
<tr>
<td></td>
<td>Ceftriaxone</td>
<td>52.3</td>
</tr>
</tbody>
</table>
We found 6 (9.2%) multidrug resistant strains (resistance to at least three antibiotic classes), distributed in 5 (10%) strains from wild animal samples and one strain from grasscutter samples (6:7%).

Despite differences of resistance proportions noticed in strains from wild animal and those from reared grasscutters, no statistical differences were found between the two categories of samples. This is likely due to the fact of low use of antibiotics in the farming of grasscutters. Indeed, the use of antibiotics in farming is a key factor promoting the emergence of resistant bacteria strains in animals. In Côte d’Ivoire, wild animal species rearing is still little advanced (Goue and Yapi, 2015), but has the potential to supply the population with bushmeat and reduce the hunting pressure in wild animals. However, limited use of antibiotics in this sector and animal welfare guidelines also need to be encouraged, particularly in view of the increasing numbers grasscutter farms.

References


Evaluation of Biomarkers of Udder Health in Cow Milk

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Abstract

In the mammary gland, number and distribution of leukocytes are important for the successful defense against invading pathogens and the resolution of infectious disease. Somatic cells count (SCC) have been used for a long time as indicator of udder health. However, this parameter is temporally and individually more variable during lactation and it always is not a clear indicator of a potential infection.

Besides the determination of SCC, the characterization of milk leukocyte populations was considered a valid and most useful indicator of inflammatory reaction in udders of apparently healthy cows (Schwarz et al. 2011; Pilla et al., 2013). The relative proportions of lymphocytes, macrophages and polymorphonuclear leukocytes (PMN) play an important role in the immunity of mammary glands. The CD11b is expressed as a non-covalently linked heterodimer with CD18 (Mac-1) and is involved in the trans-endothelial migration of monocytes and neutrophils through the interactions with stimulated endothelium to the site of inflammation.

The aim of the study was to evaluate immunological markers of mastitis which are easy to measure and predictive of the disease at an early stage of infection. To evaluate the different immunological conditions, the characterization of milk cell populations and the expression of CD11b on the surface of milk leukocytes was performed on purified milk cells. Composite milk samples from all quarters of 43 cows, 8 of which presenting naturally infected udders were collected, and milk cell populations and CD11b expression were evaluated by flow cytometry. Moreover, for all milk samples, SCC, milk electrical conductivity and the average flow rate, were obtained.

Milk samples from cows with healthy udders showed a significantly higher percentage of live lymphocytes (P<0.04) and a significantly lower percentage of leukocytes expressing CD11b (P<0.03). Moreover, in the group of cows with SCC ranging from 100,000 to 300,000 cells/mL (cows without evident clinical symptoms) Mean Fluorescence Intensity (MFI) of CD11b on leukocytes was significantly lower (P<0.04) in milk samples coming from cows negative at the bacteriological tests. In addition, cows with healthy udder showed significantly lower values of milk electrical conductivity (P<0.01) and higher value of milk flow rate (P<0.03) respect to cows with infected udders.

These results show that flow cytometry parameters as the percentage of specific leukocyte populations and the expression of some molecular markers of the immune system, could be used as potential biomarkers of udder health status in dairy cows.

Keywords
Mastitis; biomarkers; milk leukocytes; flow cytometry;

1 Introduction

Emphasis on genetic selection to maximize milk production has increased metabolic stress associated with milk synthesis and secretion (Sordillo and Streicher, 2002). However, high
yielding cows are more likely to have a compromised immune system and are more susceptible to infection around calving (Cai et al., 1994; Mallard et al., 1998). Recently, the World Health Organization adopted a global plan to combat resistance to antibiotics under the conceptual framework "One World, One Health". Global health of humans and animals is interconnected, pointing to the need for a "One Health" perspective. Animal welfare and antibiotic resistance are now important topics in consumers and citizens’ expectations for the livestock sector. It is necessary to regain consumer confidence by improving practices, transparency and establishing a constructive dialogue with the society at large (WHO, 2015). Solutions can be found in a renewed animal care, improving real time monitoring of animal welfare and in enhancing natural resilience and robustness of animals especially at the early life stages.

Mastitis, is the inflammation of mammary gland and represents around a third of the direct costs of all common dairy diseases (Peters et al., 2015). Mastitis has public health repercussions, particularly associated with the increased use of antimicrobials for disease treatment. Somatic cells count (SCC) have been used for a long time as an indicator of udder health status. However, this parameter is temporally and individually more variable during lactation and it always is not a clear indicator of a potential infection. Beside the determination of SCC, in recent years the differential cell count (DCC) was considered a valid and most useful indicator of inflammatory reaction in udder of apparently healthy cows (Schwarz et al., 2011; Pilla et al., 2013). The differentiation of leukocytes present on milk allows observing changes in relative cell population and the evaluation of immunological markers expressed by each population.

Actually, modern milking machines equipped with automated devices, which register daily milk weights, milk composition, milk flow rate (MFR) and electrical conductivity (EC), have been tested to detect mastitis through changes in milk production and its characteristics (Sørensen et al., 2016).

The aim of our study was to evaluate immunological markers of mastitis that are easy to measure and predictive of the disease at an early stage of infection.

2 Material and Methods

2.1 Animals and milk samples collection

The management and care of the experimental animals was carried out in compliance with the 86/609EEC European Union directive guidelines. Milk samples were collected from lactating cows kept at the Research Centre for Animal Production and Aquaculture. Composite milk samples were obtained from lactating cows and besides the SCC, measured with a DeLaval Cell counter DCC instrument (DeLaval International AB), the measurements of the EC and the MFR were obtained from farm milking machine (DeLaval).

2.2 Purification of milk somatic cells and experimental design

The composite milk samples (100 mL) from all quarters were collected from 43 cows, 8 of which presenting naturally infected udders, ascertained by bacteriological analysis. Immediately after collection, the milk samples were centrifuged at 800g for 20 min at 8°C and the fat layer and supernatant were discarded. Pellet of purified cells were washed twice with cold PBS. The experimental design was established to divide the milk samples first into 2 groups: infected and non-infected, based on bacteriological analysis, and then into three groups based on the SCC values: Group A (n=15) samples with SCC≤100,000 cells/mL, Group B (n=11) samples with SCC=100,000-300,000 cells/mL and Group C (n=17) samples with SCC >300,000 cell/mL.

2.3 Flow cytometric analysis of milk somatic cells

Purified milk cells were stained with a four-color panel of the following antibodies: FITC-conjugated anti CD11b, PE-conjugated anti CD45, APC-conjugated anti CD14 and Live/Dead® Fixable Dead Cell (Thermo Fisher). Beside dead cells exclusion, live PMN and
monocytes/macrophages were identified by gating strategy as LD/CD45+/CD11b+/CD14-/-low cells and LD/CD45+/CD11b+/CD14+ cells respectively; live lymphocytes were identified by size and granularity (FSC-H vs SSC-H) and as LD/CD45+/CD11b+/CD14+/low/CD14- cells; milk leukocytes expressing CD11b were identified as LD/CD45+/CD11b (Figure 1). Milk cell populations and CD11b expression were evaluated on FC500 cytometer and data were analyzed with Kaluza Analysis Software (Beckman Coulter).

3 Results

3.1 Percentage of lymphocytes and expression of CD11b as potential biomarkers of udder health

Flow cytometric analysis showed that milk samples from cows with healthy udders had significantly higher percentage of live lymphocytes (P<0.04) and a significantly lower percentage of leukocytes expressing CD11b (P<0.03) (Figure 2). Moreover, in Group B, cows without evident clinical symptoms, MFI of CD11b on leukocytes was significantly lower (P<0.04) in milk samples coming from cows negative at the bacteriological tests (Table 1).

3.2 The electrical conductivity and the milk flow rate are associated to udder health

The milk EC recorded on cows with healthy udder showed significantly lower values (P<0.01), while the MFR was higher (P<0.03) (Figure 3) respect to cows with infected udder. However, any differences in these parameters were observed between infected and non-infected cows within Group B.

4 Discussion and conclusions

In our previous work, we have effectively used flow cytometry to assess the health status of the mammary gland in cattle (De Matteis et al., 2018a; De Matteis et al., 2018b) and buffaloes (Scatà et al., 2015). Therefore, in this study a flow cytometric approach was used to characterize the leukocytes subsets and the expression of the β-integrin CD11b on the surface of leukocytes purified from composite cow milk. The CD11b is expressed as a non-covalently linked heterodimer with CD18 (Mac-1) and is involved in some important processes including the trans-endothelial migration of monocytes and neutrophils through the interactions with stimulated endothelium to the site of infection. The SCC is a well-established measurement in the diagnosis of mastitis, but does not differentiate into lymphocytes, macrophages and PMN cells. The relative proportions of these cells play an important role in the immunity of the mammary gland and the resolution against invading pathogens depend on number and distribution of leukocytes (Paape et al., 2003; Rainard and Riollet, 2006).

Results of our study showed that in infected conditions, the percentage of live lymphocytes decreased and simultaneously it was observed an increasing on percentage of CD11b+ cells. Furthermore, we observed an increasing of the expression of this immunological marker on the surface of milk leukocytes in cows with SCC within the physiological range but positive to bacteriological test. The evaluation of CD11b expression allow to determining the activation of leukocytes during infection (Borjesson et al., 2002). In addition, we observed that milk indicator traits as EC and MFR showed significant association with the health status of the udder. In conclusion, this study showed that: 1) the percentage of specific leukocyte populations and the expression of immunological markers could be potential biomarkers of udder health status in dairy cows; 2) the flow cytometric parameters together with the measurements obtained during milking could be useful indicators of potentially infected udder.
Acknowledgments
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Figure 1
Flow cytometric gating strategy for the Differential Cell Count

![Flow cytometric gating strategy](image)

Live leukocytes with back gate FS vs SS = LD/CD45+  
Live Lymphocytes = LD/CD45+/CD11b+/CD14+  
Live PMN = LD/CD45+/CD11b+/CD14+  
Live Macrophages = LD/CD45-/CD11b+/CD14+

Figure 2.
Modification of the milk differential cell count (A) and expression of CD11b (B) during infection

![Modification of milk differential cell count](image)
Table 1. Differential cell count and expression of CD11b in milk samples from Group B

<table>
<thead>
<tr>
<th>Group B</th>
<th>Lymphocytes</th>
<th>PMN</th>
<th>Macrophages</th>
<th>Leukocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean Fluorescent Intensity (MFI) CD11b+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non infected</td>
<td>45.90 ± 4.59</td>
<td>39.39 ± 11.67</td>
<td>14.80 ± 14.80</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>26.30 ± 8.38</td>
<td>53.93 ± 13.05</td>
<td>23.24 ± 4.96</td>
</tr>
</tbody>
</table>

Figure 3. Changes in milk indicators Electrical Conductivity (A) and Milk Flow Rate (B) during infection mastitis

References
Alternative Approaches to Managing Demand for Antibiotic Treatment in Dairying

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Abstract
In response to concerns about the emergence of resistance, the use of antimicrobials in agriculture is being subjected to increasingly stringent regulations. These inputs are typically administered by farm personnel seeking to control a biological condition of uncertain origin, and they are not always effective in doing so. A standard formal economic model exists for production input demand where the input has uncertain efficacy. However there is reason to believe that decision-makers systematically deviate from expected behavior. We used antibiotics demand and administration data collected from about 460 dairy farmers to assess responses to the probability of avoiding a loss and the loss magnitude. The responses are studied with an eye to policy implications. In particular, a tax on antibiotics is unlikely to significantly reduce demand while increasing the potential loss that antibiotics help to manage is unlikely to increase demand by much. Farmers would like more informative diagnostics, but it is unclear how these diagnostics would affect demand for antibiotics.

Keywords Antibiotics tax, behavioral decision biases, diagnostics, mastitis, response to loss.

1. Introduction
Regulations on antibiotics in animal agriculture have expanded over the past decade. Antibiotics have been applied to food production animals in feed and water to either prevent disease or improve feed efficiency, and also as therapy. Our interest is in dairy farm use where purposes are therapeutic, i.e., restoring productivity through eliminating infections. Our focus is the managerial economics of farm-level antibiotics choices. The decision-making problem arises in barnyard conditions and can be information intense. The goal is to protect value and the management setting is to process information on a cow’s health status in the presence of substantial uncertainty about what, if anything, ails the cow and whether a treatment will prove to be effective. The cow’s genetic profile, production records and medical history, the diagnostic tests used and recent events in the herd are among relevant data that will be used to assess the risks and potential rewards from the antibiotic application decision.

Humans are prone to many decision-making biases (Just 2014) that can arise as a result of external pressure or the way the human brain perceives and processes information. Research in human medical practice reveals strong patient pressure on doctors to over-prescribe antibiotics (e.g., Linder et al. 2017). Behavioral nudges through informational reminders (Meeker et al. 2014) and feedback mechanisms (Eilermann et al. 2018) have been advocated and show promise. Evidence is also accumulating that agricultural decision-makers may, through rational inattention or irrationality, choose inputs to attain goals other than maximizing mean profit or risk adjusted mean profit (Du et al. 2017). For example, Perry et al. (2017) find evidence that United States corn and soybean growers did not adjust herbicide active ingredient application rates when only product concentration changed.

Arguably the most influential non-classical theory on economic decision-making is prospect theory. It emphasizes distinctions that individuals make between gains and losses relative to a reference point, and also patterns in how objective outcome probabilities are reformed into weights when evaluating alternative risky choices. Bocquého et al. (2014) provide experiment-based evidence that sampled French farmers distinguish between gains and losses, are more sensitive to losses than gains and behave as if unlikely adverse events were more probable than is true. More specific to the issue at hand, we are not aware of work addressing how farmers think of on-farm antibiotics decisions. Although not addressing antibiotics management choices, Hansson and Lagerkvist (2014) argue that the prospect
theory mindset toward choice evaluation shapes how Swedish dairy farmers compare mastitis management strategies.

Broadly considered, antibiotics have three potential roles in animal protein production: to promote growth and improve feed-to-product conversion; to prevent clinical manifestation of disease; and to treat an identified disease. The first use is not an issue in milk producing animals, in part because milk is tested for the presence of antibiotics and will be condemned if found. The second and third uses occur through direct treatment and not with feed as a vehicle. As with humans, there are many reasons why antibiotic therapy can arise. But the main organ treated is the mammary gland for mastitis, to prevent or eliminate infections that will temporarily reduce milk production or will permanently scar tissue. Antibiotics can be injected directly into the teat when the cow is not lactating to reduce the risk of udder inflammation. Alternatively, a lactating cow suspected of having mastitis may be treated and the milk discarded until it is free of antibiotics. Effective from 2017, U.S. Veterinary Feed Directive (VFD) regulations no longer permits growth promotion and feed conversion efficiency rationales for applying antibiotics that are labeled as important for human medicine. Our interest here is in the other two motives. A VFD goal is to ultimately shift many antibiotics from over-the-counter (OTC) availability to availability only upon obtaining a prescription from a licensed veterinarian.

Some clarifications together with a simple decomposition may be in order when comparing the extent of antibiotics actually applied in food production with what is best for society. The standard economic framework views farmers as making decisions so that some version of profit, expected profit or a related private objective is maximized. However, farmers may have little incentive to include the impact of their antibiotics actions on the development of antibiotics resistance and so ultimately on losses to society through deaths and additional costs for alternative treatments. The damage is done through widespread use is beyond an individual’s control and a farmer who refrains from private use will compete with those who do not. Thus privately optimal use is likely to far exceed what is best for society. In that case, a tax on antibiotics in agriculture or restrictive regulations would be effective instruments for curtailment but it is not clear whether an information campaign to increase awareness would have any discernible impact. Our motivation is separate. It is how actual use compares with privately optimal use. Were actual use to exceed privately optimal use then a new set of instruments become available to manage antibiotics in agriculture. For example, farmers can be made aware of private advantages of reducing input levels or of neglected technologies. Alternatively, if their inability to achieve intended goals are better understood then modifications to the decision setting, e.g., choice architecture (Thaler et al. 2013) may achieve sustainable and voluntary reductions in use. For example, requiring that the order for a prescription antibiotic be filled at a pharmacy and not by the veterinarian may remove concerns about conflict of interest and also give the grower an opportunity to reflect on all costs that would arise from applying each dose. Figure 1 illustrates the suggested input decomposition.

Our research seeks to understand both how antibiotics are used on dairy farms and whether there might be opportunities to use behavioral economics approaches to reduce antibiotics demand for mastitis therapy on these farms. It does so by asking farmers about the costs that mastitis imposes on them, how they respond to hypothetical disease management problems and what aspects of costs and benefits they emphasize most when making management decisions.

2. Materials and Methods

2.1. Formal model

A producer faced with a potential mastitis case has choice set \( n \in \{1, \ldots, N\} \equiv \Omega \) at cost \( C_n \) available to control the case. However the producer does not know whether an alternative will cure the case. Suppose that the producer taking management choice \( n \) faces loss of amount \( L_n \), and this loss can be avoided with probability \( p_n \). For choice \( n \) the expected value of loss avoided is \( p_n L_n \). The producer
can be viewed as having the problem of choosing action $n$ to maximize the expected value of loss avoided, i.e., $\max_{n\in\Omega} p_n L_n - C_n$. For a grower concerned only with expected profit any choice satisfying $p_n L_n \geq C_n$ will be considered further, and so growers should not be willing to pay (WTP) a cost beyond $p_n L_n$. Figure 2 depicts maximum WTP for this model, which we refer to as the Classic Expected Loss Model (CELM). Producers should not be WTP beyond some amount $C_{\text{WTP}}$ for $(p, L)$ pairs to the north and east of locus $C_{\text{WTP}} = pL$ with sample locus point $(\hat{p}, \hat{L})$.

2.2. Data
During spring and summer 2017, paper and web surveys were sent to dairy farmers in Wisconsin, Minnesota and Michigan, requesting information about farming conditions, investment intentions, demographics, operation scale and markets. The overall response rate was 21%, or 648 responses. A section was included that asked about antibiotics input choices on the farm, anticipated cost and revenue implications. One question presented a stylized decision environment. Four contexts were provided with Latin Square design where in each two parameters were given: probability a single cow can be cured with antibiotics, and reduction in loss if cured. Subjects were asked to provide the greatest cost they would be willing to pay (WTP) to treat the animal. Four versions were sent out differing only in this question. The number of responses to the WTP queries, which were at survey end, was 480.

We queried the nature of costs incurred per mastitis case. Median data are reported because some respondents provided unreasonably high cost levels for certain categories. Data are provided in table 1 below. These data accord well with other survey based estimates of costs, e.g., Rollin et al. (2015). Therapeutics account for a very small fraction of median costs. The cost category estimate is considerably larger than the cost of diagnosis and also larger than, though of comparable order with, the labour administration and veterinary service costs.

We also queried WTP under alternative $p$ and $L$ values. Four instrument versions were sent out, each to approximately a quarter of the survey sample and each soliciting WTP for four $(p, L)$ combinations. The query states “While observing your herd you develop the view that a cow is not performing optimally and may have an infection that can be cured by use of antibiotics. Treatment incurs a cost. The treatment may not prove to be effective. For each of Settings A through D, please provide an answer as best you can in the box to the right.” The subject is then provided with $(p, L)$ values and asked “What is the greatest cost you would be willing to pay to treat the animal?” Mean responses are provided in table 2 where cells where WTP exceeds expected loss avoided have green background. Cells where the relation is equality and where the inequality is reversed are coloured, respectively, yellow and red. Clearly for low probabilities and low losses WTP exceeds expected loss avoided. In addition, WTP appears to be more sensitive to low losses than to low probabilities.

We also asked respondents “Please identify the most and least important factors for your operation in regard to managing mastitis.” As listed in table 3, they were provided with three alternatives. Clearly more emphasis is placed on managing the probability that the treatment is successful than on managing treatment cost or on reducing loss at risk.

3. Results and Discussion
The case has been made for a tax on antibiotics (Hollis and Ahmed 2013; Van Boeckel et al. 2017). Our table 1 data suggest that a direct tax is unlikely to be effective in dairy production. It is too small a
component of overall costs and losses involved. An approach that is more likely to be successful, one which has seen general application as a by-product of new regulations, is to tie antibiotics use with other costs. This is in part how shifting antibiotics to prescription only is likely to work. Increasing time costs, including management and veterinarian time, is likely to generate a larger effective tax on antibiotics than even a large product tax.

The excess WTP finding in table 2 provides evidence that the actual application rate may indeed exceed that which would maximize producer profit. Some caution is warranted in interpretation, however. Mastitis causing bacteria can spread from cow to cow through equipment, and also into the farm’s environment to be contracted later by herd mates. We did ask the respondent to assume that the cow had been isolated and, furthermore, 70% of respondents reported that cows with mastitis were separated. If, upon detection, cows are separated then they pose no further risk to the herd. Nonetheless, herd owners may not accept this logic and may factor potential losses posed to herd mates into their WTP. Tables 2 and 3 also suggest that farmers pay more attention to increasing the probability that treatment succeeds rather than to cost or to controlling the loss at risk. This may be because they believe that probability of successful treatment is most responsive to factors at management’s control. Or it may be connected with psychological processes that underpin regularities in other data brought forth in the literature in support of prospect theory. In any case, that WTP is comparatively more sensitive to probability suggests a role for enhanced diagnostics in better managing dairy farm demand for antibiotics.

<table>
<thead>
<tr>
<th>Table 1. Median costs for mastitis case by category.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost category</td>
</tr>
<tr>
<td>Diagnosis</td>
</tr>
<tr>
<td>Therapeutics, i.e., antibiotics</td>
</tr>
<tr>
<td>Non-saleable milk</td>
</tr>
<tr>
<td>Veterinary service</td>
</tr>
<tr>
<td>Labour</td>
</tr>
<tr>
<td>Death loss</td>
</tr>
<tr>
<td>Lost future milk</td>
</tr>
<tr>
<td>Loss from premature culling</td>
</tr>
<tr>
<td>Lost from future reproduction</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Willingness to pay for antibiotics treatment (expected value in parentheses).</th>
</tr>
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<tbody>
<tr>
<td><strong>Loss that might be avoided</strong></td>
</tr>
<tr>
<td>Probability</td>
</tr>
<tr>
<td>0.40</td>
</tr>
<tr>
<td>0.55</td>
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<tr>
<td>0.70</td>
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<tr>
<td>0.85</td>
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</table>

<table>
<thead>
<tr>
<th>Table 3. Reported most and least important factors when managing mastitis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent most</td>
</tr>
<tr>
<td>Increasing probability that treatment is successful</td>
</tr>
<tr>
<td>Managing cost of treatment</td>
</tr>
<tr>
<td>Reducing loss at risk</td>
</tr>
<tr>
<td>Respondents</td>
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</table>
References
ADVANCED TRAINING THE KEY FACTOR IN INSPECTION OF MEXICAN IMPORT-AND-EXPORTATION FOOD

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Abstract
Food safety and quality certification at inspection points has become the main requirement in the international food trade. Food inspection protocols are fundamental tool that allow governments the introduction of quality and safety foodstuffs into their domestic markets. The National Service of Health, Safety and Agro-Food Quality (SENASICA) a decentralized administrative body of the Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA), is the governmental instance responsible for carrying out food safety inspections on all imported and exported food in Mexico. This paper identifies the weakness of protocols associated with the training of food inspectors, and also provides identification of international standards to be integrated into food safety management systems, to proactively deal with the risk-based approach for protecting against hazards associated with food safety. Mexican Plant Health and Security and Animal Health Officers were surveyed about the management of the cold chain at inspection stations of export-and-importation of food. Inspector’s integral management and knowledge of the cold chain, while running the food inspection should be improved as to keep the original quality and safety of the perishables products. Then, it is proposed the implementation of training programs that incorporate worldwide standards in force in international markets, in order to guarantee food safety in all perishable foods under inspection.

Keywords: Official food inspection, food safety regulation, quality shelf-life.

1. Introduction
Food safety is now still a matter of interest and concern. Largely, it is due to the global trend of food chain governed in a context of national and international efforts, to harmonize within food safety frameworks. In conjunction with the updating and harmonization of regulations, it is laid the importance of the continuous training. As a result, constant improvement is propitiated in knowledge and inspection procedures, together it is also encouraged with the experience of inspectors, officials, consultants and auditors. Legislation on food safety is based on risk assessment and transparency, as well as risk communication among the actors in the food supply chain (Smigic et. al., 2015). Bradsher et. al. (2015) indicated that within the curriculum framework of professionals in food protection, must have a continuous training during their professional phase. Government authorities are interested in addressing the challenges in implementation and effectiveness of the training programs for professionals dedicated to protection of food. For that purpose, they rely on partnerships with programs designed academically to identify the priorities in the assessment of risks in food (Fortin and Weir, 2015). For import-and-exportation operations is essential to implement regulation of food safety systems to ensure the level of protection against health risk (Malorgio et. al., 2016).
Aung and Chang (2014) showed that one factor referred to food safety and quality is spoilage. It affects costs, food waste, but also adversely affects trade and consumer confidence. To maintain food quality and safety, it is involved strict cold chain management through the supply chain. Microbial growth causes the deterioration of the food that drives the loss of food safety,
which is caused by the abuse of the temperature into the cold chain (Martin, G. 2000). Hsiao et. al., (2017) established that food quality and safety depend to a great extent on the management at each link in the supply chain. Therefore, cold chain management of perishable foods is essential to guarantee safe and high-quality products. It is then required, strict control of time and temperature during food import-export inspection operations. The aims of this paper were to identify the weakness associated during food inspection of import-and-exportation, and the identification of international standards to strengthen the risk-based approach.

2. Material and Methods
Surveys in Mexico were performed to the target population of 48 Phytozoan Plant Sanitary Security and Animal Health Officers from the National Service of Health, Safety and Agro-Food Quality (SENASICA) of the administrative body of the Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA). They mainly focusing were responsible of food verification for import-and-exportation trade activities at five entry ports located at the Mexico’s Northern border. This study focused on cold chain management and the difficulties associated during food inspection and emphasizing on the international standards that support the risk-based approach on international trade. It was set up a conceptualization of the main factors affecting compliance capacity of import-exports with food safety standards (Ait Hou et. al., 2015; Grazia et. al., 2012). It is also included the policy improvements of food inspections provided by the respondents. The data set was analysed as randomized completed design, and Chi-square test (α = 0.05), using the SAS package. The response variables were related with difficulties and suggestions of the respondents.

3. Results and Discussion
The information gathered shows that 88% respondents had a training program on a regular basis, but only 83% systematically recorded data. Of the same population, 85% indicated knowing precisely the importance and impact of cold chain on the quality and safety of perishable products. Cold chain interruptions while running food inspections were pointed out in 73% of the surveys. Table 1 shows some sources of these breaks in the cold chain. Excessive shipment handling within premises away from any refrigerated environment, workload above inspector’s number and incomplete files along confusing protocols were considered the most critical factors against a smooth and fluid inspection process. Malorgio et. al. (2016) also reported that confusing protocols, along poor match and harmonization of standards and insufficient staff were the major time-consuming conditions to overcome in the inspection of perishable foods and compliance of legislation.

Table 1. Difficulties associated with the interruption of the cold chain during the inspection process of perishable food products.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Percentage of responds (% ± SE)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excess of mobility of products in inspection site</td>
<td>20.59 ± 7.64</td>
</tr>
<tr>
<td>Excessive workload or lack of personnel</td>
<td>16.91 ± 7.82</td>
</tr>
<tr>
<td>Lack of documentation and of clarity in the regulations</td>
<td>16.18 ± 7.85</td>
</tr>
<tr>
<td>Excess in waiting time due to administrative procedures</td>
<td>16.18 ± 7.85</td>
</tr>
<tr>
<td>Failures in electrical energy</td>
<td>15.44 ± 7.88</td>
</tr>
<tr>
<td>Lack of adequate facilities</td>
<td>14.71 ± 7.92</td>
</tr>
</tbody>
</table>

* Respondents could select more than one factor. $\chi^2 = 0.8844$

Food inspectors suggested that the process of food inspection at entry ports could be improved time-wise, if reinforcement of controls to carriers, better infrastructure and adjustments in inspection procedures were carried out. In addition, up-dating and continuous training will strengthen inspector’s abilities and decision making at the time of food inspection (Table 2).
Malorgio et. al. (2016) emphasized the need to define a policy for continuous improvement in facilities and of personnel profile to consolidate fast and safe protocols for food inspection.

### Table 2. Respondents' suggestion for improving policies for food inspections.

<table>
<thead>
<tr>
<th>Proposal</th>
<th>Percentage of responds (% ± SE)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reinforcing transportation at border controls</td>
<td>24.36 ± 7.97</td>
</tr>
<tr>
<td>Improve infrastructures</td>
<td>21.01 ± 8.15</td>
</tr>
<tr>
<td>Simplifying/unifying control and administrative procedures</td>
<td>21.01 ± 8.15</td>
</tr>
<tr>
<td>Increase and systematize technical training programs</td>
<td>17.65 ± 8.32</td>
</tr>
<tr>
<td>Improve the operability of inspections on sites</td>
<td>15.97 ± 8.40</td>
</tr>
</tbody>
</table>

* Respondents could select more than one proposal. $\chi^2 = 0.6349$

Food hazards along the food supply chain could be coped with collaboration and diligence of all the actors involved are required. Therefore, food protection professionals must know the complexity and interrelation that all components of the supply chain play as well (Fortin et. al., 2015). Additionally, Malorgio et. al. (2016) recorded that the most important benefits in compliance with food safety standards are associated with increasing efficiency in interrelations among agents, improving commercial practices, market access and competitive advantage, combined with reduction of health and commercial risks. The training of food staff and level of education are factors that straight affect the quality and safety of food products. The necessity of having employees with a high qualification in all the links of the food chain is really imperative and requires the incorporation of international regulations and regulatory bodies (Antić and Bogetić, 2015). Shinbaum et. al. (2016) pointed out that the governments focus on food staff training, since it reduces costs that lead to a significant decrease in foodborne illnesses and a lower recalls rate. Therefore, a higher level of education and a systematization in the training of officials dedicated to food inspection focused on risks becomes more relevant (Läikkö- Roto et. al., (2015). Training programs increase knowledge in food safety practices and improve inspection scores (Kirshner, 1993).

Smith (2009) has mentioned that Government, traditionally has the responsibility to establish mandatory norms of food safety, to guarantee safe food to consumers. Private protocols and mechanisms are developed in the supply of higher quality food. Exporters are obliged to demonstrate compliance with public regulations, especially in differentiated product markets, for satisfying requirements of niche market. Public and private standards are based on the implementation and operation of food controls, and compliance throughout the food chain. Henson and Humphrey (2009) identified that there are four key drivers that have a direct influence on agri-food value chains in regulation and structuring in international markets: real risks in food-chain-production, consumers’ and enterprises’ safety and quality interests, strategies of business proficiency, competitiveness in a globalized market and responsibility transferred to the private sector.

Then, the operability of quality management in the food supply chain requires that food staff have a continuous training program. It is based on establishing a basic attitude, and each member represents a key role in solving problems (Lehnert and Bruckner, 2014). The reasons for implementing training programs are based on various reasons (Kamiske and Brauer, 2008), such as improvement of objectives, updating legal and public requirements, having a competent educational sector, motivate employees and have a qualified staff of professionals responsible for safety of food (Riedel, 2009). Currently, training programs should be determined with proof of competency, in compliance with standards and norms in force with food and feed trade (EQAsce, 2018; FDA, 2017). Private food standards have acquired a relevant role, and address topics related to food safety and quality issues (Clarke, 2010). Therefore, standards having a substantial impact on food safety management practices throughout the international food supply chain are detailed in Table 3.
Table 3. Key characterises of Safety and Quality Standards

<table>
<thead>
<tr>
<th>KEY FEATURES</th>
<th>STANDARDS</th>
<th>End applier of standard</th>
<th>Management of GMP/GHP, GAP, HACCP programs</th>
<th>Key Elements of HACCP</th>
<th>Geographic area of application</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>International standards</strong></td>
<td>CODEX Hygiene Principles &amp; other relevant codes</td>
<td>All food supply chain</td>
<td>Partially applied</td>
<td>Fully applied</td>
<td>International</td>
</tr>
<tr>
<td></td>
<td>ISO 22000</td>
<td>All food supply chain</td>
<td>Fully applied</td>
<td>Fully applied</td>
<td>International</td>
</tr>
<tr>
<td><strong>Global Food Safety Initiative (GFSI)</strong></td>
<td>Safe Quality Food (SQF) 2000</td>
<td>Food manufacturers</td>
<td>Fully applied</td>
<td>Fully applied</td>
<td>US and Australian market</td>
</tr>
<tr>
<td></td>
<td>International Food Standard (IFS)</td>
<td>Food manufacturers</td>
<td>Fully applied</td>
<td>Fully applied</td>
<td>German, French and Italian market</td>
</tr>
<tr>
<td></td>
<td>Food Safety System Certification (FSSC) 22000</td>
<td>Food manufacturers</td>
<td>Fully applied</td>
<td>Fully applied</td>
<td>Europe</td>
</tr>
</tbody>
</table>


There is a similarity between most standards, in the sense that all propose a safety-integrated management, whilst different stakeholders and geographical regions (Mensah and Julien, 2011) associate with differences owned. However, benefits are related to access to markets, development of personnel, improvement in communication of information and continuous improvement in food quality and safety. It is pointed out that national and international Governments are imposing new and tighter legislations and regulations, for ensuring food safety with quality assurance systems at each level in the food chain (Trienekens and Zuurbier, 2008). In this case, the Mexican government has the challenge of improving operational inspection activities, both in the continuous training programs for inspector staff, as well as in the harmonization of the administrative operations in compliance with international standards, and in the infrastructure improvement to be able to respond to inspection requirements in import-and-exportation food imposed on the international markets.

References


DETERMINANTS OF PET FOOD PURCHASING BEHAVIOR

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Abstract
Within the growing pet food market, the expanding number of options for pet food, strong marketing messages, and confusing (and often conflicting) information on the Internet have made purchasing decisions difficult for pet owners. Given these challenges, it is important to understand the underlying determinants of pet food choice so that the veterinary healthcare team can better educate pet owners about what and how to feed their pets for optimal health. The study enrolled 2,181 participants (1,209 responding about their dogs; 972 responding about their cats). Results suggest that pet owners want to provide their pets with the best nutrition possible, but face numerous challenges in determining the best diet to feed their pets. It is important for animal health providers to understand owners' underlying motivations for pet food purchases and why a pet is being fed a certain diet, and use this information to provide specific evidence-based recommendations that optimize patients' health.

Keywords
Pets, dogs, cats, pet food, purchasing decisions

Introduction
Pet ownership in the United States has been steadily growing, with 68% of households having at least one pet in 2014 (American Pet Products Association, 2015). As pet ownership has grown, overall consumer spending on pets has risen drastically from $17 billion in 1994 to $58 billion in 2014(1). A substantial component of this spending has been for pet food, with U.S consumers having spent an average of $194 per year in 2013 for pet food (Statista, 2015). With the growing number of pet owners and increasing consumer spending on pets, the pet food industry has expanded in recent years to include new retail outlets for pet food, new marketing strategies, and new varieties of pet food. In addition, the growing trend of humanization and anthropomorphism of pets has spurred strong marketing messages, ingredient claims, confusing
, and often conflicting, information on the Internet about the best food for pets (American Pet Products Association, 2015; Statista, 2015; PetFood News, 2016).

Consumers face a dizzying array of pet food choices and a growing wealth of misinformation regarding pet nutrition. Understanding how consumers make pet food purchase decisions and what factors of pet food are most important is essential information for veterinarians to help pet owners make more objective decisions about their pets' food. The purpose of this study, therefore, was to identify determinants of pet food purchasing decisions.

Material and Methods

A survey was designed to gather information about pet food purchase decisions, including the type of pet owned, factors influencing pet food purchases, owners' relationship with their pets, and demographics. Survey questions were created based on past consumer behavior research surveys and established scales (Laflamme et al., 2008; Michel et al., 2008; Chen et al., 2012; Boya et al., 2015). Most responses were made using a 7-point Likert scale. A Health Prioritization Gap measure was calculated by subtracting the importance of healthy food for pet ratings from the importance of healthy food for self-ratings.

The survey was administered through commercial survey software and was available from July 2015- February 2016. The survey was designed so that the respondent entered the names of all of his or her pets and then the software randomly selected one of the respondents' pets (if they owned more than one) and asked the questions as they pertained to that specific pet. The survey was designed to take approximately 10-15 minutes to complete. A “snowball” survey recruitment approach was used to invite cat and dog owners to participate in the study. The study was approved by the Tufts University Institutional Review Board.

Results and Discussion

The online survey was accessed 2,484 times with a total of 2,181 respondents completing at least 80% of the survey. Fifty-five percent (1209/2181) of respondents answered questions about their dog and 45% (972/2181) of respondents’ answers were about their cat. There were no significant differences in demographics for cat and dog owners (data not shown) so these were combined for all results. Respondents were predominately female (1838/1974) and the age of all respondents ranged from 18 to 82 years (mean = 46 years). A notably large proportion
(564/1975) of respondents were employed in the veterinary healthcare field or animal industry, and most respondents (1981/2181) were the primary decision makers in pet food purchases.

**Characteristics of Foods Purchased**

Respondents were asked several questions regarding what types of food they feed their pets and where they purchase pet foods. Eighty-eight percent (1943/2188) of respondents indicated that they feed commercially-prepared foods to their pets and over half (1194/2182) of respondents indicated that they fed primarily dry food to their pet. The only significant difference between cat and dog owners was that cat owners were significantly more likely to feed canned food compared to dog owners (713/977 for cats versus, 419/1211 for dogs; P<0.001). Twelve percent (278/2182) of respondents indicated that they fed other types of food to their pet, including raw food, dehydrated food, or supplements. Fifty-one percent (997/1939) of respondents indicated that they purchased their pet food from either large specialty stores, or from other sources such as such as Internet retailers (e.g., Amazon), directly from the manufacturer, or from local agricultural feed stores.

**Pet Food Labels**

In order to understand the role of calorie labeling in pet food purchase decisions, respondents were asked about their use and knowledge of calorie labeling on pet food. Most respondents (1446/1931; 74.9%) indicated that they are aware of calorie labeling on pet food but only 1013/1935 of respondents (52.4%) indicated that they use or notice the calorie labels on pet food. To further understand the role and use of pet food labels, respondents were also asked to rate their agreement with two statements about pet food labels. 63.02% (n=1256) agreed with the statement, "Information on pet food labels is misleading". For the statement, "Information on pet food labels is easy to understand," 41.1% (n=817) agreed and 47.2% (n=937) did not agree.

**The Health Prioritization Gap**

Respondents were asked to rate how important buying healthy food was for themselves and how important buying healthy food was for their pet. The owner-pet "Health Prioritization Gap" (i.e., the difference between scores) showed that 1023/1926 (53.1%) of respondents had equal priority for themselves and their pet (Health Prioritization Gap = 0, Equal Priority group).
Eight hundred forty of 1926 respondents (43.6%) had scores indicating a higher importance for buying healthy food for their pet (Higher Priority Pet group), and only 63/1926 respondents (3.3%) had scores indicating a higher priority on buying healthy food for themselves compared to the pet (Higher Priority Self group). Mean age of respondents in the Higher Priority Pet group was significantly younger (44 yrs versus 47 yrs; p = 0.0063) and this group was significantly more likely to actively seek out information about pet food (as indicated by higher rankings to the question “I actively seek out information about pet food”; p<0.001) compared to the Equal Priority group.

The focus on ingredients and good nutrition may reflect how trends in human health and nutrition have begun to spill over into the pet health world. As consumers have become more interested and concerned about what is in their food, they have begun to also focus more on the ingredients and production of their pets’ food. This is supported by the fact that 53% of respondents had a similar rating for the importance of buying healthy food for their pet versus themselves. However, it was surprising that 43.6% of respondents indicated that buying healthy food was more important for their pet than for themselves- a phenomenon that we describe here as the Health Prioritization Gap.

Ensuring that pets receive proper nutrition requires understanding consumer behavior in regards to pet food purchase decisions. However, increasing marketing claims and misinformation about pet nutrition and the spillover of trends from the human health and nutrition realm into the pet food market further complicate the already challenging task of educating consumers on how to best feed their pets. However, the strong bond owners have with their pets, their priority for providing their pets with the best nutrition possible, and their use of the veterinary healthcare team for nutritional information provides an excellent opportunity. In order to provide sound nutritional advice to their clients, members of the veterinary healthcare team need to understand the underlying motivations of pet food purchases and why a pet is being fed a certain diet, and use this information to provide specific evidence-based recommendations that optimize patients' health.
References


A procedure to approach interdisciplinary issues in One Health initiatives

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Abstract

Globalized world is increasingly showing problems of complexity together with the holistic and multidisciplinary aspect of its nature. The authors, involved in the COST EU funded “Network for Evaluation of One Health” (NEOH), after having worked in different fields, experienced the need to find an intuitive methodology to connect and integrate already existing elements and methodologies used in diverse disciplines. This work aims to provide a most practical and simplified way to integrate, combine and hybridize existing models from different disciplines, maintaining at the same time coherence with the interdisciplinary approach and system thinking. Two food-related case studies (food packaging and aquaculture) were selected according to their known or potential complexity to apply the proposed protocol in real, empirical situations. The objective of the protocol is to guide the potential users in the accomplishment of three relevant tasks in the One Health approach: (a) the description of the case study as a system within its boundaries (system identification); (b) the hybridization of knowledge from different disciplines; (c) the practical use of existing methodologies to help governance and find solutions.

These tasks are closely linked in the development of the cognitive process needed to organize the work and build up an interdisciplinary team to face complex problems. The cognitive process takes the shape of a “matrix of knowledge”. The matrix lists in vertical the knowledge and in horizontal the type of disciplines involved, the expertise required and the main methodologies needed to solve complex problems. In parallel with this process, a representation of the system and sub-systems (image of the system) related to each case study is developed and updated iteratively. The use of simple graphical symbols allows the translation of the matrix of knowledge into the image of the system. The protocol presented is an exercise that researchers are challenged to apply in other domains and situations to test its viability and effectiveness. On the other side, it could be considered a first step for beginners in system thinking and interdisciplinarity to achieve a user-friendly approach towards more complex routines. After verification, it could serve a wide range of needs and applications in project planning, implementation, management, and evaluation.

Keywords: Interdisciplinarity, system thinking, knowledge, integration

1 Introduction

One Health (OH) is increasingly getting the attention of health institutions at any level, from policy makers to managers, from field operators to researchers. OH approach is challenging for several reasons, not least the change it requires in the mind of people who get involved in OH initiatives. As in many other fields of human activity, specialization tends to prevail among operators, actors and scientists as the result of long process of scientific reductionism, which reflects in the institutional organization of knowledge, competences and operation (World Bank report 2018). OH is placed at the crossroad of human, animal and environmental domains (Baum et al., 2017): The
complexity of technical, socio-economic and environmental interrelations must be carefully understood at the scientific level and managed at the operational level (Boriani et al., 2017). Facing this complexity requires inter-trans-disciplinarity and multidisciplinarity, as defined by Choi & Pak, (Choi & Pak 2006). Interdisciplinary networks and collaborations describe the nature of One Health interventions, which create synergies among knowledge, models, and expertise related to human, animal, and environmental health to tackle a number of diversified and complex issues. System vision and inter/trans-disciplinarity are thus closely interlinked.

In this work the Authors exercise to provide a simple protocol to allow absolute beginners to move the first steps toward the practice of system thinking and interdisciplinarity. Several reasons may support this position: a long scientific tradition in the way scientific problems are approached favoured the affirmation of specialization in any domain. Furthermore the reductionist approach in science may fail to grasp problem complexity (Syberg and Hansen, 2016). Capturing problem complexity requires that specialists in a scientific or operational domain recognize the borders of their own specific vision and accept to place themselves in a wider scenario, dialectically interacting with other actors, with different specializations and points of view. Despite individual aptitude and willingness, interdisciplinary approach cannot be faced individually, it requires team working. This task is difficult at team level and at institutional level, where changes in the way problems are approached and solved requires organizational adaptation, with modified procedures and protocols within and among different institutions. At any of the above-mentioned level (team, institutions), managing the flux of resources (information, competences) and individual and organizational behaviours, requires shared rules and protocols. This poses the need for governance tools which, in relation to the complexity of the problem, may differ from established approach but should be enough simple to learn from people of different background.

2 Materials and Methods
Starting from real food related case studies, packaging and aquaculture, we developed an exercise to build up a general protocol to approach system thinking and interdisciplinarity in a wider range of contexts and situations. The objective of the protocol is to guide the potential users in the accomplishment of two relevant tasks in the OH approach:

1) the identification of the system and its boundaries
2) the hybridization of the knowledges

These tasks are closely linked as they are critical steps to build up an interdisciplinary team to face complex problems. The method adopted is an iterative cognitive process which required a preliminary effort of simplification and synthesis to extract some general rules of conduct. Authors first dedicated time to the identification of case studies potentially showing complex situations because of the implication of the different dimension of OH. Two case studies were selected because of the involvement of animal-human health problems, environment and food. The cases where treated individually in depth and finally a general protocol was extracted through the conceptualization of a general methodological model, which is in fact the result of the exercise. In this paper, for space reasons, we will only describe the base methodology and leave the application to the selected case studies for further description.

3 Results
The protocol we propose basically stands on two pillars, mutually linked and iteratively operated: (a) System identification and (b) Hybridization of knowledge.
a) System identification

For the identification of the system we started from some simple, quite common assumptions: Any problem can be described through its basic components (e.g. an object, as distinguished from other objects). We called these components the building blocks (BBs) of the problem. A building block is a fundamental element of the problem we want to tackle and that we use to represent the basic or initial perception of the problem itself.

Simple descriptors can be used to identify the uniqueness, singularity or relevant characteristics of any BB (such as nature, status, role, etc.) in relation to other BBs. Descriptors of the BBs should identify their role in an ideal causal chain and the result that they determine, so that the next step of the process (identification of the relationships) is simplified. Descriptors may thus distinguish for example among action, process, input, product, outcomes/result.

Relationships of any kind may intervene among BBs. A relationship is any kind of functional link (e.g. causation, co-determination, correspondences, etc.) between BBs. Relationships may thus have directions (e.g. direct causation or feedback), effect (e.g. increasing or decreasing) and intensity.

Starting from the original BBs and relationships, other BBs and relationships may be identified which expand the perception of the original problem. The discovery of these additional BBs and relationships defines the ways through which knowledge expands from the originally perceived problem. We called them ways of expansions which allows for the discovery of the complexity of the original problem. The identification of the ways of expansion is crucial. It depends on the answer to two basic questions: - what should be known more about the problem? - how can this be done? Logic and deduction, acquired knowledge and competences of the actor(s) of the process may help answering the first question, but the real solution lies in the answer to the second one: more knowledge can be acquired (and ways of expansion better identified) by involving more actors and competences in the process. This is the basis to build up an interdisciplinary team (see below under phase (b)).

Figure 1 shows how the original problem may appear according to a visual approach (a) and how it is represented through the identification of the ways of expansion (b). BBs are qualified according their nature (e.g. input, process, output) together with the causal relationships and the effect. Rows show the direction of causation and algebraic signs their effect. Figure 1a may exemplify the steps of building blocks, descriptions, relationships of the process; Figure 1b visualizes the way of expansions.

Figure 1 – Building up complexity and system vision
A further problem is how to identify system boundaries. System limitation mainly depends on scientific, economic and organizational constraints (i.e. existing knowledge, available resources, institutional limits, which can change over time also in relation to the result of the cognitive process). On the other side, assuming that a focus appears on a problem inside the system (e.g. managing process B in order to minimize output C’), system limits can be determined by selecting BBs and relationships according to their relevance in view of the prioritized objective.

b) Hybridization of knowledge
As mentioned above, the development of phase (a) requires that different actors involved in the process (researchers, institutions, social bodies, etc.) work together to reveal the complexity of the system, each actor bringing specific knowledge that concurs in the identification of the system according an interdisciplinary/transdisciplinary/multidisciplinary approach. Some factors can influence the individual vision of a problem. Namely: - disciplinary approach, in the academic sense; - specific expertise, stemming from the professional practice; - an institutional approach to the problem of a social body or an organization. In this sense, “knowledge” includes the disciplinary delimitation of a scientific field and the related investigation methods in the researcher mind-set, and the understanding of field people and stakeholders and their protocols, methods and standards. Integrating individual visions leads to the knowledge hybridization which translates the complexity of the system into the complexity of knowledge where each actor of the process has a place and a role assigned.

The process is articulated in two steps: 1) the development of the knowledge matrix; 2) the hybridization process. Knowledge hybridization is meant to serve and accompany the iterative development of phase (a) explained above. The practical result of this step is the association between specific problems and competencies, and the identification of counterparts or partners in the process, finally assessing possibilities and limits of each set of knowledge. In the matrix of knowledge, each domain of knowledge (e.g. human health, production management, health policy,…), corresponds to: (i) the domain of expertise (what aspects are taken in charge by the knowledge); (ii) the advancement in knowledge it creates (what useful knowledge can be obtained or expected which can contribute to the understanding of the problem); (iii) the method(s) to obtain it.

<table>
<thead>
<tr>
<th>Knowledge</th>
<th>Expertise</th>
<th>Advancement in knowledge</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food technology</td>
<td>Process engineering</td>
<td>Explain the causes of C'&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Production process analysis</td>
</tr>
<tr>
<td>Environmental science</td>
<td>Environmental externalities</td>
<td>Explain the relationship between C&lt;sub&gt;1&lt;/sub&gt; and A&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Bio-chemical analysis</td>
</tr>
<tr>
<td>Health institutions</td>
<td>Human health</td>
<td>Explain output D due to A&lt;sub&gt;2&lt;/sub&gt; on humans through process B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Epidemiologic and clinical analysis</td>
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</table>

The building up of the matrix is a dynamic process: lines (knowledges) can be added to or erased from the table according to the stage of the cognitive process and the development of the system.
4 Discussion

The protocol presented here aims to facilitate the approach to system identification and interdisciplinarity for people who are used to work according to a specific point of view (a disciplinary or technical approach, an institutional mission or a professional practice) but do want to enlarge their interest and view for any reason. It is not meant to be conclusive nor exhaustive but it wants to attract the attention of researchers and practitioners for testing and verification. Limiting to outline the basic steps of the process, we intentionally avoided to go into details of specific means and methods which can be used in the basic steps of the process, that is either the system identification and delimitation (System thinking, Theory of change, Stakeholder analysis,…) and the knowledge hybridization (interdisciplinary and transdisciplinary approaches, interdisciplinary team working, focus groups, participatory methods, matrix of competences, …). All of these conceptual and practical devices can be used to develop the exercise according to specific situations and needs. We are aware of the limitation of the present work but we believe it is a further step toward the possibility to integrate synergies coming from different expertise and existing operational methodologies. The result we look forward is that this protocol is further applied in different fields and situations, criticised and improved.

References


Abstract

The Federal Office of Consumer Protection and Food Safety (BVL) recently developed a watching and warning system for crisis prevention. The main goal is to prevent crises by the early supply of information.

In an one health approach sources reporting on risks (e.g. rapid alert systems), sources that deliver information on the crisis potential (e.g. media) and sources containing scientific information (e.g. new opinions or risk assessments) are screened on a regular basis. Although the focus is on food, the range of the sources includes all topics along the food chain affecting humans, animals or the environment which hold a crisis potential.

Relevant “crisis prevention” information is stored in a knowledge database. Outputs are reports and a newsletter for the risk managers. Informations are exchanged via an internet platform with restricted access for the prevention network members from competent authorities. A frequent exchange with the Emerging Risks Exchange Network (EREN) of the European Food Safety Authority (EFSA) is also established.

Keywords

food safety, early warning, crisis prevention, emerging risks.

Introduction

The BVL performs various tasks in food safety at federal level. These include for example the national contact points for the European Rapid Alert System for Food and Feed or for the Robert Koch-Institute in case of food-borne outbreaks, data transmissions to the European Commission and coordination in crisis situations. Following the EHEC Outbreak in Germany in 2011, crisis management was revised at BVL and a unit was created to take over crisis prevention tasks. The past has shown, that crises in food safety are associated with a lack of timely information. Examples are new developments or consumer trends, the lack of full information to consumers or missing data for risk assessment. The BVL is using the watching and warning system for crisis prevention (BeoWarn) to resolve these information deficits on the food side (Schewe et al. 2017). The goals of BeoWarn are identification of issues or risks with a high crisis potential, prevention through early and targeted compilation of information for risk management authorities and rapid provision of information for authorities in emergencies and crises.

Material and Methods

Sources

The sources of observation include, for example, information of other competent authorities along the food chain, scientific institutes, rapid alert systems, epidemiological surveillance reports and inquiries from consumers and journalists. In addition, there are numerous internet portals, blogs, journals, newsletters from NGOs and associations, RSS feeds or websites from official and non-official, national and international institutions as well as the TV program.
Furthermore queries for certain keywords are carried out in search engines to continuously identify newly published relevant documents.

**Process**

The identified information sources are reviewed, recorded, stored in a database and prioritized by an interdisciplinary team with expertise in food microbiology, food chemistry, food technology, food control, biology, veterinary medicine, epidemiology and nutritional science. Once a week, the observations are evaluated by the entire team. Together, the team discusses the relevance or crisis potential of the individual issues within Germany. As a result of the meeting, the topics that are judged to be relevant are documented separately. In this context, further action will be decided upon. If a matter is judged to be sufficiently relevant, the research work begins. Experts will be consulted as necessary. All information is collected and clearly arranged. The aim is an easy-to-understand summary report that provides an overview of the relevant facts and, if necessary, it also provides background information.

**Communication**

The reports are made available to the competent authorities in the field of food safety via an internet platform with restricted access (Schewe et al. 2017, Becker et al. 2017). BeoWarn also organises regular meetings with experts from the German Federal States for information exchange in the range of a prevention network. In the time between the meetings, the members of the network are informed about current observations via a newsletter. On European level there is a frequent exchange of observations and signals with EREN. This European network serves the exchange of information between EFSA and the member states on emerging or new risks in the field of food and feed safety, as well as on the health of humans, animals and plants. (EFSA, 2016, 2017).
Results and Discussion

In the following a few examples of BeoWarn observations and reports with a one health character are presented.

Increase of campylobacteriosis outbreaks due to raw milk consumption

BeoWarn has been observing a trend towards increased consumption of raw milk in Germany since 2015. The reasons for the changed consumer behavior are, on the one hand, the easier availability of raw milk due to the increased distribution at raw milk vending machines and publications on the health-promoting effects of the consumption of raw milk. A research and analysis carried out on the basis of the observation showed that there is a higher risk of campylobacteriosis in urban and rural districts with raw milk vending machines than in urban and rural districts without raw milk vending machines (Becker et al. 2016).

The link between campylobacteriosis outbreaks and the increasing trend towards raw milk consumption was also evident in the BVL-led nationwide system for collecting data on foods involved in outbreaks of disease. In Germany, the outbreaks caused by Campylobacter spp. accounted for the largest proportion of foodborne outbreaks with high evidence in 2015 and 2016. Compared to 2014, the number of outbreaks of campylobacteriosis caused by raw milk has increased significantly (Rosner et al. 2016, 2017).

When raw milk is supplied to consumers, the requirements of the Regulation on hygiene requirements for the production, treatment and placing on the market of certain foodstuffs of animal origin must be met. As a risk management measure to reduce the number of campylobacteriosis outbreaks, a federal statewide project group prepared a leaflet to inform farmers and operators of raw milk vending machines. To protect and inform consumers, the risks posed by raw milk and the need to boil raw milk before consumption have been communicated.

Possible contamination of fruits, vegetables and mushrooms with Echinococcus multilocularis

E. multilocularis is a tapeworm, which may cause human alveolar echinococcosis, one of the most dangerous parasitic zoonoses, characterised by a high fatality rate. The incubation time of the disease can vary between less than 5 and 15 years. The initial phase is always asymptomatic. Human alveolar echinococcosis is an emerging disease (Lass et al. 2015).

In 2016 BeoWarn observed the results of a study which was conducted in a Polish region with the highest level of E. multilocularis prevalence (50%) in foxes and the highest number of human alveolar echinococcosis. The study showed that over 23% of food samples, taken by a risk-orientated approach, were positive for E. multilocularis DNA. Especially raspberries from plantations were found to be contaminated (Lass et al. 2015). Due to the trend of raw consumption of vegetables and mushrooms with a simultaneously growing and spreading fox population as a reservoir of E. multilocularis, an increased exposition is to be expected.

However, there is no validated standard method for the detection of tapeworm eggs on food. The methods used in the first studies so far are critically discussed among the scientists. The method development is therefore urgent. Furthermore, no statement on the infectivity of possibly existing tapeworm eggs can be made by the sole DNA detection. The BeoWarn team therefore created an information package for the prevention network and contributed the topic to EREN. The discussion in the network revealed that the issue of a possible transmission of tapeworm eggs by at ground level growing fruits and vegetables was classified as an emerging problem. EFSA is currently reviewing the epidemiological and geographical relationships between human and animal echinococcosis and the analytical methods available for food. Furthermore, the aim is to develop a validated method for the analysis of food for E. multilocularis eggs (EFSA, 2017).
Residues of insecticides from the control of Zika virus on vegetable foodstuffs
After a request from a German Federal State in the prevention network, BeoWarn became active in this area. Since the end of 2015, the Zika virus has been spreading massively in Latin America and Southeast Asia. For this reason, the World Health Organization (WHO) declared a Public Health Emergency of International Concern in 2016 from February 1st to November 18th. As a result, the WHO and the Center for Disease Control and Prevention of the United States (CDC) recommended the use of pesticides - especially insecticides - to combat the Zika virus vectors in the relevant epidemic areas. The pesticides recommended by the WHO and CDC are not novel active ingredients. They are already being used to eliminate other vectors. In this context, the increased use of the aforementioned means for combating the Zika epidemic is particularly important. However, there was no certainty as to the regional application of the pesticides and the harvests possibly affected by them. Even if these chemical substances were not explicitly used as pesticides, they can still be introduced into the food chain.

Due to the constantly growing demand for plant foods and the desire for year-round availability, these are exported without restrictions from Latin American countries and thus from the affected epidemic areas. Through global trade, exports reach the European Union (EU) and German trade. A comparison of the recommended agents of WHO and CDC with those authorised in the EU showed that eight out of a total of 23 pesticides do not comply with current EU legislation. Within the scope of a BeoWarn research, the analysis of the seven most important export countries for plant-based food in Latin America for the year 2015 was carried out for Germany. In order to check which of the pesticide residues occurred in the exported plant foods of these countries, the official pesticide test results of Germany for the year 2015 were analysed. In 2015, only one non-authorised pesticide was detected on foods from Brazil. However, this was before the Zika epidemic.

The compiled information package was made available to the prevention network. The topic was also passed on to EREN as a signal. As a result EREN extended its research to the European level. Due to the incomplete data situation, no conclusion could be drawn for 2016 at the time of the EREN meeting. EREN members have been asked to provide data on nine pesticides in plant foods that before had not been tested under the European pesticide control plan or at border controls. If EFSA detects an increase in exposure, a proposal will be made to extend the European control plan on pesticide residues.

References


Targeting Effective US Antibiotic Stewardship Programs: What Data Do We Need and What Can We Learn from Data We Have?

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The US Center for Disease Control (CDC 2013) estimates that each year at least 2 million people are infected with antibiotic-resistant bacteria resulting in 23,000 deaths, $20 billion (2008 dollars) of excess direct healthcare costs, and $35 billion of lost productivity. The CDC identifies over-prescription of antibiotics as the ‘single most important factor leading to antibiotic resistance in the world,’ (p. 11). Up to 50\% of all antibiotics prescribed for people have been either unnecessary or not optimally effective, and antibiotics prescribed for food animals have served purposes beyond managing infectious diseases, such as promoting growth. Consequently, the CDC recommends improving stewardship of antibiotics as the ‘single most important action needed to greatly slow down the development and spread of antibiotic-resistance infections’ (p. 31).

1. **What data do we need?**

To formulate an effective antibiotics stewardship program, the CDC must accurately measure the burden of antibiotic-resistant outbreaks, and attribute them to antibiotic use in human health or food-animal production through time (Figure 1). This requires time series records measuring: (1) the use of a particular antibiotic in human health and food production; (2) the populations of resistant and susceptible bacteria in each use while accounting for interactive dynamics whereby some bacteria can give their resistance to other bacteria; (3) the transmission of resistant and susceptible bacteria through community interactions (human health) and the food chain (food-production animals); and (4) the burden of human-health and foodborne/wasteborne outbreaks linked to antibiotic resistance or susceptible bacteria.

Surprisingly, given supposed severity of the antibiotics-resistance problem, critical data gaps exist at all of these linkages, and data have not been, or are being, systemically collected (Centner 2016; CDC 2013).

2. **What can we learn from available data?**

To help prevent infections and the spread of resistance, the CDC monitors trends in foodborne infectious outbreaks (CDC 2013) by compiling “Annual Summaries of Foodborne Outbreaks” (1966-2015). The annual summaries consistently collect data on total outbreaks, total cases, outbreaks attributed to beef, pork, dairy, and plants (i.e., vegetables, fruits, nuts, salads), and outbreaks by specific etiologic agent. The outbreaks are not broken down into whether they were due to resistant or susceptible agents. We
downloaded individual CDC reports (https://www.cdc.gov/fdoss/annual-reports/index.html), and recorded the data into an Excel spreadsheet.

We also lack time series records on antibiotics use in animal agriculture, but have data on other market variables. We downloaded USDA records on food disappearance into the marketing system (million pounds), often used as a proxy for consumer demand (https://www.ers.usda.gov/data-products/livestock-meat-domestic-data/livestock-meat-domestic-data/#All%20supply%20and%20disappearance). We also downloaded USDA/NASS beef and hog prices ($/cwt) (http://www.nass.usda.gov/index.asp). The annual CDC and USDA records span 50 years (1966-2015).

Available data do not suffice for us to directly investigate the spread of antibiotic resistance in animal agriculture, and its impact on human health. However, we can investigate two related policy questions: First, do consumers reduce demand for products responsible for foodborne disease? The law has been slow to regulate antibiotic use in agriculture, does the market provide an alternative private regulatory control? If so, we have reason to expect that consumers might respond positively to labeling of antibiotic-free meat products—a popular policy option (Centner 2016). Such empirical evidence also would corroborate survey evidence regarding consumer’s willingness to pay for antibiotic-reduced foods (Goddard et al. 2017). Second, which foods are chiefly responsible outbreaks by particular etiologic agents, and thus should be policy targets? Recently, the CDC began publishing ‘Food-Germ Pairs Causing Outbreaks’ for a given year. We investigate the long-term dynamics of such pairings.

3. Phase Space Reconstruction

We must wring the answers to these questions from relatively short time-series records that are volatile and appear more random than systematic (Fig. 2, black curves). A conventional empirical assumption is that volatility is due to stochastic forcing of linearly-stable real-world systems by exogenous random shocks (Uusitalo et al. 2015). However, this is not necessarily the case—results from nonlinear dynamics prove that highly irregular and complex system dynamics can be endogenously forced by deterministic nonlinear behavior. The source of volatility in our time-series records makes a profound difference in our analysis. In a linear-stochastic world, we look for randomly-drifting interactions among foodborne-disease system variables. In a nonlinear-deterministic world, we look for state-dependent interactions that depend mechanistically on the levels of system variables—the systematic interactions of interest to the cause-and-effect emphasis of public policy. Since neither world is compelled by theory, we let the data guide the selection. This approach is compatible with the initial inductive stage of the classic scientific method in which “[scientists] are presented with observations and asked to build theories…to go backward, to solve for [the system] that made them” (Ellenberg 2015).
The discipline of nonlinear dynamics has mathematically solved the *backward* problem of reconstructing system dynamics from observed output when the underlying equations are unknown. *Nonlinear Time Series Analysis (NLTS)* applies this empirically to reconstruct real-world system dynamics from observed time-series records when the real-world system is unknown (Kantz and Schreiber 1997; Huffaker et al. 2017). The major result is *Phase Space Reconstruction*: Phase space dynamics—which early practitioners thought required all system variables—can be reconstructed from only a single system variable with its lagged copies serving as surrogates for omitted system variables (Takens 1980). We construct an *embedded data matrix* from an observed time series whose first column is the time series and remaining columns are lagged copies. For example, the 3x3 embedded data matrix for the time series $x(t) = (1,2,3,1,2)$ with a time delay of a single period (embedding delay) and three lagged copies (embedding dimension) is shown in Fig. 3 (shaded observations are lost in the lagging process). The rows of this matrix are the multidimensional points of a trajectory on the reconstructed phase space attractor. Phase space reconstruction has been generalized so that real world attractors can be reconstructed from combinations of observed co-variates and their lagged copies (Deyle and Sugihara 2011). We statistically tested the likelihood that apparent structure in reconstructed attractors is due to stochastic forcing rather than deterministic nonlinear dynamics with *surrogate data* (Kantz and Schreiber 1997). Finally, we used multivariate attractors reconstructed from our foodborne-disease dataset to empirically detect interactions among co-variates over time with *Convergent Cross Mapping* (Sugihara et al. 2012), and characterize the nature of detected interactions with *Empirical Dynamic Modeling* techniques (Deyle et al. 2018).

### 4. Data preprocessing

To improve the performance of phase space reconstruction, we filtered noise from standardized time series records with *Singular Spectrum Analysis* (Golyandina and Korobeynikov 2014). This is a signal processing method that separates structural variation (signal) from unstructured variation (noise) in time series records, measures the strength of isolated signals, and tests strong signals for nonlinear stationarity required by *NLTS* (Itoh and Marwan 2013). The signals that we isolated in the data are shown in Fig. 2 (red curves). Of these, only beef and pork prices were found to be nonstationary for NLTS purposes, and thus were dropped from further analysis. We used remaining stationary signals to reconstruct real-world foodborne-disease dynamics from our dataset.

### 5. Volatility in the foodborne-disease data is mostly likely due to deterministic forcing

In Fig. 4, we show a real-world foodborne-disease attractor reconstructed from the beef-outbreak record and two lagged copies separated by a one-period delay. The attractor passed surrogate testing, and thus provides empirical evidence that volatility in the data is most likely forced by nonlinear deterministic dynamics. The attractor comprises the low and high frequency oscillations characterizing time series records in Fig. 2.

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1 Phase space graphically portrays deterministic system dynamics. System variables serve as coordinates, and trajectories connecting their levels in each time period (the *states* of the system) depict their co-evolution through time. In dissipative dynamic systems, these trajectories evolve long-term along an *attractor* bounded within a subset of phase space.
6. Network interactions among foodborne-disease co-variates

In Fig. 5, we show a community network diagram that summarizes detected interactions. The nodes of the diagrams correspond to stationary co-variates found to be forced by deterministic dynamics, and the arrows to detected interactions. Of particular interest to the questions that we pose above, beef and pork outbreaks drive consumer demand for pork but not for beef in the data. We also see that beef and pork outbreaks drive outbreaks by E.coli, allowing us to investigate the historic contributions of beef and pork to E.coli outbreaks. Interestingly, we also detect interactions among the etiologic co-variates, providing empirical evidence that outbreaks can possibly be caused by multiple pathogens (Smith et al. 2004), and pathogen-pathogen interaction (Singer 2010).

7. Impact of beef and pork outbreaks on consumer demand for pork

Figure 6 shows dynamic interactions characterized with partial derivatives numerically computed in each time period from embedded data matrices reconstructing phase dynamics from various perspectives. The first question that we posed above is whether consumers reduce demand for products responsible for foodborne disease. Fig. 6a scatterplots the marginal response of pork demand in each time period to beef outbreaks (vertical axis) against its marginal response to pork outbreaks (horizontal axis). We see that the marginal response to beef outbreaks is negative for most time periods, indicating that pork demand marginally decreases in response to an incremental increase in beef outbreaks. We might intuitively expect the opposite—that pork demand would increase as consumers substitute away from beef during beef outbreaks. However, consumers might substitute beef for pork in response to falling beef prices during beef outbreaks with the expectation that stores will protect them from illness by removing tainted products from their shelves. Fig. 6a also shows that the marginal response of pork consumers to pork outbreaks is both positive and negative over time, and that this response does not exhibit any systematic relationship with the response to beef outbreaks. Positive response could be driven by falling pork prices that overwhelm health concerns, and negative response could reflect the opposite. A negative response provides a clear incentive for pork producers to avoid outbreaks and thus provides a private market-based regulatory control. Whether a positive response provides the same market control depends on whether
total industry revenue rises in response to the offsetting impacts of increased sales and reduced prices, which in turn depends on the price elasticity of demand for pork. Moreover, we would not expect consumers putting more weight on falling pork prices than health concerns during pork outbreaks to be willing to pay more for antibiotic-reduced foods, while consumers weighing in the opposite direction might be willing to pay such a premium. These are empirical questions.

The second question that we posed is: Which foods are chiefly responsible outbreaks by particular etiologic agents, and thus should be policy targets? The interactions detected between E.coli and beef and pork outbreaks in the community interaction diagram allows us to ask this question for E.coli outbreaks. Fig. 6b scatterplots the marginal response of E.coli outbreaks to beef outbreaks (vertical axis) against its marginal response to pork outbreaks (horizontal axis) in each time period. We see that the responses are negatively correlated ($R^2 = 0.38$), indicating a tradeoff between beef and pork as sources of E.coli outbreaks. We also see that most scatter points rest in the quadrants of the plot for which incremental increases in pork outbreaks marginally increase E.coli outbreaks, providing evidence that E.coli has a larger marginal response to pork than to beef outbreaks.

**References**


Goddard, E., Hartmann, M., & Klink-Lehmann, J. Public acceptance of antibiotic use in livestock production in Canada and Germany. In *11th International European Forum on System Dynamics an Innovation in Food Networks, Innsbrook-Igls, Austria, 2017*


Chair: Griffith, Garry Richard (University of New England, Australia)
Discussant: Goddard, Ellen (University of Alberta)

Food value chains consist of businesses that collaborate to progressively create value for the final consumer. With increasing affluence and education consumers have higher expectations and requests with respect to the product and process quality of their food. In some situations, it pays an individual firm to invest alone to meet those demands, or the chain governing firm will make the investment and recoup that investment through contractual arrangements with suppliers. However in other situations, certain members of the chain consciously and deliberately decide to take joint action to provide particular types of goods or services. Such goods are at least partly excludable and at least partly congestible and they are provided jointly because it is too costly to do so individually.

In the wider economy, these goods are called club goods or collective goods and there is a vast literature describing and characterizing these types of arrangements, ranging from local sporting clubs to multinational institutions. Recent work has examined how these concepts of club theory can be used in describing and assessing the structure and operation of food value chains. The concepts of chain failure, chain goods and chain externalities have been proposed as the value chain equivalents of market failure, public goods and social externalities found in the wider economy.

In this session, we first provide an overview of club theory and its value chain equivalent, followed by findings from a recent analysis of food markets in Australia and Germany in which we assess whether this theory can provide an approach to recognizing and overcoming chain failure in these markets. We show that there are many cases of past investments in food value chain innovations, and in current food industry responses to consumer and community concerns such as food safety or animal welfare, that could be explained by the theory. That is, individual businesses have formed a club within a value chain to collectively design and provide a chain goods solution to a chain failure. We found this was more likely to occur where there are significant chain and external costs, and hence where collective solutions are required. Such action was also more likely to occur at upstream rather than downstream stages of the chain, where there was relatively less regulatory control of the chain, and where the governance structure of the chain was relatively loosely rather than tightly controlled.

We propose that food safety incidents can be considered as examples of chain failure, and that the systems and mechanisms within food value chains that assure adequate levels of food safety are examples of chain goods. In the remainder of this session, we apply the concepts of chain failure, chain externalities and chain goods in two different retail environments, Australia and Germany, to understand how food safety assurance mechanisms and processes can be implemented in a co-ordinated action among food chain actors to assure consumers that the food they buy is safe.
Food Safety Assurance Systems as Chain Goods
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University of New England

The theory of clubs provides the rationale for why certain members of a population consciously and deliberately decide to take joint action to provide particular types of goods or services. Such goods are at least partly excludable and at least partly congestible and they are provided jointly because it is too costly to provide such goods individually. These goods are called club goods or collective goods. In recent work we have been examining how these concepts can be used in describing and assessing the structure and operation of agricultural and food value chains. The concepts of chain failure, chain goods and chain externalities have been proposed as the value chain equivalents of market failure, public goods and social externalities found in the wider economy.

We have systematically analyzed key food markets in Australia and Germany and elsewhere to test whether club theory can help provide an alternative approach to recognizing and overcoming chain failure in these markets. We found that there were many cases of past investments in food value chain innovations, and in current food industry responses to consumer and community concerns such as animal welfare, that could be explained by the theory. That is, individual businesses have formed a club within a value chain to collectively design and provide a chain goods solution to a chain failure. We found this was more likely to occur where there are significant chain and external costs, and hence where collective solutions are required. Such action is also more likely to occur at upstream rather than downstream stages of the chain, where there was relatively less regulatory control of the chain, and where the governance structure of the chain was relatively loosely controlled rather than tightly controlled. We also found that there are two particular issues that require further consideration. One is the nature of risk in agricultural value chains, and how it is related to member preferences, and the other is the specific form of collective action to be taken by agricultural value chains in the future.

Food safety is of concern to all food value chains. In this paper we assess whether these concepts of chain failure, chain externalities and chain goods can assist value chain participants to understand how food safety assurance mechanisms and processes can be implemented within a club format to convince food consumers of the health quality status of the purchases they are making. Subsequent papers apply these principles to particular food safety issues in Australia and Germany.

Implications of chain failures when marketing meat with production and process attributes in Australia
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In Australia, demand is growing for food products marketed with claims about ‘where’ and ‘how’ food was produced, processed and distributed; as well as how humans, animals, and the environment were impacted. This is particularly true for meat and other livestock products. The demand growth for food products with these attributes may be driven, in part, by consumers’ perceptions about both private benefits and public/social benefits.

The marketing of meat products with these credence attributes (attributes that the consumer cannot evaluate unless information is provided), creates policy and food chain governance issues for both the private and public sector. Consumers must trust and rely on the seller to provide truthful information and for the information to be passed through the chain. As a
result, there is a high degree of information asymmetry. Searching for information can be costly for an individual consumer or firm. Sharing information about food production and procurement is not a simple task, particularly for livestock products that are processed or exchange ownership many times before making it into the shopping baskets of the consumer. Furthermore, because food products marketed with credence attributes are often valued higher by consumers, they are generally sold at a premium price over conventional products. Thus, without appropriate chain governance systems in place to establish production and process criteria and methods for verifying credence claims, some producers may take advantage of market opportunities without changing their production systems.

In this paper we add to the discussion on whether club theory provides insight to explore alternative governance approaches to overcoming chain/market failure issues in Australian meat markets. We analyse nationally representative data collected from over 2300 consumers during November and December in 2015 and 2017 to provide insight on the type and extent of chain failure that exists due to the marketing of various credence claims. We explore consumer perceptions of various claims, with particular focus on claims (e.g. organic and free range) that at least some consumers perceive to offer enhanced food safety and to address social concerns. This perception information is helpful to understand the extent of the chain failure. Then, through a unique econometric analysis of purchasing behaviour and socio-demographics we provide policy advice on governance systems that may help to address chain failure issues.

Australian meat markets present an interesting case study as there is increased marketing of meat products with credence claims that are perceived by consumers to address private food safety and quality concerns, as well as public or social concerns, but there are only government (public) standards for two credence claims, ‘organic’ and ‘free range’, and products may be sold with these claims without providing proof of certification.

Establishing higher private standards for residues by market forces

Simons, Johannes
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Food safety as a credence attribute is difficult to access by consumers themselves. Moreover their knowledge about residues, their impacts on human health and the public food safety regime is generally low. Nevertheless food safety is a very emotional issue in public debate. Science can hardly prove that food is safe. Based on scientific studies it is possible to provide evidences or no evidence for risk but no evidence for risk does not mean that food is safe. Public debates about BSE and genetically modified food as well as several WTO disputes are good examples of conflicts in this area and of the limits of science in the context of food safety policy.

Food safety policy can take scientific uncertainties into account by applying the precautionary principle. In this regard public standards reflect a societal compromise which applies for all market participants. In German fruit and vegetable markets however retailers established higher standards than the public ones. Given that the overwhelming share of fruits and vegetables is marketed via these retailers, the private standards have a big influence on the organisation of the respective value chains.

The development of higher private standards was driven by different, interdependent factors. First, for some consumers governmental food safety policy is not safe enough. They argue that “no scientific evidence for a risk” does not mean “no risk” and as a consequence the precautionary principle should be applied to a greater extent than in public standards. Second, NGOs fuel the uncertainty of consumers by imposing standards in their food quality and safety tests. Moreover they name and indirectly blame retailers and well-known brands if the
products they sell are not in line with the higher standards. Third, retailers in Germany face a high level of competition. Moreover, a high level of fixed costs in the retail sector leads to a disproportional large change in profits as a result of a change in market share. Therefore customer retention and acquisition play an outstanding role in the strategy of retailers and their reputation is considered as a relevant competitive factor. As Germany is a large market for fruits and vegetables and retailers are focal companies in the fruit and vegetable value chain they have to power to enforce higher private standards and to influence the organisation of the value chain.

In conclusion it becomes clear that market forces can lead to residue standards that are stricter compared to public ones and almost mandatory for suppliers. The development in Germany is driven by interplay between consumers’ uncertainty, NGOs activities, competition between the retailers, and the power of retailers to enforce the higher private standards. Still it is contentious whether the private standards lead to more food safety.
INNOVATIONS TO REDUCE BACTERIA AND ANTIMICROBIAL RESISTANCE FROM FARM TO FORK

Introduction:
Dr. Hanns-Christoph Eiden (President, Federal Office for Agriculture and Food)

Moderation:
Dorothea Weißengruber (Federal Office for Agriculture and Food)

Abstract

The German food industry is under competitive constraints on both national and international markets. To drive forward technical progress and, thus, improve the market position of small and medium-sized enterprises, the German Ministry of Food and Agriculture funds research and development projects within its innovation support programme.

This programme supports technical and non-technical innovations in Germany with a special focus on food, agriculture and consumer health protection.

In the last years, several projects have been funded in the fields of reduction of antimicrobial resistances and improving food safety by the reduction of bacteria in the food chain.

In the first part of the session the German innovation support programme will be introduced. Subsequently three projects focusing on innovative approaches to reduce bacteria and antibiotic resistance along the food supply chain are presented:

- EsRAM: Development of measures for reduction of antibiotic resistant bacteria along the entire poultry production chain.

- Evitar: Development and implementation of an evidence-based therapy and consultancy concept to reduce the use of antibiotics and to minimize antibiotic resistance in dairy farming.

- 3Plas: Plasma-based decontamination of dried plant-related products for an enhancement of food safety.

As a project initiator, the Federal Office for Agriculture and Food manages the above projects on behalf of the German Ministry of Food and Agriculture.

Keywords

Project funding, Innovation Support Programme, Federal Office for Agriculture and Food, food safety, antimicrobial resistance
DEVELOPMENT OF MEASURES FOR REDUCTION OF ANTIBIOTIC RESISTANT ESCHERICHIA COLI ALONG THE ENTIRE POULTRY PRODUCTION CHAIN

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Abstract

Antibiotic-resistant bacteria (e.g. Extented spectrum btea-lactamase producing E. coli; ESBL) can occur at all stages of poultry production after horizontal or vertical transfer. The goal of the joint research project "EsRAM" is the development of products, processes and measures for the reduction of transfer and of development of antibiotic resistant bacteria along the entire poultry production chain. The overall product of the EsRAM project is "poultry meat with a reduced burden of antibiotic-resistant bacteria, particularly ESBL". Individual measures or products to be developed are: i) new and improved methods and technologies for disinfection of hatching eggs and for hatchery management to reduce the vertical transfer of ESBL, ii) Methods for decontamination of faeces from poultry farming in terms of antibiotic-resistant bacteria, iii) Development and optimization of housing factors, hygiene measures and feeding regimes and their combinations to reduce the prevalence of ESBL in broilers, iv) Development of an effective defined Competitive Exclusion Culture for significant reduction of colonization of ESBL in broilers, v) Development of prebiotics, probiotics and phytogenic additives to reduce the colonization in the intestine of broilers with ESBL, vi) Optimization of existing and development of new methods and technologies for the slaughter and processing of poultry, as well as optimization of the slaughter and processing management to reduce the vertical and horizontal transfer of ESBL, and vii) Development of an data-based electronic assessment tool for the assessment of procedures and measures for a synergistic production of antibiotic-resistant pathogens (e.g. ESBL) throughout the entire poultry production chain. The effects of these individual measures and their combination are validated with regard to the contamination of poultry meat. The results achieved so far will be presented.

Keywords

Antibiotic-resistant bacteria, ESBL, poultry production, horizontal and vertical transfer, food additives
Introducing an Evidence-Based Mastitis Therapy Concept to a Conventional Dairy Farm

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Abstract

The EVITAR project (Development and implementation of an evidence-based therapy and consultancy concept to reduce the use of antibiotics and to minimize antibiotic resistance in dairy farming) was conducted to achieve a long-term change in antimicrobial treatment (AT) patterns of dairy farmers to decrease antimicrobial usage and to minimize the risk of potential antimicrobial resistances. Basis for this was an evidence-based mastitis treatment (EBMT) concept for which a novel rapid culture system was developed.

The new on-farm culture test (mastDecide®, QUIDEE GmbH) was established in eight dairy farms in Northern Germany from September 2016 until February 2018 coupled with an EBMT concept. Study design was a comparison of bacteriological cure rates (BCR) and antimicrobial doses before and after introduction of the novel concept. During the preliminary phase, all clinical mastitis cases were cytomicrobiologically analyzed and the current therapy concept was recorded. Aim of the study was to investigate the effect of the implementation of a rapid test-based therapy concept on antimicrobial consumption, BCRs and antimicrobial resistance development.

The new test system mastDecide® consists of two tubes containing different culture media. The first one verifies the growth of Gram-negative (esp. coliforms) and Gram-positive cocci, the second one exclusively the growth of Gram-positive cocci, illustrated by a decoloration after 12 to 14 hours. The therapy concept implied a differentiated intramammary AT (IAT) based on the test result. Only mastitic udder quarters with a Gram-positive test result received an IAT. Furthermore, therapy unworthy cows (third mastitis in lactation; somatic cell count (SCC) thrice above 700,000 cells/ml) did not receive IAT. Systemic AT was used only in cases of severe mastitis.

Quarter foremilk samples were taken immediately after the appearance of clinical mastitis signs (day 0) as well as on days 14 and 21 (+/- 3) as control samples for cytomicrobiological analysis. A quarter was recognized as bacteriologically cured if the mastitis-causing pathogen was absent in both control samples. Farm personnel performed and evaluated the results of mastDecide® independently and directly on farm. Test results and the following AT were recorded. Dairy producers were free to choose the pharmaceutical agents.

The implementation of the concept varied between farms. Before implementation of the EBMT concept to Farm A, which was characterized by the greatest compliance to the study protocol, 90 % of the affected quarters received IAT and the BCR was 81 % (n=186). After implementation of the EBMT concept, the intramammary administration of antimicrobials decreased to 33 % of the affected quarters, while the BCR remained unaffected at 83 % (n=431).
The EVITAR project was supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.

Keywords
Clinical mastitis, selective treatment, on-farm culture, evidence-based therapy, therapy concept

1 Introduction

In the dairy industry, bovine mastitis is the most common cause for antimicrobial consumption (Kuipers et al., 2016). The painful disease does not only affect animal welfare but also causes great economical losses mainly due to milk yield lost and increased culling rates (Pol and Ruegg, 2007). Over the last decades, low SCC and a maximum cure rate were the only aims to pursue, which lead to relatively low treatment criteria and a mostly blanket AT for every cow with clinical signs (Vaarst, 2002). Although present research has shown no alarming increase of antimicrobial resistance of mastitis-causing bacteria (GERMAP, 2016), there is an exerted pressure through public concerns to reduce antimicrobial usage in the dairy production equal to other medical sectors (Krömker and Leimbach, 2017). Achieving long-term changes in treatment decisions of farmers, new strategies and tools are needed to support and motivate producers (Lam et al., 2012). An EBMT concept can be an effective option to save antimicrobial doses compared to conventional therapy concepts while keeping cure rates constant (Mansion de Vries, 2016).

Basis of the EBMT concept is to skip antimicrobial substances if the application is useless. Identification of those cases demand knowledge concerning the individual cow as well as the mastitis-causing pathogen. Cows with mastitis caused by Gram-positive pathogens, esp. streptococci and staphylococci, benefit from an AT significantly (Roberson et al., 2004). Mastitis, caused by Gram-negative bacteria, especially coliforms, are characterized by a high self-cure rate and consequently do not necessarily require antimicrobials in mild to moderate cases (Suojala et al., 2010). Moreover, 30 % or greater of clinical mastitis samples exhibit culture-negative outcomes. An AT in those cases should be questioned (Oliveira and Ruegg, 2014). As a laboratory examination takes at least 48 hours identifying the mastitis-causing pathogen, on-farm rapid tests are a necessary tool for implementing a selective AT. Preceding clinical trials compared outcomes of a selective AT in a test group with those of a blanket AT (Lago et al., 2011; Mansion de Vries et al., 2016). Saving up to 60 % of the amount of antimicrobials due to a selective treatment in the test group, animals showed no differences in bacteriological cure in comparison to animals with blanket AT.

Aim of this present study was the implementation of a practical EBMT concept using a rapid on-farm test. Focus was on reducing antimicrobial consumption in the long term while keeping the BCRs unchanging. On this account the study design was a comparison of BCRs and antimicrobial doses before and after introducing the EBMT concept to the farms. The novel rapid tube test system mastDecide® (QUIDEE GmbH, Homberg, Germany) was performed and evaluated directly by farm personnel. After an incubation of 12 hours a classification of the mastitis-causing pathogen with respect to Gram-positive, Gram-negative or no bacteriological growth was feasible.
2 Material and Methods

2.1 Farm, Mastitis Definition, Sampling, Preliminary Phase

The study was conducted on eight free-stall dairy farms in Northern Germany from November 2015 to February 2018. Farms differed in herd size, farm structure (family business, dairy personnel), milking system (conventional, milking robot), and type of production (organic, conventional).

Classification of the mastitis severity score (MS) was done as followed: MS 1 if there was only change in the appearance of milk, MS 2 in the case of additional local clinical signs of the udder and MS 3 for cows with general clinical signs (fever, lack of appetite).

During the preliminary phase from November 2015 to September 2016, all clinical mastitis cases and the respective treatment of the cows were recorded. After detection of a clinical mastitis, farmers took a foremilk sample antiseptically. Post-treatment quarter samples were taken after 14 (+/-3) and after 21 (+/-3) days. Samples were stored refrigerated and were send to the laboratory of the University of Applied Sciences and Arts Hannover, Germany, for the conventional microbiological diagnostic examinations according to the GVA (2009). Antibiotic resistances were determined by agar diffusion tests and by analysis of the minimum inhibitory concentrations of a panel of isolated pathogens (Staphylococcus aureus, Streptococcus uberis, Escherichia coli, other coliforms).

2.2 Evidence-based Therapy Concept

Three decision points result in the recommended AT: first point is the clinical appearance, second the animal-related factors, and third the pathogen-related factors.

1. Mastitis score: Cows with serve mastitis (MS 3) immediately get systemic antimicrobials and supportive fluids. IAT of mild to moderate cases (MS 1, MS 2) delay while the result of the rapid test is pending. A decision concerning the IAT of all grades is done after receiving the result of mastDecide®, 12 hours after diagnosis (point 3).

2. Therapy worthiness: Cows with high SCC (>/= 700,000 SCC/ml) in the previous three monthly dairy herd improvement data or with more than two mastitis cases in the actual lactation are covered by the definition of therapy unworthy cows. Those animals receive no further local AT because of their small chance of bacteriological cure.

3. Mastitis-causing pathogen: Out of the remaining therapy-worthy cows, only those with Gram-positive test result receive IAT, while udder quarters with Gram-negative test result or no verified bacterial growth stay untreated. Furthermore, every cow receives a NSAID treatment immediately after detecting the clinical mastitis for up to three days. [Image 1; Decision Tree]

2.3 Rapid on-farm Test mastDecide®, Test-Phase

From September 2016 to February 2018 the new treatment concept and the rapid test were tried and tested by the dairy employees. Sampling was done as supplied in the preliminary phase. Farms were visited by a veterinarian, explaining background knowledge and the recommended therapy concept. For performing mastDecide®, clean working areas were set up in separate office rooms. Every person who would former use mastDecide® tested it several times under direction. Herd manager filled in a protocol about every mastitis case, containing the mastitis severity score, the test result, information about the therapy worthiness of the animal, and the conducted treatment. Usage of the on-farm test and evaluation of the results were done directly on farm by the trained dairy personnel.

2.4 Definitions

Bacteriological cure was defined if the mastitis-causing pathogen of the mastitis sample was absent in both post-treatment quarter samples 14 (+/-) and 21 (+/-) days after diagnosis.
3 Results and Discussion

Due to great differences between the EBMT concept and the previous therapy concepts compliance of implementing the new concept varied between farms. Therefore, only results of farms will be presented where not only the rapid test was implemented but also all aspects of the EBMT concept. In the preliminary phase, 90% of the affected quarters received IAT. The BCR was 81% (n=186). In the test phase the intramammary administration of antimicrobials decreased to 33% of the affected quarters, while the BCR was 83% (n=431). No changes of other udder health key performance indicators (culling rate (90 d), new infection rate, rate of incurable animals) occurred in the period of the study. There are clear indications for an increasing antimicrobial susceptibility of mastitis-causing bacteria, esp. Gram-negatives, to the tested antibiotic agents.

The results of this study suggest that implementation of an EBMT concept on a conventional dairy farm can safe antibiotic doses with unaffected BCRs. Besides those positive outcomes regarding the behavioural change of treatment decisions, the implementation implies extra efforts due to checking the animal related factors, taking milk samples, performing mastDecide®, and intense monitoring of sick cows. Furthermore, delayed treatment while pending results of the rapid test can be challenging if multiple persons are involved in the treatment. Conditions for a successful implementation are constant documentation and communication. Another difficulty exist through most farmers believes that a cow’s prognosis for cure will get worse while waiting 12 hours for test results (Neese et al., 2006). For years blanket AT seemed to be the only option for a high standard of udder health, removing those doubts must be seen as a longer process.

References


GVA (German Veterinary Association), 2009. Leitlinien zur Entnahme von Milchproben unter antiseptischen Bedingungen und Leitlinien zur Isolierung und Identifizierung von Mastitisrerregern. [Guidelines for aseptic milk sampling and guidelines to isolate and identify mastitis pathogens], 2nd edn. Gießen, Germany: German Veterinary Association


**Image 1: Decision Tree**

Reference: Schmenger, Leimbach
PLASMA-BASED DECONTAMINATION OF DRIED PLANT-RELATED PRODUCTS TO ENHANCE FOOD SAFETY

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Abstract
Cold atmospheric pressure plasma (CAPP) has been used in the food industry since the 19th century for disinfecting water based on the generation of ozone. In recent years, the interest in cold plasma processing as an emerging non-thermal technology in food production has increased. Plasma is defined as an (at least partially) ionized gas and is sometimes called the fourth state of matter. Depending on the system configuration and the feed gas used, plasma consists primarily of different reactive components: such as ions, free electrons, photons and atoms. Due to the wide variety of cold plasma systems, CAPP opens up fundamentally new possibilities for food processing and can be applied at different points along the food chain; for production, modification, and preservation, as well as in packaging of plant- and animal-originated food.

The objective of the research project ‘Plas is to ensure the safety of dry products using CAPP, while maintaining product quality. Besides the microbiological safety, this includes the physico-chemical assessment of the treated products. A prototype containing a microwave based plasma source is constructed and will be tested on a pilot scale regarding feasibility. To initialize the subsequent industrial implementation of the overall project, the two major plot lines are: i) process design and technical implementation in pilot scale (INP Greifswald, Theilen Maschinenbau GmbH and CZIOTEC GmbH); ii) process monitoring and product safety (ATB Potsdam, First Sensor AG). Both sub-projects are carried out in close interaction between industry and research partners, thereby a high practice relevance of the solutions is ensured. Among others, wheat grains, pepper, and oregano are exemplary tested products for the research study on tailored plasma processes and the recent status of the project work will be presented. Additionally, the regulatory issues and further requirements for successful implementation of cold plasma technologies in food processing will be discussed.

Keywords
Cold atmospheric pressure plasma, food safety, dried food disinfection

References
INNOVATIVE APPROACHES FOR EXPLORING THE APPRAISAL OF NOVEL TECHNOLOGIES

Chair(s): Baum, Chad M. (University of Bonn, Germany)
Discussant(s): Baum, Chad M. (Institute of Food and Resource Economics, University of Bonn)

“How safe is safe enough?” This ostensibly simple question from Starr (1969) is credited with launching the research agenda on technological risk. In particular, if we judge the potential for nuclear catastrophe to be too high or possible damages to key ecosystems too large, how many of the potential benefits from technological advance are we willing to forswear? Given this framing, the ensuing literature has tended to aim at a “socially acceptable and essentially optimum” balance of risks and benefits (Slovic 1987). For instance, by attempting to quantify using objective measures such as annual mortality, Starr could offer predictions like the public would be “willing to accept “voluntary” risks roughly 1000 times greater than “involuntary” risks” and that “acceptability appears to be crudely proportional to the third power of the benefits” (ibid., 1237).

However, this reliance on objective measures is also notable for what it omits: the varied ways in which societies actually respond to novel technologies. As reflected by the case of genome-editing technologies, acceptability cannot be guaranteed by the potential of a technology, e.g., to improve personal health and safety or promote greater sustainability. Moreover, it cannot be assumed that perceptions of risks (and benefits) are harmonious within a society. For instance, belying the conventional reliance on experts, we observe that expert and public priorities need not necessarily converge since, first, scientists are more optimistic overall (Loewenstein et al. 2001; Slovic 1987) and, second, they tend to under-weight socio-economic consequences like job losses and privacy considerations (Scheufele et al. 2007; Sarewitz 2015). Consequently, whereas trust in science remains generally high, there are fields like synthetic biology for which public opinion is strongly negative. As this skepticism represents a sizable obstacle for the further development and commercialization of any related products (Hacker and Köcher 2015), acceptance represents a vital pre-condition for marketing new high-value products and technologies.

Over time, the frequency and complexity of the research into individual determinants of acceptance has increased (e.g. Ronteltap et al. 2007; Tenbült et al. 2008; Frewer et al. 2011). However, just a handful of determinants (perceived risks and benefits, attitudes, knowledge, sociodemographic factors) command the lion’s share of attention (Gupta et al. 2012). Accordingly, this session leverages a variety of methodological approaches to engage with stakeholders and offer insights into acceptance. This includes attempts to employ the integrative potential of neurologically based research to understand decision-making, along with qualitative approaches such as group concept mapping. Firstly, such methodological diversity helps to identify potential stakeholder concerns and offer insight into messaging strategies that can effectively communicate with the public. Moreover, the qualitatively oriented studies have the benefit of extending attention to viewpoints across the value chain. Indeed, consumers are far from the only stakeholders which influence the market potential of novel products and technologies. By focusing on the entire supply chain (e.g. production, processing, packaging, retailing), this session thus enables us to compare and contrast viewpoints against one another, ensuring that more salient considerations are taken into account and any differences better understood.
Consumers’ evaluation of 3D-printed food

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New food technologies, such as genetically modified, functional foods and processing technologies, have promising potential to enhance food supply and increase nutritional security for a growing global population (Floros et al., 2010; Augustin et al., 2016; Misra et al., 2017; De Steur et al., 2017). As new technologies are unlikely to succeed in the marketplace without consumer acceptance (Floros et al., 2010; Augustin et al., 2016), various studies have investigated this topic. In specific, a recent systematic review on food technology acceptance behavior along the supply chain shows that only 17\% applied common theories, namely Theory of Planned Behavior (Ajzen, 1991) and Protection Motivation Theory (Rogers, 1975) (Kamrath et al., mimeo). Instead, the majority of consumer studies on food technology acceptance used tailored, “study-specific” models, making the comparison and synthesis of results difficult. As a consequence of the lack of an overarching and comprehensive theory/model (see Lusk et al., 2014; Bredahl et al., 1998; Bearth & Siegrist, 2016; Frewer et al., 2016; Kamrath et al., mimeo), a theoretical framework is therefore needed to integrate existing theories with variables used in the research on food technology acceptance.

In this study, we therefore take a further step toward building a theoretically grounded model of new food technology acceptance by examining consumers’ evaluations of new food technologies (that is, 3D-printed food) as a function of individual, social, physical and macro-level factors – motivated by the social ecological framework of Bronfenbrenner (2009) –, based on results of an extended literature review with 169 papers (Kamrath et al., mimeo). This integrative food technology acceptance model will be compared to the well-known theory of planned behavior. A survey, developed in combination with Qualtrics (www.qualtrics.com), will target 500 German consumers and be conducted from October 2018. In developing the survey, a questionnaire and scales were developed, reflecting insights from both social-scientific theories and the extended literature on food technology acceptance research. To conduct the analysis, covariance based structural equation modeling (CB-SEM) using STATA will be applied to assess path coefficients and determine of the explanatory power of all the variables from the various theories.

This paper not only makes a methodological contribution as far as the development of an integrative theory that is relevant for future food technology acceptance research, but also builds upon the current understanding of consumers’ acceptance of 3D-printed foods in Germany. In this manner, we are taking the first steps in the direction of a food technology evaluation model, though its final development will require more effort.

Based on preliminary research, it is hypothesized that the Theory of Planned Behavior will have predictive relevance. Further, the proposed food technology evaluation model based on factors identified by an extensive literature review will increase the explained variance, measured by $R^2$. It is expected that consumers’ responses toward 3D-printed food is similar to their reactions to other new food technologies that have added convenience and health-enhancing properties. In this sense, consumers will hesitate about the benefits of 3D-printed foods and will evaluate more the perceived
risks. Thus, a well-designed information campaign is needed for a successful implementation of 3D food printers.

References


Do consumers think that purchasing bio-based products matter? The role of systems thinking and connectedness to nature

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Several consumer products such as cosmetics, detergents or plastic packaging are produced on the basis of fossil fuels contributing to the increase of GHG emissions and climate change (Besi, McCormick 2015). In order to alleviate negative consequences for the environment, these products can also be produced sustainably without fossil ingredients using biomass as raw materials (Bugge et al. 2016). However, Onwezen et al. (2017) found that consumers associate positive as well as negative feelings with bio-based products. One reason for this ambivalence could be that consumers’ beliefs about the environmental benefits of these products differ. Indeed, a crucial determinant for pro-environmental consumption behavior has been found to be the extent to which people believe that their individual efforts make a difference in solving environmental problems (Ellen et al. 1991, Coelho et al. 2017; Ghvanidze et al. 2016; He and Zhan 2018). The concept of perceived consumer effectiveness (PCE) is in line with psychological theories stating that the decision to perform an action depends on the expected results (Ajzen 1991; Bandura 1982). In order to promote pro-environmental behavior, it is crucial to understand how consumers’ beliefs influence behavior and help to alleviate negative consequences for the environment (Cojuharencu et al. 2016).

However, very little research has examined factors shaping PCE. For example, Vermeir and Verbeke (2006) found that PCE cannot be induced by short term manipulations (e.g. by giving examples how consumption behavior affects the environment) indicating that PCE might be strongly inherent to a person (like worldviews). Based on this assumption, we suggest that PCE can be rather described as a worldview encompassing values, beliefs or general assumptions about reality such as a systems-thinking mindset and a sense of connectedness to nature. More specifically, we argue that people who believe that the ecosystem is a complex whole with several interdependencies and feel connected to nature have a stronger PCE because they recognise that their actions and ecological phenomena are interrelated (Davis, Stroink 2016; Davis et al. 2009). In order to test our hypotheses, we developed a quantitative survey which adapts scales from the literature to measure systems thinking (Thibodeau et al. 2016), connectedness to nature (Mayer et al. 2004) and PCE (Antonetti, Maklan 2014) as independent variables as well as consumers’ intention to purchase bio-based products as the dependent variable. Our data are collected from 500 German consumers.

The study is conducted in cooperation with Qualtrics which allows for external validity of the sample. We expect that systems thinking and connectedness to nature positively affect consumers’ intentions to purchase bio-based products and that this impact is at least partially mediated through the impact of these variables on PCE. Understanding these relationships helps to develop policy or marketing initiatives to increase the consumption of bio-based products. For example, communication strategies should indicate how the consumption of bio-based products indeed contributes to protect the environment. Furthermore, public institutions like schools or universities should teach a systems-thinking mindset and a sense of connection to nature in order to increase public PCE.
Restrictions in the diffusion of active and intelligent packaging-innovations at the German retail level

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Active and intelligent packaging (AIP) is a heavily discussed innovation in the food packaging sector, and one gaining attention due to the ability to satisfy consumer demands and provide solutions in times of changing packaging requirements. In spite of its many advantages, e.g., extending shelf-life and providing information about product quality and packaging integrity, little to no food packaging has incorporated the abovementioned technologies, nor are they commercially available at the retail level in Germany.

This work examines the origins and reasons for the low diffusion rates of packaging innovations in Germany. By combining theoretical factors obtained from the literature with empirical data derived from the Group Concept Mapping (GCM) methodology, important latent influence factors can be identified and also rated by stakeholders according to their respective importance.

Apart from the fact that AIP innovations are able to reduce food waste and increase transparency and food safety, successful commercialization efforts can be observed in countries outside Europe, such as Japan, Australia and the US (van Dongen and Kruijf 2007; Restuccia et al. 2010; Aday and Yener 2015). Many researchers have therefore examined why consumer acceptance of AIP differs in Europe. For example, Aday and Yener (2015) found that the majority of consumers in their sample were willing to use innovative food packages to prevent microbiological spoilage. However, other studies show a rejection of active packaging if nanotechnology is used (Bieberstein et al. 2012). Due to the divergent findings in the literature, closer attention should be paid to the reasons for resistance. European legislation is specifically mentioned as a potential barrier, but it seems that higher acceptance in the aforementioned countries cannot be completely explained by differences in the legislative framework alone (Ahvenainen and Hurme 1997; van Dongen and Kruijf 2007). Day (2008) thus considers whether a country's climate is hot and humid climate, thus promoting mould growth, as the main reason for successful commercialization in Japan. But without valid justifications for different adoption rates, it remains unclear why German stakeholders struggle to benefit from the advantages of AIP. Indeed, little research has been undertaken analyzing, in a holistic approach, the opinions of stakeholders across the value chain (Kerry et al. 2006; Dainelli et al. 2008). While the number of studies exploring consumer acceptance of AIP is relatively high, other important actors have received less attention (Chen et al. 2013; Petersen et al. 2014; Pennanen et al. 2015; O’Callaghan and Kerry 2016). To examine these research gaps, reasons for limited diffusion are explored and the different positions of packaging suppliers, food manufacturers, retailers, researchers, and consumers considered. The analysis seeks to answer the following questions:

- Why is AIP not yet implemented at the retail level in Germany?
- Where are possible sources of resistance within the food value chain?

The paper represents a new approach to investigate diffusion and adoption barriers of AIP. GCM shall be used to incorporate all stakeholders, thus aiming to extend the current literature about consumer acceptance and, moreover, identify resistance factors for the whole innovation system.
Assessing internal validity and predictive power of attitudes towards genetically-modified foods using neuroscientific approaches

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One of the challenges in understanding real-world (market) behavior for controversial technologies, like genome-editing technologies, is the assessment of the validity and predictive power of participant explicit measurements (e.g., surveys). Individuals tend to tune their beliefs about the risks of potentially harmful activities towards the views of the people they commonly interact with (Leiserowitz, 2005). If individual explicit responses can be impacted by environmental expectation, explicit responses may not be representative of real-life preferences. Therefore, assessment of factors influencing marketing behavior on genetically-modified foods (GMFs), using a variety of approaches, can ascertain the level of internal validity in explicit responses in marketing analysis. For the German market, an individual’s acceptance and willingness to pay for GMFs may be influenced by their knowledge of genome-editing technologies and attitudes (Albarracin & Shavitt, 2018; Huffman et al. 2007; Klein et al. 2009; Lusk et al., 2015; Wunderlich and Gatto, 2015). Using neuroscientific approaches to measure attitudes towards GMFs indirectly, such as the single-category implicit association test and functional magnetic resonance imaging, this study attempts to address the representativeness and predictive power of the explicit attitudes for GMF against affective behavioral and neurological correlates for consumer willingness to pay.
SUSTAINABLE AGRICULTURE AND ANIMAL WELFARE: CONFLICT OF GOALS BETWEEN ANIMAL HEALTH AND ENVIRONMENTAL HEALTH?

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Abstract

Animal welfare, food safety and the protection of the environment are three concerns consumers have about modern food production systems. Production systems considering these three issues can be in conflict, and finding ways to reduce this conflict is essential as international standards governing sustainable food production develop.

These conflicts can be reduced if flexible animal welfare standards are animal-based and attempt to directly assess the state of the animals themselves rather than prescribing the conditions under which the animals are reared. Animal welfare is often treated as an ethical issue, and is therefore difficult to deal with under current trade agreements. A greater appreciation of the link between animal welfare and animal health makes the link with food safety clearer. Improvements in animal welfare have the potential to reduce on-farm risks to food safety, principally through reduced stress-induced immunosuppression, reduced incidence of infectious disease on farms and reduced shedding of human pathogens by farm animals, and through reduced antibiotic use and antibiotic resistance. Health problems of farm animals continue to be serious threats to animal welfare, and measures of disease incidence can serve as animal-based measures of animal welfare. Continued development of hazard analysis and critical control point-based approaches to animal welfare would allow a smoother integration of animal welfare and food safety standards.
TOOLS AND INDICATORS TO DESCRIBE SUSTAINABLE ANIMAL HUSBANDRY

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Abstract

Nowadays, the food and agricultural sector is often subject of debate. Frequently discussed are the husbandry conditions as well as potentially negative impacts of animal production on the environment. For this reason it can be expected that discussions will continue in the future. Production systems will change as well as consumption patterns. This development will have an influence on the existing structures. Due to the growing pressure (from different stakeholder groups) towards a more sustainable way of husbandry and production, for instance in Germany and its international trade partners, there is an actual need to develop new concepts and practical tools in order to improve the situation in the food and agricultural sector. The aim of this contribution is to illustrate and evaluate the applicability of the Hot Spot Analysis methodology as a reliable and low-cost tool for the assessment of ecological and social problems along the entire product life cycle. For a better understanding of the methodology and its meaning, the Hot Spot Analysis is demonstrated by an example within the area of pig production. The methodology itself was developed by the German Wuppertal Institute. Based on a literature review in addition to results from discussions with experts, the main Hot Spots occurring in the specific life cycle can be identified. For this, the defined social and ecological criteria firstly have to be weighted. The weighting is based on the evaluation by the author. Apart from this, the different phases of the life cycle, depending on their social and ecological impacts in general, also have to be weighted, too. Thus, the main social and ecological factor loads can be discovered in the course of the value chain. After drawing up the results, these can be discussed with various stakeholders involved in pig production and meat consumption, helping to identify potentials and overcome weaknesses in a participative way. The results of the Hot Spot Analysis on pig production showed that the main weaknesses along the value chain are animal welfare and the negative environmental impact. This result reflects the ongoing discussions in the agricultural sector and emphasizes the need for action. In conclusion, the Hot Spot Analysis is a feasible and attractive tool to face potentials and obstacles in the value chain of a product. Companies can use it as an opportunity to identify possible and reasonable aspects to implement or improve corporate sustainability standards.

Keywords

Hot Spot Analysis, Sustainability, Animal Welfare, Environmental Impact

Introduction

In view of current developments on the pork markets, competitive and cost pressures on pig farmers are expected to remain high (Spiller et al. 2005). This has consequences for agricultural livestock in economic, social and environmental terms. However, further intensification of production is difficult to communicate. In many places, animal husbandry is already confronted with grave social acceptance problems (Heise 2017). In its report of March 2015 the Scientific Advisory Council for Agricultural Policy (WBA) at the Federal Ministry of Food and Agriculture also attests "significant deficits, especially in the area of animal welfare, but also environmental protection" to livestock farming (Wissenschaftlicher Beirat für Agrarpolitik und Deutschland 2015). The dominant topics at
present are the widespread use of antibiotics and zootechnical interventions such as the customary tail docking and tooth clipping of piglets.

However, solutions that cover this area and tackle the need for sustainable production or consumption decisions are difficult to communicate. Test marks and quality seals are often not sufficiently recognized. Consumer surveys show that a low price is often the dominant criterion for demand. Sustainably produced pork with sensually distinguishable properties such as colour, texture or intramuscular fat is not yet established on the market in North-Rhine-Westphalia. However, this is the target of the EIP-project entitled “Roiporq- Eignung einer speziellen Schweinekreuzung für alternative Haltungsformen bei voller körperlicher Integrität der Tiere für eine Differenzierung im Markt”.

The objective of this paper is to demonstrate the methodology of the Hot Spot Analysis (HSA) as a practical tool to identify hot spots along a value chain of a product or service. Therefore the individual steps will be explained, followed by a short description of the results of the HSA for the Roiporq-Project. In the conclusion the advantages and disadvantages of the HSA will be discussed.

**The concept of the Hot Spot Analysis**

The Hot Spot Analysis (HSA) was developed by the Wuppertal Institute (Liedtke et al. 2010; Biege et al. 2010; Wallbaum und Kummer 2006). In recent years the HSA developed into an established tool to evaluate the sustainability of products or services. The emphasis of the HSA is on the examination of ecological and social criteria along a value chain of a product or service. Therefore the most important life cycle phases of the examined products are assessed with regard to ecological and social criteria. Through this procedure the relevant Hot Spots along the value chain can be emphasized. This makes the identification of effective solutions for potential problems easier.

If a company has decided to evaluate the sustainability along the value chain of a product, the HSA is a very practical and reasonable tool. The analysis is based on publicly available data and literature in addition to information from experts and stakeholders.

The following illustration explains the individual steps of the HSA.

**Figure 1:** The Steps of the Hot-Spot Analysis (Rohn und Biege 2011)
At the beginning of the HSA the life cycle phases have to be defined (step 1). In general these are the following:

1. Extraction of raw material
2. Processing
3. Usage
4. Waste disposal

Depending on the object of investigation, other lifecycle phases may be adapted. The life cycle phase ‘usage’ for example can be divided into the provision of supermarkets and the usage in private households.

In order to the defined life cycle phases, the ecological and social criteria have to be examined (step 1). These criteria will be evaluated for each life cycle phase. Similar to the definition of the life cycle phases, the defined criteria can variate in dependence of the object of investigation.

**Table 1:** The main ecological and social criteria of a HSA (Bienge et al. 2010)

<table>
<thead>
<tr>
<th>Ecological criteria</th>
<th>Social criteria</th>
</tr>
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<tbody>
<tr>
<td>Abiotic raw materials</td>
<td>General working conditions</td>
</tr>
<tr>
<td>Biotic raw materials</td>
<td>Social security</td>
</tr>
<tr>
<td>Energy resources</td>
<td>Training &amp; Education</td>
</tr>
<tr>
<td>Water resources</td>
<td>Workers’ Health &amp; Safety</td>
</tr>
<tr>
<td>Land use</td>
<td>Human Rights</td>
</tr>
<tr>
<td>Waste</td>
<td>Living Wages</td>
</tr>
<tr>
<td>Emissions to air (incl. greenhouse gas emissions)</td>
<td>Consumer Health &amp; Safety</td>
</tr>
<tr>
<td>Emissions to water</td>
<td>Product Quality</td>
</tr>
<tr>
<td></td>
<td>Animal Welfare</td>
</tr>
</tbody>
</table>

Table 1 lists the main ecological and social criteria that are applicable to the analysis.

Based on the defined life cycle phases and criteria, the first step is to analyse the existing literature regarding to the individual criteria for each life cycle phase (step 2). The analysis of existing literature includes scientific papers as well as general information about relevant firms/partners and studies without scientific background. The collected information can be supplemented by expert knowhow. It is advisable to summarize the information in a synoptic table. This provides an overview about the most important information on the criteria of a life cycle phase and helps to structure the results.

After screening and analysing the relevant literature, each criteria has to be evaluated in dependence of its impact on the environment or society.

**Table 2:** Evaluation of the relevance in each phase (Rohn et al. 2014)

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<tbody>
<tr>
<td>0</td>
<td>No relevance</td>
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<tr>
<td>1</td>
<td>Low relevance</td>
</tr>
<tr>
<td>2</td>
<td>Average relevance</td>
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<tr>
<td>3</td>
<td>High relevance</td>
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<td>n.s.f.</td>
<td>No source found</td>
</tr>
<tr>
<td>n.a.</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>
After weighting each criteria according to a life cycle phase, the life cycle phases themselves have to be evaluated (step 3). This is a preliminary stage before identifying the Hot Spots. Therefore the weightings of each criteria have to be multiplied by the weighting of the life cycle phase to which it belongs. Through this procedure the Hot Spots can be identified (step 4). All values with a result between ‘6´ and ‘9´ can be considered as a Hot Spot. Lower values (e.g. 1, 2 or 3) are insignificant.

The results of this analysis should then be presented and discussed with experts and stakeholders. This approach is necessary to complete the information and strengthen the results of the HSA. If necessary the evaluation can be adapted (step 5). Through this procedure the validity of the HSA can be guaranteed.

**Results regarding to the HSA of the EIP-project “Roiporq”**

The Hot Spot Analysis is one of several tools to assess sustainability in the EIP-funded research project "Roiporq". The project aims to develop a pig production concept that is equally suitable for both conventional and ecological fattening. During the breeding phase of pigs zootechnical interventions (castration, tail docking and tooth grinding) should be avoided just as the use of antibiotics.

The results of the HSA presented here relate specifically to the value chain of the project described. The results were discussed with the partners in the project, but also with external stakeholders.

The evaluation of the results has revealed some interesting aspects. It shows that the ecological and social criteria are in general concentrated in specific life cycle phases. The most significant impact on the environment has the phase of raw material extraction for example. In contrast to another existing HSA about pork (for further information see: Rohn et al. 2014), however, the influence is not considered to be too strong. The reason for this probably lies in the type of stable in which the animals grow up. One of the main features of this research project is an innovative “Pig Port Stable”, a partially open air housing system for pigs. This significantly reduces energy consumption, water use and air emissions compared to conventional pigsties.

In return the most social vulnerabilities occur in the slaughter phase. This result is congruent with the findings of the other HSA (Rohn et al. 2014). Working conditions in slaughterhouses are still very exhausting. In addition, the issue of animal welfare is discussed controversially. Although the project partner is very innovative in this area, there is still room for improvement to promote animal welfare further.

In order to validate the factors and to identify the hot spots, the results were discussed with the project partners and other relevant experts and stakeholders. The results show that along the entire value chain there are frequent hot spots where there is still need for action. These hot spots in particular relate to animal welfare and environmental protection at the beginning of the value chain.

**Conclusion**

The Hot Spot Analysis has proved to be a feasible and practical tool to identify critical social and ecological vulnerabilities along the value chain of a product or service. Especially for companies that haven’t yet dealt with the issue of sustainability, the HSA offers a practical entry-level tool. With the HSA the client can get an initial overview of the situation. In areas
where the HSA is not sufficient, other (analytic) tools can be employed to assess the sustainability of the product or service in greater depth, for instance the Life Cycle Assessment (LCA). The LCA works with databases and provides a deeper and more comprehensive analytic tool. In this context, the HSA can be viewed as a simple entry-level tool for a more in-depth LCA analysis.

For companies, that haven’t yet taken sustainability into account, the HSA can be a tool to get a fast overview about sustainability topics according to their value chain. Thus, HSA helps to ensure that the entire value chain is studied and to gain a deeper insight into the processes. Another advantage of the HSA is, that it is applicable to any product or service. By applying a HSA, a company can specifically identify and address weaknesses in environmental and social criteria in the value chain of its product.

In return the literature review of the HSA can be very time consuming. Furthermore, the results of the HSA are generally influenced by the personal attitude of the author. For this reason, it is important that the results are discussed with experts and other stakeholders in order to achieve a neutral view.

References


Rohn, H.; Bienge, K. (2011): Workshop "Diskussion zu Methodik Hot Spot Analyse und weiterer Nachhaltigkeitsbewertungsmethoden".


ICT TOOLS TO MONITOR THE ENVIRONMENTAL IMPACT ON ANIMAL AND HUMAN HEALTH

GÉ BACKUS²

²CONNECTING AGRI & FOOD

Abstract

As livestock farms are becoming larger and larger and the farms are more and more managed together with paid labour, the need for livestock farm management tools enabling more span of control increases rapidly. The farm manager needs to have overview and control, and in a timely manner. At the operational level animal care takers need support and transfer of tasks. Recent developments in Information and Communication Technology (ICT) have brought new perspectives for the use of Decision Support Systems. The Internet of Things (IoT) enables connecting the digital and physical world. And together with the ever cheaper sensor technology and netwworks (LoRA and gateways) it becomes possible to provide farmers with online access to real time data on the production circumstances in farm buildings. Real time sensor data are obtained online for temperature, relative humidity, CO2, NH3, and air pressure. The sensor data are related to the outside climate data for the specific farmer’s region. Water usage and water quality is also measured. These data allow integrated data-analysis to support identifying optimal parameter values for climate and process control. In the near future new system modules will be added with weight data using camera technology. Individual animals will be identified with RFID tags to relate slaughter data to the compartments where the animals were reared. Another promising area we are working on right now is the application of ´legal tech´. Nearby farms we will measure H2S, butyric acid and NH3, being important odour compounds, real time and give the farmers and nearby living citizens online access to these data. This way we can replace the costly procedure of applying for an environmental permit with a contract between the farmer and his neighbours based, on actual sensor data.

Starting December 2017, thirty farmers are using the software application `Smart Barn`. The farmers have 24 hour 7 day an week access to the sensor data, and receive also regularly feedback on their results benchmarked against the group performance. It is scheduled to introduce a new version of the ICT tool summer 2018. This version will apply fuzzy logic methods to transform `Big data` into a limited set of daily linguistic messages and alerts.
1. Introduction
Animal welfare is dependent on a good balance between performance and health, this directly influences product quality and safety, for that animal production is an important key factor in the “One Health Initiative”. It is known that incorrect feeding can be one of the factors of many disorders; an unbalanced uptake of nutrients or toxic components lead to a weakened immune system, therefore multiple diseases will occur and endanger animal health. In terms of health, the gastro-intestinal tract, especially the small intestine, plays a key role. Its function is not only the absorption of nutrients; it is also an immunological organ with multiple lymphoid follicles such as Peyer's plaques in the ileum. In addition to animal welfare, the quality of the animal derived food and its safety are significant factors for a modern animal production. Meanwhile it is known, that modulation of gene expression in the intestine is possibly influenced by feeding defined nutritional components. Our aim is to analyze feed additives as environmental factors, which modulates the expression of immunologic genes and can lead to an improvement in animal production and health.

Gastro-intestinal health & microbiome
Mammals, such as the pig, are dependent on maternal colostral milk in their first few hours post partum. The colostrum supplies the piglets with essential vitamins, proteins, antibodies and growth factors, which are be protective by modulating the gut microbiota and improve intestinal permeability. Without this intake, the immune system will not develop sufficiently and piglet loss increase rapidly within the first week of life. Together, innate and acquired immunity form the immune system. It protects and purifies the organism from environmental pathogens, endogenous tumor cells and old body cells. The gastro-intestinal tract is one of the most important immunological organs, e.g. 70% of circulating antibodies have their origin in the intestine. First of all, the small intestine is due to its epithelium and cellular components (e.g. macrophages and neutrophils), protecting from the invasion of pathogens. Pathogens can be recognized by the components of the immune system via Pathogen Associated Molecular Patterns (PAMPs); these are target markers on pathogen surface. The innate immune system notices PAMPs by Pattern Recognition Receptors (PRRs), e.g. toll-like receptors (Speckmann et al., 2009). Nevertheless, if pathogens were able to penetrate this protective barrier, the acquired immune system, which is activated by innate immunity, intervenes with T helper cells, T cytotoxic cells and B lymphocytes. In addition to the cellular components of the immune system, cytokines are another essential immunologic key factor. These are soluble polypeptides that work as mediators which are involved in immunological cascades, for instance inflammatory processes. The immune response is not restricted to a single mechanism, signal pathway or gene, but consists of complex, interacting networks of biochemical and cellular networks. To investigate the various components of the immune system it is possible to monitor blood parameters, for example via the level of leukocytes or acute-phase-proteins.

For more detailed investigations it is necessary to examine the expression and biosynthesis of cytokines as well as other immunological parameters, located in the blood and the intestinal tissue.
In addition to immunity, the intestinal flora, termed an external organ, has an important impact on normal gut function and animal health. Microbiome influences on human health have been studied for several years, but represent fairly new research field in animal science, especially in pig production. The microbiome, which includes all intestinal microbiota and their genes, develops after birth and it is subject to constant change throughout lifetime. The microbial composition (e.g. potentially pathogenic or health-promoting) inside the intestine depends on individual, but also on environmental factors, for example nutrition (Trevisi et al., 2015). Within 3 – 4 weeks bacterial composition reacts and adapts on feed conversion (Metzler-Zebeli et al., 2015). Briefly, the most important functions of microbiota within intestine are facilitating nutrient digestion and assimilation, maintaining a barrier against invasion by pathogens and providing immunological surveillance signals. Microorganisms express PAMPs on their cellular surface; trigger PRRs and pathogenic microbiota activate the immune system. The non-pathogenic, commensal, bacteria utilize food residues of their host and usually do not activate defensive mechanisms of animals body. But how can the microbiome of an animal affect its immune system? There are several immunological pathways that can be influenced by bacterial production of transcription and co-factors, for example production of diverse cytokines or NF-κB which is related to the integrity of the epithelium and thereby immune homeostasis (Wolowczuk et al., 2008). Another benefit is bacterial production of short chain fatty acids as propionate and butyrate. They can be absorbed by the intestinal epithelium and are used for production of immunological factors. Furthermore, they can also directly stimulate or inhibit signal pathways. Due to such influences, control of bacterial colonization in intestine can have a significant impact on animal health.

The improvement of animal welfare and health is a principal task of modern animal production. There are several approaches to fulfill these needs. Both disciplines, animal breeding and nutrition, desire to identify genes, which are related to productivity, welfare and health. The phenotype of an animal depends on both, genetic information and environmental factors, such as nutrition. Therefore, it is necessary to investigate these two pillars simultaneously. A concept to connect these tasks is nutrigenomics, which is an interdisciplinary scientific research field. It is a linkage between genetics, biochemistry and nutritional sciences.

**Functions of feed & health / animal welfare**

Besides energy supply, micronutrients and fatty acids can modulate target gene expression as a switch (Wahli, 2013). In order to estimate the nutritional benefit for an organism, the effects of physiologically-active-components must be considered at the molecular level. Those biological signatures, e.g. genes, proteins and metabolites can be detected by cellular sensors, thereby activating and stimulating multiple signal pathways in the body. Finally such biomarkers can be used for an early warning in nutrition-induced disorders of immunological homeostasis (Muller and Kersten, 2003). In human it was shown, that saturated fatty acids are able to stimulate activation of PRRs (e.g. TLR2 and TLR4), whereas intake of unsaturated fatty acids through feed leads to an inhibition of TLR-mediated signaling pathways. Furthermore, fatty acids affect production of cytokines and membrane proteins (Wołowczuk et al., 2008). Phytochemicals can regulate gene expression in pigs (Wei et al., 2017). Such phytogenic additives can be produced out of plant secondary metabolites. Phenols and flavonoids are just fragments of this huge group of substances. Some of these substances have antimicrobial, antioxidative or digestive effects. Kaput and Rodriguez (2004) found that, Phenols, e.g. Thymol and Carvacrol, have an anti-inflammatory effect and can influence gene expression. It was also shown, that Thymol and Carvacrol posses ROS-scavenging activity by their ability to split off hydrogen atoms and therefore binding of free radicals. In pig’s intestine they have an antibacterial effect against Escherichia coli and Escherichia faecalis (Mastelic et al., 2008; Wei et al., 2017).
For piglets, weaning phase represents a critical stage for gastro-intestinal health development. The switch from easily digestible milk to a more solid nutrition, the separation from the sow and the formation of new animal groups are critical stressors for piglets. Stress is also correlated with injuries to intestinal epithelium, a change in the microbial composition of the gut, a lower feed intake and therefore a reduced weight gain after weaning. Another effect is the activation of the adaptive immune system resulted in production cytokines and T lymphocytes. One goal of this project was to improve gastro-intestinal piglet’s health by using an oregano flavor additive. The reaction of elements of the acquired immune system and the microbial composition in the intestine to this additive was investigated.

2. Material and Methods

At the beginning of the experiment, 25 days after birth, 16 piglets (Landrace x Pietrain) from two sows were selected after weaning by their initial weight (7.85 ± 0.9 kg). Four piglets were directly euthanized for sample collection (day 0). The remaining 12 animals were randomly allocated to two groups with six piglets per pen. Groups were kept under identical environmental conditions, but one group was feed additionally with an oregano flavor additive (DOSTO® powder, DOSTO® FARM, Westerstede, Germany) to the standard feed (EuroStart, Agravis, Münster, Germany). The concentration was 1500 mg flavor additive per 1 kg standard feed. 20 days after weaning all remaining pigs were euthanized to collect jejunum, ileum, small intestinal digesta. Total RNA was isolated from jejunum and ileum by using the GF-1 Nucleic Acid Extraction Kit (Vivantis, Subang Jaya, Malaysia) and genomic bacterial DNA with the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) following the manufactures protocols. After measuring RNA concentration, cDNA was reverse-transcribed with the First Strand cDNA synthesis kit (Thermo Fisher Scientific GmbH, Schwerte, Germany). The rtPCR for TNF-α, ICAM1, IL-1α, IL-1β, IFN-μ, HSP60, CD4, CD8, Enterococcus genus, Escherichia coli and Lactobacillus genus, as well as HPRT1, β-actin, RPL4 and 341/534 as internal controls, was performed using their respective primer pairs. Statistical analyses were carried out using R (version: 3.3.1). Comparisons among day 0, treated and control groups were performed using the one -way ANOVA followed by linear contrasts. Differences were considered significant when p < 0.05.

3. Results and Discussion

As shown in table 1, there is a significant difference in the expression of TNF-α in the jejunum between the treated and the control group (p < 0.001). The mRNA level is lower in the treated group. TNF-α is an important cytokine that is involved in systemic inflammatory processes and is part of the NF-κB signaling pathway (Wei et al., 2017). Another part of this pathway is IL-1β, which is expressed significantly lower in the treated group (p < 0.01). In both significant differences of mean ct-values in the membrane proteins CD4 and CD8 were investigated. There are differences in gene expression between the two tissues examined in the experiment. There is a significant reduction of Escherichia coli in the treated group compared to the control (data not shown). This may be taken as an indication of the antimicrobial effect of the flavor additive used in this study.
Table 1: Mean ct-values of the investigated genes in jejunum and ileum

<table>
<thead>
<tr>
<th>group</th>
<th>genes</th>
<th>jejunum</th>
<th>ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TNF-α</td>
<td>IL-1β</td>
</tr>
<tr>
<td>day 0</td>
<td>TNF-α</td>
<td>27.46^c</td>
<td>32.09^a</td>
</tr>
<tr>
<td></td>
<td>(± 0.19)</td>
<td>(± 2.13)</td>
<td>(± 0.32)</td>
</tr>
<tr>
<td>treated</td>
<td>TNF-α</td>
<td>28.25^b</td>
<td>32.11^ab</td>
</tr>
<tr>
<td></td>
<td>(± 0.30)</td>
<td>(± 1.39)</td>
<td>(± 0.66)</td>
</tr>
<tr>
<td>control</td>
<td>TNF-α</td>
<td>27.80^c</td>
<td>29.11^c</td>
</tr>
<tr>
<td></td>
<td>(± 0.63)</td>
<td>(± 1.07)</td>
<td>(± 0.54)</td>
</tr>
</tbody>
</table>

All results are presented as mean ± SEM (n = 16)

^a,b,c Mean values within a column with different superscript letters were significantly different (p < 0.05)

Wei et al. (2017) showed that Thymol and Carvacrol are able to reduce weaning stress and have an effect on immune system related genes and several bacteria. In the present in-vivo study it was possible to illustrate that a flavor additive comprising Thymol and Carvacrol is able to alter gene expression in pig small intestine after weaning. In order to exploit the performance potential of farm animals, an optimization of the diet is indispensable. It is possible to change gene expression of immunological factors, bacterial composition and to reduce the negative effects of weaning stress by prior administration of certain additives. Feed manufacturers can use this knowledge to develop nutrition lines for specific stages of animal production for improvement of reproduction and performance, the reduction of diet-related crops and by that the reduction of drugs such as antibiotics. Questionable is whether it is feasible to preserve positive nutritional effects across generations. Translation of nutrigenomical data into precise predictions for animal breeding is a further goal for which additional research is needed.

4. References


THE THIRD WAY IN GERMANY AND CHINA – TRADE-OFF BETWEEN ANIMAL WELFARE AND FOOD SAFETY

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Abstract

Surveys have shown that about 90 percent of German consumers are willing to pay more for products which are produced under a higher standard of animal welfare. Aspects like higher standards or more transparency in animal farming also play an important role when it comes down to consumer expectation. In Europe, organic farming is increasing from year to year, but cannot be the only solution to everybody.

Grocery shopping relies a lot on trust, so food scandals shake the confidence and thus have serious consequences on the whole sector. Against this background, the idea arises not only to label foods that comply with a higher standard of animal welfare, but also to make these foods perceptible distinguishable from other foods by their sensory features. Based on specific sensory properties like smell, color or taste, the ‘Third way groceries’ are a possibility to ensure food safety and to give the consumer a valuable help for his own decision concerning the quality and the certain way a product was produced. Third way groceries are neither organic certified nor conventional produced. As well they are independent from specific labels like ‘Initiative Tierwohl’, which is an industry alliance of agriculture, beef industry and food retailing or ‘Für mehr Tierschutz’, which is given by the German humane society.

At this time the German food trade presents a few products which can be considered as a third way product; it is just the beginning of a new marketing strategy.

There are already different projects which imply high standards of food safety and animal welfare in the field of poultry. For example, the ‘Kikok-chicken’ is a slow growing breed which is fed on more than 50 percent corn in their feeding ration. This high amount of corn leads to a yellow meat color which is caused by the carotene in the corn. Additionally there is more space for the chicken compared to conventional broiler chicken, the feed exclude any genetically modified substances and there is no use of antibiotics. The yellow color of the meat makes it easily distinguishable from other poultry meat and reminds the consumer that the broiler grew up under better conditions. The Kikok-chicken is more expensive than conditional chicken but still cheaper than chicken that is produced in an organic way.

To support consumer in buying products with higher animal welfare standards, to show them a response on keeping conditions and to give them the opportunities to buy higher standard products we should consider third way products as an important and remarkable factor in the future.
The HyReKA Project: Dissemination of Antibiotic Resistant Bacteria in the Aquatic Environment and Strategies Designed for Their Removal

Chairs: Bierbaum, Gabriele (IMMIP, University Clinic Bonn, Germany), Exner, Martin (IHPH, University Clinic Bonn, Germany)
Discussant: Exner, Martin (IHPH, University Clinic Bonn)

The project HyReKA investigates the dissemination of antibiotic resistant bacteria into the environment and tests strategies that are designed to inhibit this dissemination.

Antibiotic resistant bacteria may enter the aquatic environment via different paths and the first talk will show how bacteria enter the environment through the wastewater treatment plant into the receiving surface waters. The study compares rural and clinical/urban wastewater and surface waters. Enrichment of bacteria showed, that bacteria producing extended spectrum betalactamases and bacteria resistant to three classes of antibiotics were isolated from nearly all sample sites in both systems. In contrast extensively resistant bacteria producing carbapenemases, part of which were susceptible to only one remaining antibiotic, were mainly isolated from the clinical wastewater. Both classes were not fully retained by wastewater treatment.

The second talk will show the occurrence of antibiotic-resistant pathogens in wastewater from the poultry slaughterhouses and their emergence following wastewater treatment by in-house wastewater treatment plants. Here multidrug resistant producers of extended spectrum lactamases and methicillin resistant staphylococci were found and, with the exception of staphylococci, were also detected in the effluents of the wastewater treatment plants.

The third talk will then introduce five advanced wastewater treatment processes, that were tested for the removal of antibiotic resistant bacteria: (1) ozone treatment, (2) UV treatment, (3) the combination of ozone and UV treatment, (4) ultrafiltration and (5) activated carbon filtration combined with sand filtration. The removal of the resistance genes was measured by qPCR. The results will show that a synergy between ozone and UV treatment was detected. The best removal was obtained using ultrafiltration, whereas activated carbon filters were not effective.

“HyReKA” is part of the BMBF (Federal Ministry of Education and Research) funding measure “Risk management of new pollutants and pathogens in the water cycle (RiSKWa)” in the funding priority “Sustainable Water Management (NaWaM)”, Germany (FKZ 02WRS1377).
Occurrence and Dissemination of antibiotic resistant bacteria in the aquatic environment by clinical and municipal wastewater

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Wastewater as a source of antibiotic resistant bacteria has been discussed repeatedly. It is crucial to survey the potential sources of antibiotic resistant bacteria and how they are released and disseminated into the environment. Within a sub-study of the joint research project “HyReKA” the pathways by sewage from clinical/urban and rural/municipal wastewater are investigated. The aim of this study is to investigate the dissemination paths of healthcare-associated resistant bacteria in an urban setting, in comparison to a rural/municipal catchment area of a small river.

Water samples of the clinical/urban system were taken from sanitary facilities of patient rooms in a university hospital, its wastewater, at different sampling points within the connected sewer system and the local sewage treatment plant (STP) as well as from the receiving surface waterbody. The samples of the rural/municipal catchment area were taken at eight different surface water sampling sites influenced and not influenced by municipal wastewater, including influents and effluents of four municipal STPs (not influenced by hospitals or industry). Antibiotic resistant bacteria were isolated on selective agar plates to identify ESBL-producing bacteria, methicillin resistant Staphylococcus aureus (MRSA) and vancomycin resistant enterococci (VRE). Grown colonies were differentiated and pre-selected by morphological and physiological characteristics and final species identification was done using MALDI-TOF MS. Antibiotic resistance was confirmed by testing the minimal inhibitory concentrations (MIC) and resistant strains were epidemiologically classified using molecular typing methods. In addition, beside qPCR of target resistance genes themselves, culture-independent Microbial Source Tracking (MST) tools for identification of fecal pollution sources (human, ruminant and pig) were applied. Therefore, qPCR assays that target host-specific Bacteroidales 16S ribosomal RNA genes were performed. Both areas were characterized by site inspections, mapping and geographical information systems (GIS) methods.

In all samples resistant bacteria could be detected in both catchment areas (urban, rural), whereas the urban wastewater showed concentrations one to three log steps higher. MRSA was only found sporadically. Molecular typing indicated 26 XDR bacteria (extensively drug-resistant) with susceptibility to only one or two antibiotics in the clinical/urban system. The rural system yielded no XDR strains. MST analysis revealed the presence of human gene markers within the rural catchment area as well as light signals of ruminant and pig specific Bacteroidales sequences.

Urban/clinical wastewaters were charged with a higher load of multidrug resistant bacteria than the rural/municipal wastewater. Although most of these bacteria were eliminated during wastewater treatment, dissemination into surface waters is possible as single resistant bacteria were still present in the effluents of the wastewater treatment plants. Human wastewater is the main source of fecal contamination - and consequently most likely the origin of ARB and ARGs – in the rural catchment. But also animals themselves or manure can have a negative impact on the water quality, albeit to a lesser extent. Future analysis in regard to infection risks for the population using the receiving surface water bodies and identification of possible critical control points will take these investigations into account.
Antibiotic-resistant pathogens in wastewater inside poultry slaughterhouses and their fate in in-house wastewater treatment plants

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This study investigated the occurrence of antibiotic-resistant pathogens (ARP) in wastewater from the poultry slaughterhouses and their emergence following wastewater treatment by in-house wastewater treatment plants (WWTPs).

Wastewater samples were taken from transport trucks and along the production chain inside two poultry slaughterhouses in Germany with slaughtering capacities of 125,000 (S1) and 440,000 (S2) chickens/day. Wastewater samples were taken also from a farm (S3) with slaughtering capacity of 30 chickens/month. Inflows and outflows of the in-house WWTPs with daily treatment capacities of 600 m³ (S1) and 3300 m³ (S2) were sampled as well.

Samples were screened for the presence of ARP using CHROMagar selective media. Up to 6 colonies of appropriate morphology per sampling point were selected. The final identification was done by MALDI-TOF MS and resistance was confirmed by determining of MICs using the ID/AST testing system. The resistant strains were further characterized by different molecular typing approaches. The colistin-resistant strains were screened for the presence of mcr-1, mcr-2 and mcr-3 by rt-PCR.

A total of 201 resistant strains were isolated from 48 samples from S1 and S2, and 142 strains were isolated from 24 samples from S3. 88% of all strains from S1 and S2 were represented by E. coli, MRSA and A. baumannii complex, whereas their abundances in the outflows from in-house WWTPs were the lowest. 12% of the isolated strains belonged to Klebsiella spp. (6%), Enterobacter spp. (4%), Citrobacter spp. and Pseudomonas spp. each 1%. E. coli and A. baumannii complex were the most widespread (93% of isolated strains) in S3. The percentages of K. pneumoniae and C. freundii were low and lay at 4% and 3% respectively. No VRE were detected.

37,1% of the isolated strains from S1 and S2 (n=170) were MDR and 18,8% colistin-resistant, whereas 37,5% thereof were mcr-1 positive and none mcr-2/mcr-3 positive. 52,3% of the E. coli and 5,4% of the strains from A. baumannii complex were MDR. The percentages of MDR among Klebsiella spp. and Enterobacter spp. were 69,2 % and 75% respectively. 6,3% of the isolated strains from S3 were MDR and 2,6% colistin-resistant (all negative for mcr). All isolated strains were carbapenem susceptible.

Phylogenetic typing of E. coli showed that the most frequent phylogenetic groups of strains from S1 and S2 (n=71) were C (49,3%) and B1 (23,9%), followed by E and F (each 9,9%) as well A (5,6%). Only 1,4% of strains belonged to the pathogenicity-associated extraintestinal group B2. 71,9% of E. coli from S3 belonged to group E, 25% to groups B and C as well 3,1% to group A. No strains from groups B2 or D were isolated.

The MRSA strains (n=15) were known livestock-associated types and revealed following types: t034 (60,0%), t011 (20,0%), t899 (13,3%) and t8588 (6,7%).

In conclusion, antibiotic-resistant pathogens are widely spread in the wastewater inside the investigated poultry slaughterhouses. Despite the reduction by the WWTPs, they could still be found in the outflows, posing a threat to human health, and a need for further investigation.
Evaluation of advanced wastewater processes to eliminate opportunistic pathogens and antibiotic resistant bacteria

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Wastewater treatment plants are important barriers against the dissemination of opportunistic pathogens and antibiotic resistant bacteria to the aquatic environment. In contrast to chemical pollutions bacteria from condition wastewater can persist and proliferate under suitable situations in downstream aquatic habitats. To reduce the amount of opportunistic bacteria and the dissemination of antibiotic resistance genes (ARG) through wastewater discharge, an elimination/disinfection application or a highly effective reduction is needed. The current conventional wastewater treatment is not designed to efficiently reduce opportunistic and antibiotic resistant bacteria. Also the more commonly used active carbon filters, which offer a high removal of micropollutants, are insufficient.

Based on different bacterio-physiologic characteristics, 5 advanced wastewater treatments processes were investigated for their microbiological removal potential. The first process utilized Ozone (1 g ozone per g DOC) to treat wastewater after biological treatment. The second was a UV-treatment (400 J per m²). The consecutive application of Ozone- followed by UV-radiation was the third investigated process. An ultrafiltration as well as an active carbon filter followed by sand filtration for comparison represented the most recently studied advanced wastewater processes.

The detection of opportunistic bacteria and ARG was performed by qPCR utilizing taxonomical marker genes for intestinal enterococci, Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii, and Escherichia coli. Beta-lactamases/carbapenemases (blaTEM, blaCTX-M, blaCTX-M32, blaNDM-1, CMY-2, blaVIM2, OXA48), macrolide resistance (ermB), tetracycline resistance (tetM), sulfonamide resistance (sul1), and ARGs against last line of defense antibiotics like vancomycin (vanA) and colistin (mcr-1) were investigated by qPCR. For all analyses life/dead discrimination was applied to remove dead/injured bacteria and free/released DNA prior to qPCR quantification.

A synergy-like effect in the reduction of opportunistic bacteria and ARG was observed by combining ozone treatment and UV irradiation. Here an increased reduction was analyzed up to 3 orders of magnitude. Particularly P. aeruginosa displayed robustness towards ozone but the subsequent UV irradiation improved its reduction. A similar synergy-like effect of ozone and UV was observed in case of the investigated ARGs. Most of the beta-lactam/carbapenem resistance genes were reduced below the detection limit including the ARGs against last line of defense antibiotics vancomycin (vanA) and colistin (mcr-1). But higher abundant ARGs like blaTEM, ermB, tetM, and sul1 were still found in residual abundances in the effluent of the combination treatment.

In general the combination of ozone treatment and subsequent UV irradiation displayed a greater effect in bacteria reduction compared to each individual process. The highest removal of bacteria was achieved by ultrafiltration, reducing up to 5 orders of magnitude of enterococci. Other opportunistic bacteria were either reduced close to, or below detection limit (K. pneumoniae, A. baumannii). In case of ARGs ultrafiltration reduced all but blaTEM below the detection limit. Activated carbon filtration showed no effective removal for all opportunistic bacteria and ARGs (less than 40%).

But what is not well understood is the fate of these determinants after discharge from WWTPs plus their release via untreated wastewater into the environment and what impact they have on various ecological communities, including humans.
Antibiotic free meat: (im-)possibilities from a supply chain point of view

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Background
Antibiotics (AB) are widely used in livestock production, particularly pigs and poultry. The risk of development of resistance to antibiotics (ABR) has been recognized in the last decades. Main fear is that infections in humans, which currently can be treated rather easily with available ABs, will become uncurable in the future. In response, initiatives are taken at various levels to reduce or even abandon use of ABs (ABU). Such initiatives have various (conflicting) impacts on the different actors in the value chain network, as well as on its organisation. Hence, reduction of ABU involves various aspects and many stakeholders, and requires a comprehensive inventory of (im-)possibilities and reasons thereof.

Objective
The objective is to inventorize the important issues regarding production, processing and marketing of meat produced with reduced or no ABU.

Program of the organized session
The session (approximately 90 minutes) starts with an introduction (HW Saatkamp) and ends with a short wrap-up and outlook (M Hartman). Short presentations and activities include:
1. Antibiotic use: the farm economic perspective (JL Roskam)
2. Antibiotics: perspective and demands from consumers (J Simons)
3. Reduction of ABU: the viewpoint from slaughterhouses and retail (D Oorburg)
4. Selection of 5 key-factors (HW Saatkamp)
5. Discussion of the 5 key-factors (moderation: J Simons)

Short contents of the presentations
Antibiotic use: the farm economic perspective (JL Roskam)
ABs are a relatively (very) cheap damage abatement input for livestock production. Moreover, in case of structural inefficiency and/or incidental diseases, ABU has a high marginal production value. This high economic value is a main incentive for ABU. Additionally, ABU can be a cheap (disease) risk management instrument. Hence, the economic impact of reducing ABU is determined by many ...

... (quite often) farm-specific factors, e.g. animal-disease interaction, farm health management and the risk-attitude of the farmer. Between farms, a large variation in ABU and production results occur (Figure 1). This offers prospects for reduction of ABU. However, ABU reduction without compromising farmers’ income requires well-focused and farm-specific measures. This could harbor conflicts with harmonized sector-wide measures, i.e. acceptance and compliance by individual farmers. Summarized results of quantitative economic research will be presented.

Antibiotics: perspective and demands from consumers (J Simons)
Many consumers consider antibiotics as an integral and necessary part of an animal husbandry system that is perceived as rotten, nightmarish and only profit oriented. Resistances and as a consequence an increasing defenselessness against infections appear as a result of a condemnable treatment of animals. But even though the husbandry system in general and antibiotics in particular is experienced as threatening, many consumers succeed in suppressing and splitting off while purchasing, handling and eating meat. However, antibiotic free meat may have market prospects as – it is supposed to have a direct link to personal health.

Reduction of ABU: the viewpoint from slaughterhouses and retail (D Oorburg)
The adverse effects of a complete ban on ABU are unacceptable (e.g. impaired animal health and welfare) as well as unnecessary (i.e. in view of the development of AB resistance). Essential is aiming at the right balance of ABU, i.e. responsible ABU. This could serve the interests of both the consumers and producers. However, this requires a carefully developed implementation, particularly with regard to protocols of ABU, safe-guarding and auditing. Moreover, communication to both producers and consumers is vital for acceptance and compliance.

Aims of the structured discussion
This requires an active involvement of the audience! The aim is to:
• Critically evaluate major determining issues for reduction of ABU during production and marketing of products
• Discuss, and thereby inventorize, the (im-)possibilities for implementation (not only economical, but also organizational, institutional, etc.)
• Identify strong and weak points of future brand concepts for meat with reduced or no ABU

Acknowledgements

Figure 1. Average farm performance (weight at 42 days, Y-axis) and ABU (X-axis) for 35 Belgian broiler farms.

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Introduction
It is a global challenge to refashion food systems (Heller et al., 2013), to ensure the human nutritional health and avoid the ecosystem degradation (Dernini et al., 2017). Dietary choices are the central driver behind the environmental impacts (Heller et al., 2018). For the enhancement of sustainable and healthier diets, there is a need to integrate nutrition and environmental sciences in a sophisticated manner (Heller et al., 2013). The aim is to assess the impacts on the human nutritional health and ecosystem health of self-selected diets from individuals in a German metropolitan region, using a nutrition-environmental epidemiological cross-sectional life cycle assessment (LCA) study, to evaluate the level of sustainability and healthiness of diets within local and urban contexts. This study helps to integrate sustainable diets and healthier food systems, complementing the already existing One Health framework.

Material and Methods

Expected Outcomes
This study expects to integrate human nutritional health and ecosystem health, complementing the already existing One Health framework, underpinning domains concerning human-animal-environment interactions within the food systems. It is imperative to conceal health and sustainability aims, and understand how they interact in real diets, in order to promote healthy and environmentally sustainable dietary shifts in urban areas.

References

Acknowledgments
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FOOD FINGERPRINTING - AN ANALYTICAL APPROACH TOWARDS COMPREHENSIVE FOOD AUTHENTICATION

INVESTIGATION OF HARD CHEESE, EDIBLE SEED OILS AND SPIRITS

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INTRODUCTION
The non-targeted analysis (so called fingerprinting) is increasingly in the focus of scientific interest. This approach is providing the capability for comprehensive characterization of food matrices. The generated data of the fingerprint are used for statistical data analysis (univariate or multivariate) to investigate different authenticity issues. One aspect examined in the project FoodAuthent is the comparability of Fourier transform-infrared (FT-IR) fingerprinting spectra of a variety of edible oils recorded at different devices.

FINGERPRINTING APPROACH
- sample preparation
- analysis
- evaluation
- data acquisition
• authentic sample
• non-authentic sample (e.g. adulterated food)
• authentic range of reference values (database)

Fig. 1: General workflow of fingerprinting analysis.
• non-targeted simultaneous detection of as many features as possible ⇒ “spectral fingerprint”
• comparison with an existing database (authentic range of reference values)
• possibility to identify deviations from authentic samples (e.g. addition of forbidden substances, false declaration)

CHALLENGES IN FINGERPRINTING
The routine use of fingerprinting is currently restricted to certain products (e.g. juice, wine and honey) often in conjunction with commercial solutions.
• analytical requirements for routine use are:
  - consistency instrumental variation
  - consistency spatial variation
  - consistency temporal variation
  - validation of analytical procedure/quality assurance measures

CONSISTENCY INSTRUMENTAL VARIATION
- one step towards harmonization is to ensure the comparability, inter alia, of identical devices
- significant differences between intensities recorded at different instruments are evident

Fig. 2: Raw spectra of replicate measurement of one rapeseed oil sample recorded at FT-IR spectrometer 1 (n=46) and 2 (n=46).

EXPLORATIVE DATA ANALYSIS COMPARISON

Fig. 3: Scores-Plot (PC 1; PC 2) of PCA with 150 oil samples recorded at FT-IR spectrometer 1 and 2 (as examples: green box – linseed oil, orange box – rapeseed and pumpkin seed oil).
• investigation of sample set (n=150) by PCA to assess devices difference
  ➢ without correction the spectra of two identical spectrometers are not comparable

Approaches for Optimization
1. determination of a specific correction factor for the instruments
2. optimization by data pre-processing
  ➢ improvement of comparability by applying the two correction approaches

CONCLUSION
• application of non-targeted analytical approaches in routine analysis is limited
  ➢ need of measures towards standardization
• BMEL project FoodAuthent investigates some aspects of validation and harmonization:
  ➢ ensuring the comparability of measurements, e.g. using different analytical devices from the same manufacturer

*The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.
Blockchain Provides Food Safety

**SENSORS**
To trigger alerts in cases of rule violations

**SMART CONTRACTS**
To enable immediate and automated payments

**STANDARDIZED TRANSACTION FORMAT**
To provide uniform, real-time tracking data

**MARKETPLACE**
To automate the allocation of transportation orders

**APP / WEBSITE**
To enable customers to track their food through the supply chain

**WHAT’S THE ADVANTAGE?**

**Transparency**
- Real-time information
- For supply chain partners
- For customers

**Immutability**
- Transaction documentation
- Avoidance of food scandals

**Market**
- Transportation allocation
- Foster competition

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Fraunhofer FIT
Sandra Andrea Klein, Wolfgang Prinz, Wolfgang Gräther
Abstract: Antibiotics are randomly used in Bangladesh for quick fattening of different meat producing animals (including poultry chickens). Although WHO Guidelines on Use of Medically Important Antimicrobials in Food Producing Animals released in 2017 strongly recommended complete restriction of use of all classes of medically important antimicrobials in food producing animals for growth promotion. This research, in its primary empirical analysis found that farmers, consumers and even regulators are not aware of the fact that widespread use of antibiotics in meat producing animals might create antimicrobial resistant infections that could be transmitted to human health through food or through direct contact with food-producing animals or through environmental spread. In absence of the effective regulation and adequate qualitative legal scholarship on the use of antibiotics on food producing animals and its possible impacts on public health, the current study will attempt to find out the regulatory changes that could be done by the government of Bangladesh without further delay. First, the present study will analyze available literatures from various disciplines, such as law, public health, veterinary sciences and so forth. In the next stage animal farmers, meat consumers, public health professionals, veterinary scientists and regulators will be interviewed to examine the reasons of use of antibiotics in food producing animals. Finally, based on the mixed methodological data a set of recommendations will be made for the government of Bangladesh on how random use of antibiotics can be stopped to the food producing animals effectively. In doing so the “One Health” approach suggested by WHO will be taken into consideration.

Why farmers use antibiotics in meat producing animals

- For fattening the cows and poultries so that it looks healthy and so that the farmers can sell them in higher price
- For producing more meat to meet the demand of protein
- For ignorance of updated knowledge regarding poultry and animal feed
- For the higher cost of alternative feeds
- Antibiotics are not optimally prescribed up to 50% of the time
- At almost 100% pharmacies sell antibiotics without seeing any prescriptions
- For regulatory drawbacks regarding animal feed and meat production

What are the consequences of using antibiotics in meat producing animals

- The antibiotic use in meat producing animals in Bangladesh creates antimicrobial resistance which threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi. (WHO, 2018)
- Antibiotics are not working on 56% population of the country
- Public health situation worsening day by day
- Environment is contaminated
- Effectivity of the exiting regulation got questioned

Principle regulation to restrict the use of antibiotics meant producing animals

The Fish Feed and Animal Feed Act, 2010. Section 14 talks about the banning of the use of antibiotic in animal feed. S 14(1): Any harmful chemicals including antibiotic, growth hormone, steroid and insecticides cannot be used in fish feed and animal feed. S 14(2): Any body violating the provision of sub-clause (1) will come under the purview of crime under this law.

Legal drawbacks of the Fish Feed and Animal Feed Act, 2010 for combating use of antibiotics in meat producing animals

1. Problems in criminalizing the antibiotics use through injection:
   - The word as “Animal Feed” has been defined as “... various nutritious feedstuff and mixtures thereof prepared artificially or through other means for the purpose of raising and saving the animals from malnutrition...” - s 2(6). The word “Adulterated fish feed and animal feed” means any fish feed or animal feed containing poisonous or harmful substances which are harmful for fish, livestock or other animals or environment or such fish feed or animal feed which is not in consistent with the matters as mentioned under clause 11 and 13 or proved in the Quality Control Laboratory as adulterated or poisonous or harmful fish feed or animal feed or as injurious substances” - s 2(11). Feeds are generally taken through mouth. So if an animal is injected antibiotic for growth purpose – it would not mean the “feed”. Hence, the injecting antibiotic for growth purpose has not been criminalized in the law.

2. Hindrance to Access to Justice:
   - S 18 of the Act talks in regard to the acceptance of trial for crime committed. It says “without any written complaint of the Director General or empowered officer, no Court shall accept any case for trial under this law.” – This embargo is creating limitation to access to justice for a victim of unwanted use of antibiotics.

Problems in the enforcement mechanism to eradicate or to reduce antibiotic use in meat producing animals

- Adequate number of labs are not available to test the existence of antibiotics in foods
- Adequate number of mobile courts are not available in the rural area to monitor antibiotic use on meat producing animals
- As per the annual report of 2016-17 by the Ministry of Fisheries and Livestock – only 44 mobile courts have been administered in a year throughout the country which is extremely insignificant
- Sometimes corrupt officials let the culprits continue their business where laboratories use antibiotics are random.

3. Bailable offence:
   - S 20 says that the crimes committed under this Act will be non-cognizable and bailable.

This paper argues that considering the long standing effect of antibiotics on human health and civilization, this should be a non-bailable offence for the time being until the happening of this offences come under control.

4. Very Low Punishment:
   - As per s 20, if any person commits any crime under this Act, that person will undergo imprisonment of one year or penalty up to Tk 50,000/- or will be convicted in both the cases simultaneously. The current paper considers that this punishment is very low. The civil penalty is insignificant since the businesspeople are extremely rich and they make a huge amount of profit by violating the law. 50,000 taka (equivalent to 500 Euro) is not a remarkable civil penalty considering the value of currency in Bangladesh.

Problems in administrative enforcement mechanism

- Many farmers are not aware of the danger of antibiotic use. The author has found that more than half of the farmers do not possess knowledge about the risk of unauthorized antibiotic use in meat producing animals
- The Govt has discovered a poultry feed made with several herbal leaves in Bangladesh (GOB report, 2016-17) which can fatten the poultry very quickly. But few farmers are aware of this fact.
- Antibiotic use for fattening of meat producing animals are not scientifically proven. But as the other farmers use it- many of the farmers are using it following the fellow or predecessor farmers. The awareness creating campaign by the Government is not enough.

‘One Health’ approach to combat antibiotic use in meat producing animals

- Bangladesh developed a Strategic Framework for One Health Approach to Infectious Diseases in 2012. (GOB, 2017). Later it was recognized by the Ministry of Fisheries and Livestock.
- Government has established an Inter-Ministerial Steering Committee for One Health in 2016. (WHO, 2017)
- There is a notable One Health network established in 2008 namely, ‘OH Bangladesh’. It regularly arranges international OH conference in Bangladesh. (McKenzie et al, 2016)
- ‘The OH Hub, Bangladesh, established in 2013, with focal points from the Institute of Epidemiology Disease Control and Research and the Department of Livestock Services and a membership of 44, is integrated with and supports the OH Bangladesh organization’. (McKenzie et al, 2016)
- However, adequate researches are not found following One Health approach for reducing or eliminating antibiotic use in meat producing animals in Bangladesh.

Recommendations

- The Fish Feed and Animal Feed Act, 2010 should be updated taking into account the above-mentioned regulatory loopholes
- More research should be conducted following the One Health Approach
- Farmers and concerned businesspeople should be trained properly to avoid antibiotic use in meat producing animals
- Alternative feeds for fattening the meat producing animals should be invented and made available at cheaper price
- Government should concentrate on creating awareness among consumers to avoid meats where antibiotics have been used
- Government should create laboratory facility available where existence of antibiotics could be easily tested.
Introduction:
Selenium was invented in 1817. It has been regarded as a toxic and carcinogen element, but in 1957 Schwartz and Foltz published that selenium could avoid the necrosis of the liver\[^1\]. From 1973, we know that selenium is the main element of glutathione-peroxidase which fights against harmful free radicals\[^2\]. Selenium deficiency increases the risk of depression, heart and vascular diseases, brain hemorrhage, thyroid malfunction and the formation of some type of cancer. It connects to Keshan-disease, Keshan-Beck syndrome, Down’s syndrome and the infant cretinism\[^3\-5\].

The risk of selenium deficiency in case of people on a healthy, various diet is low, but in Europe the soil is poorly selenized, which could indicate health hazard. The RDA of selenium is 55 μg/day, the upper limit is 400 μg/day according to the WHO. The selenium sources are the followings: liver, seafood, meat, milk, etc. The average selenium content of the milk is 25 μg/l, and it supports our daily selenium intake in 6-10%\[^6\]. That’s the reason why we decided to produce functional, selenium enriched milk.

Materials and methods:
In our experiment, we intended to produce selenium enriched milk by feeding three ‘Hungarian Simmentaler’ type cows with different dose of selenium (1. picture). Every dose of the supplementation took 2 weeks long. We gathered milk samples weekly, and we froze it until the ICP measurements. Freezing has no effect on selenium content of the milk.

We made dairy products to prove that oral selenium supplementation appears in the products as well.

Results:
On the 2nd picture you can see the average selenium content of the milk. Our experimental milk contain more selenium than the control sample. The selenium content of the experimental milk is more than the control in every dose. The difference is significant, so we proved that we can produce functional milk with feeding cows with selenized fodder.

Summary:
Milk as a basic nutrient is a selenium source for humans. Adding selenium into milking cows’ feed gives an opportunity to increase the selenium content of the milk. According to our measurements, the Se-enriched dairy products may be suitable for satisfying the daily Se-needs.

References:
CHARACTERIZATION OF STAPHYLOCOCCUS AUREUS ISOLATED FROM RAW MILK FROM VENDING MACHINES

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Introduction

Using raw milk vending machines makes possible for customers to buy raw milk delivered from dairy farms directly. Raw milk from vending machines should be boiled before consumption, since raw milk may contain various microorganisms, including pathogens such as Staphylococcus aureus (Galičić et al., 2015). S. aureus is one of the most important microorganisms in dairy farms that can cause mastitis in cows (Kovács et al., 2013). The aim of this study was to investigate the occurrence of S. aureus in raw milk from 13 vending machines which can be found in Hajdú-Bihar County, Hungary, and after identifying the S. aureus strains isolated from the samples by the coagulase test, the characteristics (tellurite reducing ability, lecinthinase activity, hemolysin production ability) and the antibiotic resistance of isolates were determined.

Materials and Methods

During our research, we collected raw milk samples from 13 vending machines in Hajdú-Bihar County (Hungary) in February 2016. After sampling, the samples were delivered within two hours in a cooler bag equipped with ice batteries to the Microbiological Laboratory of the Institute of Food Science at the University of Debrecen, where the samples were processed as soon as possible. The preparation of the samples was carried out in accordance with the MSZ EN ISO 6887-1:2000 standard. The determination of S. aureus was performed according to MSZ EN ISO 6888-1:2008 standard. The isolated strains were stored at -80 °C until further studies, which were performed in January and February 2018. Hemolysis tests were performed on Columbia blood agar (Biolab Ltd., Hungary) and the antibiotic resistance of isolated strains was performed by agar diffusion method according to the Clinical and Laboratory Standards Institute guidelines (2017).

Results and Discussion

Based on the results, S. aureus was occurred in raw milk samples from all 13 vending machines; the mean values ranged from 0.8 to 2.9 log₁₀ CFU/ml (Figure 1). The difference between the mean S. aureus counts of samples taken from vending machines was not significant (P>0.05). In the samples of 3 out of 13 vending machines, the S. aureus count exceeded the limit (n=2.70 log₁₀ CFU/ml) set in the regulation of the Hungarian Ministry of Agriculture and Regional Development and the Hungarian Ministry of Health, Social and Family Affairs 1/2003 (I. 8). However, the refusal limit (M=3.3 log₁₀ CFU/ml) was not exceeded.

A total of 26 S. aureus strains were isolated from raw milk samples. All strains produced coagulase enzyme, had tellurite reducing ability and lecinthinase activity. Among the 26 strains, 9 strains were nonhemolytic, 12 strains showed weak hemolysis, 3 strains showed α and β hemolysin, and 2 strains showed β hemolysis on blood agar.

In antibiotic resistance testing, it was found that all strains were susceptible to cefoxitin, gentamicin and trimethoprim/sulphamethoxazole. In this study, 9 strains (35%) were resistant to penicillin, which is higher what Peles et al. (2007) detected in their study (30.5%). In our study, 6 strains (23%) were resistant to tetracycline, which is higher than in the study of Visciano et al. (2014) and Peles et al. (2007). Furthermore, based on our results, 1-1 strains (4-4%) were resistant to chloramphenicol, clindamycin and erythromycin, as shown in Table 1, where R is resistant, I is intermediate, S is sensitive.

Conclusions

The results of our studies confirm the necessity to boil raw milk from vending machines before consumption. Since S. aureus was present in all samples, the consumption of raw milk from vending machines without heat treatment could be unsafe from a public health point of view. Our results also corroborate other findings in other literature, which show that in recent years the proportion of occurrence of resistant strains is rising continuously in the world and in Hungary.

References


Figure 1: S. aureus count in samples taken from raw milk vending machines

Table 1: Antibiotic resistance of S. aureus strains (n=26) isolated from raw milk

Antimicrobials (concentration) | R | I | S
---|---|---|---
Cefoxitin (30 µg/disk) | 0 | 0 | 26
Chloramphenicol (30 µg/disk) | 1 | 4 | 0 | 25
Clindamycin (2 µg/disk) | 1 | 4 | 0 | 25
Erythromycin (15 µg/disk) | 1 | 4 | 0 | 25
Gentamicin (10 µg/disk) | 0 | 0 | 0 | 26
Penicillin G 10U | 9 | 35 | - | 17
Tetracycline (30 µg/disk) | 6 | 24 | 0 | 20
Trimethoprim/ Sulphamethoxazole(1.25+23.75 µg/disk) | 0 | 0 | 0 | 26

Acknowledgements

The work/publication is supported by the EFOP 3.6.1-16-2016-00022 project. The project is co-financed by the European Union and the European Social Fund.
Vétérinaires sans Frontières Germany has implemented various projects in collaboration with local private and public stakeholders in East Africa for 27 years, under the One Health approach that is now gaining recognition in health policies and brings together multiple disciplines to achieve optimal health for people, animals and environment. Somalia is deeply affected by a civil war which is lasting for more than 20 years. Additionally, food security is currently threatened due to consecutive seasons of poor rainfall affecting 5.7 million people (World Food Programme, 2018). In pastoral communities like in Somaliland, animal products provide essential high-quality proteins and micronutrients for the growth and health of the population (Zinsstag, et al., 2002) and livestock related activities account for 60-70% of the gross domestic product and employ 80% of the workforce.

**Main Objective**

- Improve food-security, -safety and livelihoods of the milk and meat value chain members - producers, processors, vendors and Community Animal Health Workers (CAHWs) - (direct beneficiaries) and local population (indirect beneficiaries) in Somaliland.

**Results:**

- Improved animal health of the herds: total of animals receiving animal health services by 20 CAHWs trained by this project 28,701 (hence 3,220 reached households)
- Better quality and safety of local animal products scaling up the access of the population to hygienically improved animal protein
- Improved income of producers, vendors and processors price of fresh milk went from 0.8 Dollar to 1.25 Dollar / Liter
- Fifteen Income Generating Women Groups (IGA) improved business associations, receive support in finances and management
- 26 staff members from Ministry of Livestock and 75 members from three project districts received policy formulation, implementation and evaluation trainings improved capacity to regulate and provide services in the milk and meat sectors by governmental institutions
- Participatory development of a context-relevant policy framework for the milk and meat sectors

**References:**


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One Health – Transdisciplinarity at ZB MED

Eva Seidlmayer, Christoph Poley
ZB MED – Information Centre for Life Sciences

1. Background

Ebola, SARS, bird flu, food safety scandals, antibiotic resistance, and microplastics in water and organisms all have one thing in common. They are clear examples of why pathologies and diseases can no longer be explained in isolation without reference to how we feed ourselves, how we produce food, and how we assist the vulnerable and needy. Our understanding of medicine in today’s world requires us to take a far more complex view of human beings within the ecosystems they inhabit. Since the last decade, this holistic understanding that environmental factors can impact human health has been referred to as One Health.

ZB MED – Information Centre for Life Sciences offers tailor-made tools for research questions concerning One Health and related fields.

2. One Health as a transdisciplinary research principle

One Health is an integrated, systemic research approach. It recognizes that human health is closely connected to the health of livestock, wild animals and nature (Cumming 2015). One Health is an age-old concept that is implicitly referenced in ancient Greek philosophy. It is currently taking on new relevance in science and policymaking as a result of climate change, globalisation, and the increasing mobility of people, animals, and goods.

3. Interdisciplinarity or transdisciplinarity at ZB MED?

ZB MED takes a transdisciplinary approach to its provision of literature and services (Mittelstrass 2007). Transdisciplinarity focuses on determining the most relevant problems by integrating diverse forms of research. It breaks down the historically conditioned limitations of conventional research strategies while also applying a critical eye to the received content. Incorporating both disciplinary and interdisciplinary styles of research, transdisciplinarity does not imply the dissolution of individual specialist disciplines, but rather their integration, if and when this is conducive to addressing research questions.

4. One Health tools at ZB MED

- Structured literature from the full range of life sciences in the ZB MED Knowledge Environment: more than 60 million records from 65 databases, 58% of which cover medicine/health, 31% agricultural science, 14% environmental science, and 11% nutritional science (some databases appear in more than one category)
- Data enrichment through the addition of research data, information on availability, and other forms of enhancement

4.1 Support for transdisciplinary research

- Search queries and metadata on the LIVIVO search platform are expanded to include vocabulary from the specialist thesaurus MeSH (medicine), AGROVOC (agriculture) and UMTHEs (environment)
- Enhances search queries
- Assigns items to specialist groups
- Identifies related terms
- Transdisciplinary filtering and frame of reference for search results
- Availability of open access full texts and research data, plus access to licensed full texts from any location

4.2 Unique perspectives on holdings based on ZB MED research

- Use of ontologies to link literature to databases facilitates disciplinary and transdisciplinary searches in LIWWD (Müller et al. 2017)
- ZB MED develops its own tools for the interoperability of ontologies, making it possible to link records, display graphical relationships, rank individual items by relevance, run complex searches of related terms, and link to the specialist thesaurus MeSH (medicine), AGROVOC (agriculture) and Drugbank (pharmaceutics)

4.3 Publishing on transdisciplinary topics

- Publication of disciplinary and interdisciplinary journals, handbooks, congress abstracts, research data and grey literature
- Gold and green open access publishing
- Advice on open access and research data management

5. Summary

ZB MED – Information Centre for Life Sciences offers a range of services that support research strategies for questions in the field of One Health and in the fields of medicine and nutritional, environmental and agricultural science, catering to both disciplinary and interdisciplinary perspectives.

References


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EPFA - Software to enhancing field application entomopathogenic fungi against insects

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Introduction - EPFA general concept
Entomopathogenic fungi (EPF) are naturally occurring insect pathogens that have the potential to be used for pest control. Different species isolates of EPF only kill specific species of insect, making the use of these organisms’ eco-friendly and sustainable pest management options for improving crop production. EPFA is a decision Support System (DSS) for modeling and mapping the virulence of fungal-based biopesticides when applied against a specific insect pest. The software uses time-dose-mortality data to establish temperature dependent models for the virulence of a fungal-based biopesticide against a selected agricultural pest; and by step by step, the virulence models are linked to regional and continental climate data for spatial mapping of the potential zones of efficacy of the biopesticide in pest suppression.

Objectives
• To establish temperature dependent models for the virulence of a fungal-based biopesticide against a selected agricultural pest using time-dose-mortality data
• To link the virulence models to regional and continental climate data for spatial mapping of the potential zones of efficacy of the biopesticide in pest suppression
• To estimate the optimum required distance between traps or number of auto dissemination device to be placed per unit of space (ha, km²) in an agro-ecosystem to control the insect pests

Materials and methods

Results and Discussion
EPFA is a module based DSS for mapping zones of efficacies of fungal-based bio-pesticides by predicting the zones of their potential virulence. This tool could be of great help for scientists, pathologists and IPM practitioners in their attempt to increase the use and application of EPF in a more efficient way in agriculture IPM context.

Cameroon map of potential efficacy of ICIPE 62 isolate when used against Mustard aphid modelled with the EPFA software. The level of efficacy varies between 0 and 1. Locations with zero percent (0%) probability of efficacy are displayed in white values between 0 and 0.5 in blue, values between 0.5 and 0.75 in green and values between 0.75 and 1. In red indicating the highest efficacy levels.
Distinguishing between MRSA (Methicillin-Resistant Staphylococcus Aureus) and MSSA (Methicillin-Sensitive Staphylococcus Aureus) with MALDI-TOF MS

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Introduction
MRSA infections in the hospital or other associated environments often lead to serious symptoms or in extreme cases death. These microbes can also be isolated from community; livestock associated sources and food products.

Aim
The aim is to be able to identify these multi-resistant microbes faster, cheaper and more reliably in a routine diagnostic laboratory - to develop a method that accomplishes this goal by using the MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry) technique.

Materials and Methods
The MALDI-TOF MS technique is based on coupling a laser ion source and a time-of-flight mass spectrometer. The energy of the laser emitted is absorbed by the matrix, which in turn ionises the target compounds of the sample, and thus they can enter the vacuum of the mass spectrometer and finally reach the detector. The obtained mass spectra provide information on the protein and macromolecule profiles of the sample. This fingerprint pattern serves as a base for the routine identification of the microbes, compared to a validated database. In addition, this fingerprint pattern can be potentially use to differentiate MRSA and MSSA (methicillin sensitive Staphylococcus aureus) isolates.

First step: Determination of resistance with cefoxitine disc (Fig. 1.)
Applied culture media:
- Blood
- Mueller-Hinton
- Baird-Parker
- 24 h, 37°C

Second step: Sample preparation
- Direct mounting
- Modified direct mounting
- Ethanol sample preparation
- Direct mounting with FA

MALDI-matrixes:
- α-HCCA (α-Cyano-4-hydroxy cinnamic acid)
- SA (sinapinic acid)

Third step: 1μl sample on the target (Fig. 2.)
Automatic run method with MALDI-TOF MS (Fig. 3.)

Results
The method development includes an assessment of the effects of the culture media, sample preparation (direct mounting, ethanol/formic acid), and the use of different MALDI matrixes (HCCA, SA) on the obtained mass spectra. The strains used are characterised biochemically and genetically by 16S rDNA sequence and MLST (multi locus sequence typing) techniques. Based on the results of the MLST we worked with 6 different sequence types. One MRSA and one MSSA strain are of the same sequence type. The optimal sampling methods were the modified direct mounting with SA-matrix, on Blood media. In the experiment we found 9 peaks which are suitable for the separation of MRSA and MSSA. Of these 9 signal peaks, the 2414 m/z ion peak was the only one present in all MRSA strains.

Conclusions
The MALDI-TOF MS technique can be suitable for distinguishing between certain MRSA and MSSA isolates. Among the sample preparation methods, modified direct mounting was found to be the most suitable, because this method combined with SA-matrix presents biomarkers for separation. We found 9 signal peaks based on which MRSA and MSSA isolates could be separated.

Acknowledgements
The work/publication is supported by the EFOP 3.6.1-16-2016-00022 project. The project is co-financed by the European Union and the European Social Fund.
APPROPRIATE MEASURES FOR OUTPATIENT ANTIBIOTIC USE IN EUROPE

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5. Department of Primary and Interdisciplinary Care (ELIZA), University of Antwerp, Antwerp, Belgium

Introduction

Antibiotics: drugs used to treat bacterial infections
- Used too often
- Used incorrectly

Antimicrobial resistance: ability of bacteria to survive antibiotic treatment
⇒ Part of solution:
  - gather trustworthy information

Methods

Modelling antibiotic use:
- Repeated measures
- Seasonal variations
  ⇒ Non-linear mixed model
- Construction of final model:
  ⇒ Drop non-significant random effects
  ⇒ Drop non-significant fixed effects

Linking use – resistance:
- PNSP: penicillin and cephalosporin use
- ENSP: macrolide and tetracycline use
- Time-lag of 0, 1 or 2 years:
  ⇒ Generalized linear mixed model
- Construction of final model:
  ⇒ Drop non-significant DID and/or PID

Results

Modelling antibiotic use:

Table 1. Parameter estimates for the final model.

<table>
<thead>
<tr>
<th></th>
<th>time</th>
<th>amplitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>DID</td>
<td>0.1037*</td>
<td>0.0187*</td>
</tr>
<tr>
<td>PID</td>
<td>-0.0061</td>
<td>-0.0033</td>
</tr>
</tbody>
</table>

⇒ Significant increase in DID
⇒ no significant change in PID

Data

ESAC project (2000-2007):
- Number of defined daily doses per 1000 inhabitants per day (DID)
- Number of packages per 1000 inhabitants per day (PID)

EARSS project (2000-2009):
- *Streptococcus pneumoniae*
  ⇒ Penicillin non-susceptibility (PNSP)
  ⇒ Erythromycin non-susceptibility (ENSP)

Connecting use – resistance:

PNSP:
⇒ No time-lag
⇒ PID only

ENSP:
⇒ One year time-lag
⇒ PID and DID

Table 2. Odds ratio estimates for final models.

<table>
<thead>
<tr>
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<th>DID</th>
<th>ENSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNSP</td>
<td>-</td>
<td>0.779*</td>
</tr>
<tr>
<td>PID</td>
<td>1.959*</td>
<td>3.683*</td>
</tr>
<tr>
<td>Time</td>
<td>0.997</td>
<td>1.005</td>
</tr>
</tbody>
</table>

⇒ Odds to be non-susceptible:
  - increases with PID
  - decreases with DID

Conclusion: valid surveillance needs information on DID and PID!
Open access publishing is a good way for the scientist to disseminate their research results all over the world. The publications can be updated within a short period of time and with a minimum of effort.
Introduction:
Antibacterial resistance is recognized as an important public health problem. Antimicrobial resistance is associated with increased length of hospital stay, and morbidity, whereas carrier state (Watts, et al., 2017) and prevalence on hands among subjects exposed to hotspot has not been completely elucidated despite the importance of the hands as a vector for the transmission of pathogens; (Centers for Disease Control and Prevention, 2017,Edmonds-Wilson, et. al, 2015), therefore, the aim of this research was to determine isolation incidence of ESKEAPE members in handwashing samples of subjects with known exposure, and to assess risk factors for ESKEAPE isolation.

A case-control study was conducted among farmers, healthcare personnel, and subjects without environmental exposure to antibacterial resistance (IRB; FM/DI/046/2017). We quantified the number of CFU / mL of hand wash samples in chromogenic, selective media (Chromoagar® (ORIENTATION, KPC, ESBL, VRE and mEA) to assess for resistance against vancomycin, β-lactams, carbapenems. The results were analyzed using the SPSS Software (Version 21.0, August 2012) to evaluate significance differences between groups by U-Mann-Whitney. Finally, logistic regression model was explored to characterize association of exposure pathways, of specific resistant microorganisms.

Material and methods:
Eight groups of presumptive microorganisms had significant differences with respect to the study area. Here, we show the influence of "hotspots" that represented the resistant microorganisms. Denoting that all of them belong to the ESKEAPE group, so it would indicate that the exhibition area has an influence with the presence of them.

The hospital group showed the highest concentration of Pseudomonas resistant to KPC and ESBL when compared to group who is exposed to well water or residual water. Although, the hospital group has a lower concentration of Enterococcus, KEC compared to residual water group these microorganisms are used as indicators of contamination. The administrative group has a lower concentration of resistant microorganisms with statical significantly with respect to the others groups (Pseudomonas resistant to ESBL and KPC).

Results & Discussion

Graph: Concentrations of the different type of microorganism for each worker in different media, each group is compared with the others and where there is a significant difference, a square of the color corresponding to the comparison is placed.

Reference


**Requirements of the GlobalG.A.P. Integrated Farm Assurance Standard on the Responsible Use of Antimicrobials and the Implementation of Antimicrobial Reduction Policies at Farm Level for Livestock and Aquaculture Production.**

Dr. Roland Aumüller, Veterinary Surgeon, Standards Management Livestock and Feed  
Elmé Coetzer, PhD, Vice President, Chief Standards Officer  
GLOBALG.A.P./FoodPlus GmbH, Spichernstr. 55, 50672 Köln, Germany

**The Integrated Farm Assurance Standard (IFA) V 5.1**
A Modular Approach to G.A.P. Certification

G.A.P. stands for Good Agricultural Practices and GLOBALG.A.P. is the worldwide standard that assures it.

The principles for the use of antimicrobials according to the GLOBALG.A.P. livestock and aquaculture standards.
1. As little as possible, as much as needed.
2. Adherence to the principles of antibiotic stewardship.
3. Commitment to the principles of WHO as integrated in the “One Health” program.

Common Requirements of Livestock and Aquaculture Standards for Antimicrobial Reduction Policies as outlined in the specific Control Points and Compliance Criteria.
1. Records on purchase and application of antimicrobials.
2. Exclusive use of officially approved antimicrobials.
3. No use of antimicrobials for prophylactic purposes.
4. Compilation of a written veterinary health plan (VHP) outlining policies for the reduction of antimicrobials and the avoidance of critically important antibiotics for human health.

Specific Requirements of the Livestock Standard:
1. Workers health & safety - creating the awareness amongst workers in intensive livestock production systems of the higher risk and possibility of being a carrier of MRSA and/or ESBL bacteria.

Specific Requirements of the Aquaculture Standard:
1. Exclusion of banned compounds under FAO/WHO Codex Alimentarius (e.g. Chloramphenicoles, Nitroimidazoles).
2. Prohibition of antimicrobials for the purpose of growth promotion.

Conclusions: The GLOBALG.A.P. Integrated Farm Assurance Standard demands from livestock and aquaculture producers to implement clear policies and proper documentation on the responsible use of antimicrobials in order to achieve compliance with the requirements of the standard and to become a GLOBALG.A.P. certified producer. It is committed and contributes to the overall policy of the One Health and Food Safety policy.

Reference documents: GLOBALG.A.P. Integrated Farm Assurance Standard Version 5.1, Livestock Standard V 5.1, Aquaculture Standard V 5.1  
www.globalgap.org/documents
Simulation of biofilm development in dairy cattle troughs for the development of strategies to prevent the spread of infectious bacteria in the environment

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Introduction

Water is a very important factor concerning animal welfare and the spread of pathogenic bacteria. However, legal regulations and official guidelines regarding the quality of water for animals are rather unspecific. The role of biofilms as a source for infections and their dispersal into the environment is particularly understudied.

![Figure 1: Different cattle troughs with biofilms](image1.png)

Aim

The aim of this study was to simulate a cattle trough and determine if and how a biofilm develops and detaches from the surface, depending on different factors such as trough material, temperature or nutrition availability.

Materials & Methods

- A naturally-occurring bacterial culture from a cattle trough (Figure 1) was cultivated and different cattle trough materials were inoculated.
- It was investigated whether a development and a detachment of a biofilm occurred depending on:
  - the material (polyethylene or stainless steel)
  - the inoculation temperature (5°C or 25°C)
  - the availability of nutrient medium (low or high level) in a period of seven days.
- Assumption: detachment of biofilm causes rise of the bacterial count in the surrounding medium.
- The medium was tested on total bacterial count (TBC) agar and renewed on daily basis to identify the detachment of the biofilm.
- The developed biofilm was investigated for TBC, Enterobacteriaceae spp., Methicillin-resistant Staphylococcus aureus (MRSA), Extended-Spectrum Beta-Lactamasen (ESBL) and Adenosintriphosphat (ATP) on the seventh day.

Results

- After four days of incubation, a mature biofilm developed on polyethylene at a temperature of 5°C.
- The detachment was verifiable through a significant increase of over 1,4 log₁₀ (cfu/ml) in the surrounding medium.
- Material and temperature influenced detachment (P<0.05) (Figure 2).

![Figure 2: Development of the total bacterial count (TBC) in the surrounding medium depending on material and temperature.](image2.png)

- The bacterial growth on the surface depends on the surface material (polyethylene > stainless steel) (P<0.001), the temperature (25°C > 5°C) (P<0.001), as well as on the nutrient level (higher > lower) (P<0.05) (Figure 3).

![Figure 3: Total bacterial count (TBC) and Enterobacteriaceae spp. on the surfaces after seven days.](image3.png)

- ESBL-forming bacteria were verifiable in some biofilms and seem to be connected to the biofilm’s maturation.
- The ATP-test was highly correlated to the bacterial growth on the surfaces (r=0.87, P<0.001).

Conclusion

- The results of this study show that a mature biofilm can develop in a cattle trough over a period of four days even at low temperatures (5°C).
- Therefore it is necessary to clean cattle troughs in an interval shorter than four days to prevent the spread of pathogenic bacteria through biofilm detachment.
- More research is needed to determine other influences on the development and detachment of biofilms in cattle troughs.

One Health & Food Safety Congress 2018
#Poster 155#

Acknowledgements: The authors thank the participating farms and the funding by the core budget of Prof. Petersen
Monitoring of the cleanliness in livestock stables and training of the staff can interrupt infection chains of pathogenic and the spread of antibiotic resistant bacteria

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Background

To interrupt infection chains in all-in-all-out pig production systems and the spread of pathogenic bacteria along the food chain, a proper cleaning and disinfection system must be established in the farm management. In a former study we compared five different methods to assess the cleanliness and identified critical control points for hygienic conditions on pig farms (Heinemann et al., 2017).

Aim

The aim of this study was to sensitize and train the farmers and the staff for critical control points and evaluate the effect of training.

Material & Methods

Half a year after the training of the staff, farms were visited again for sample collection. Swab samples were taken in randomly chosen pens of six different fattening farms. Additionally sock samples and water samples were taken. Samples were analyzed for protein and ATP content as well as for the parameters total viable count (TVC), total coliforms (TC), E. coli, methicillin-resistant Staphylococcus aureus (MRSA) and extended-spectrum beta-lactamase builder (ESBL). Additionally, visual cleanliness (VC) of the sampled areas was recorded using a 4-score grading scheme. Data were analyzed with a mixed model in SAS with farm and time as fixed factors.

Results

Figure 2: A significantly reduction of TVC was found between initial sampling (blue) and sampling after training (green) (P<0.001). Other microbiological parameters, ATP and protein content gave similar results.

Figure 3: Beside high values of swab samples from drinkers (6.63±1.03 log₁₀ cfu/ml; n=24) water samples showed a high bacterial load (5.21±0.84 log₁₀ cfu/ml; n=20), which can be seen as dirt accumulation in a cross-section of a drinker (A) and after sampling with a swab (B). Bacterial load exceeds the BMEL recommended value for animal drinking water of 4.0 log₁₀ cfu/ml.

Figure 4: Training successfully improved the hygiene management as exemplary shown by a decrease of positive findings for MRSA and ESBL.

Table 1: A training effect depends on the willingness of the farmer to change his hygienic management. There seems to be a lower limit for reducing the microbiological condition in pig stables with slatted floors.

Conclusion

Monitoring of the cleanliness in livestock stables and farm-specific training could successfully prevent or limit the spread of infectious diseases and antibiotic resistant bacteria and should be immediately integrated in consulting service. Attention should be taken for hygienic condition of nipple drinkers to prevent an easy spread of diseases by animal drinking water.

References

Evaluation of measures to reduce β-lactam residues and bacterial load in waste milk for feeding

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Introduction

- Pathogens and drug residues in waste milk can be transmitted by feeding, which can cause health problems and lead to the spread of antibiotic resistances.
- Practicable measures to reduce pathogens and antibiotic residues in waste milk for feeding are needed but not available yet.

Aim

The aim of this study was to evaluate different measures to reduce the β-lactam concentration and bacterial load in waste milk.

Materials & Methods

**Sampling:** Waste milk samples were collected from 12 mastitic lactating dairy cows from two different farms that were treated with benzylpenicillin or cefquinom, respectively. The samples were directly subjected to eight different measures (Figure 1).

**Treatments:** Stirring similar amounts of waste milk with adsorbent materials (activated carbon, carbon supplement, plastic materials) for 1h, thermal treatment (microwave, autoclave and freezing), change of the pH with sodium hydroxide to pH 10 for 1h and back to pH 6,7 with peracetic acid.

**Analysis:** Active β-lactam residues were analyzed using the Devoltest® (BR Brilliant, DSM Food Specialties B.V., Delft, Netherlands; Figure 1) as well as the total aerobic bacterial count (TBC).

**Statistics:** Data was analyzed with a mixed model with farm, active component and different measures as fixed factors.

Results

- Thermal treatment with the microwave significantly reduced the bacterial load but not the β-lactam activity. A reduction of bacterial load was obtained by sterilization (121°C, 15 min). The other treatments had no significant effect on bacterial load (Figure 2).

![Figure 2: Total bacterial count (TBC) of waste milk in regard to eight different treatments.](image)

- A high degradation of benzylpenicillin (62.8%) and cefquinom (70.8%) was achieved by sterilization. The change of the pH to pH 10 for 1h tended to reduce the β-lactam activity (Figure 3).
- Antibiotic residues in waste milk seemed to be more affected by the form of application and the dose given than by the farm than the active component that was used.

![Figure 3: Active β-lactam components of waste milk after eight different treatments.](image)

Conclusion

- Autoclaving waste milk significantly reduces β-lactam residues as well as the bacterial load.
- PH changes may also have a potential effect.
- Whether or not these (or similar) methods can be used on-farm to process larger amounts of waste milk needs further exploration.
- Further studies are also needed to quantify reduction with improved measures and methods and evaluate nutritional quality.

Acknowledgement: The authors thank the two participating farms and the funding by the core budget of Prof. Petersen.
Strength and weakness of the „therapeutic frequency“ to minimize antibiotic consumption in German farm animal production

Institute of Animal Science, University of Bonn, Germany

Background
With the renewal of the German Drug Act (16th AMG-Novelle) in 2014, antibiotic consumption in farm animal production is monitored in order to reduce its usage. Exceedance of the farm-specific biannual therapeutic frequency beyond the 75% quantile obligates livestock owners to submit a written plan of measures to the public veterinary authority.

Aim
Scientific evaluation of the plans of measures

Material & Methods
• Analysis of the course of therapeutic frequency for swine from 2014 to 2017.
• Identification of strength and weaknesses of the monitoring by media analysis, surveys and expert interviews (Figure 1).

Results

Table 1: Although the invitations to the two online surveys were actively spread, return rates and completion rates were too low to draw any conclusions.

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<tr>
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<th>Livestock owners</th>
<th>Veterinary officials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invited</td>
<td>210</td>
<td>100</td>
</tr>
<tr>
<td>Return rate</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Completion rate</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

- Willingness for participation disappeared due to biased discussion and negative news coverage.
- Reasons for rejection of plans of measures: inconsistency, incompleteness or imprecision.

Figure 3: Potential measures identified by the media analysis.

Figure 2: Median and 75% quantile of the therapeutic frequency of piglets (left) and fattening pigs (right) since the beginning of the monitoring [1].

- Experts agreed that the course of the farm-specific therapeutic frequency over time allows to distinguish between improvable animal health management and spontaneous appearance of infectious diseases.

Conclusion
- A more transparent uniform procedure with an automated data processing would help to increase acceptance, reduce workload and uncover pattern as piglet supplier with impaired health status.
- Number of for health reasons scarified animals and treated dairy cows should be included in the antibiotic monitoring to complete the health information with an One Health approach.

Quellen

One Health & Food Safety Congress 2018, Bonn, Germany
Abstract #159
Effects of waste milk intake on large intestinal microbiome of newborn farm animals

E. Heuschen¹, J. Thiele¹, B.G. Schulze Dieckhoff¹, C. Heinemann¹, S. Stewart¹,² and J. Steinhoff-Wagner¹

¹ Institute of Animal Science, University of Bonn, Germany; ² Department of Animal Science, Oklahoma State University, USA

Introduction

• After treatment of mastitis, milk contains antibiotic residues and must be withdrawn from the market. Because it is available at no charge, this milk is predominantly fed to calves even though a subtherapeutic dosage might promote the development of resistant pathogens or provoke a shift in the intestinal microbial community.

• Especially in the first days after birth, the microbial community is known to be very unstable and effects of waste milk during feeding are shown in calves.

Research question

• Do the results obtained in preweaned calves represent the effects in other newborn monogastric mammals?

• Does the effect of waste milk on large intestinal microbiome persist during bulk milk feeding?

→ Conduction of this study with a small number of animals to verify methods, identify confounding variables and get values for a power analysis.

Material & Methods

Sampling:

• Spontaneously from three different commercial farms when mothers were treated.

• Feces and rectal swabs were taken.

Table 1: Pre-study design with piglets and calves who suckle waste milk (WM) or control milk (CON)

<table>
<thead>
<tr>
<th>Group</th>
<th>WM</th>
<th>CON</th>
<th>WM</th>
<th>CON</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at sampling, days</td>
<td>7</td>
<td>7</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Diet</td>
<td>suckling treated cow</td>
<td>suckling untreated cow</td>
<td>waste milk from day 5 to 10</td>
<td>bulk milk from day 11 to 14</td>
</tr>
<tr>
<td>Number of animals</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

Analysis:

• DNA was extracted from fecal samples and phylogenetic analysis of the 16S rRNA gene sequence was performed by a specialized commercial lab (Wisplinghoff, Cologne, Germany).

• Swab samples were plated on selective agar for MRSA and ESBL.

Table 2: Individual fecal flora: Firmicutes was the most abundant species (28-76%), followed by Proteobacteria (8-58%) and Bacteroidetes (0.2-44%).

Table 3: Heatmaps show that effects of waste milk feeding after 5 days bulk milk feeding to calves were rare. Few phylotypes only appeared after waste milk feeding: Bacteroides coprophilus, Butyricoccus pullicaeorum, Clostridiaeaeae and Eubacteriaceae.

Table 4: Heatmaps for fecal samples of piglets. Streptococcus were only found after suckling of WM.

Table 5: Resistances (positive finding/ total samples) in regard to waste milk feeding. Fecal samples did not contain MRSA. ESBL-producing bacteria were spread within calves which might be a result of close contact between animals of both groups, antibiotic residues in colostrum and/or reduced hygiene standards in cattle industry compared to pig production.

Conclusion

Future experiments are needed to address duration of feeding, active components and farm-specific origin.
Zinc and Copper attenuates transfer of antimicrobial resistance genes

Witso, Ingun Lund; Buberg, May Linn; L’Abée-Lund, Trine; Wasteson, Yngvild
Department of Food Safety and Infection Biology, Faculty of Veterinary Medicine, NMBU

Introduction
Heavy metals present in the environment could induce antibiotic resistance, and Zn and Cu have been proposed as drives of antibiotic resistance (Becerra-Castro et al. 2015; Yazdankhah et al. 2014). *Escherichia coli* displaying resistance to extended-spectrum cephalosporins (ESC) was detected in the Norwegian broiler production (Mo et al. 2014; NORM/NORM-VET 2012). The conjugative IncK plasmid harbouring *bla*~CMY-2~ was found in the majority of the isolates (Mo et al. 2016). There is a need for more research on how non-antimicrobial substances such as Zn and Cu contribute in the spread of AMR- genes. Thus, the aim of this study was to investigate the effect of Zn and Cu on conjugation of a *bla*~CMY-2~ carrying plasmid.

Material and Methods
An in vitro conjugation assay was conducted to study the effect of Zn and Cu on conjugation of *bla*~CMY-2~ carrying plasmid. Quantitative real time PCR was used to study the effect of Zn and Cu on expression of genes involved in conjugation.

Table 2: Bacterial strains used in this study

<table>
<thead>
<tr>
<th>Donor</th>
<th>Resistance</th>
<th>MIC Zn (mg/mL)</th>
<th>MIC Cu (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli 1292 IncK</td>
<td>Cefotaxime (Ctx)</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>E. coli DH5α</td>
<td>Nalidixic acid (Nal)</td>
<td>0.3</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Results

**Figure 1:** Conjugation of plasmid harbouring *bla*~CMY-2~ gene were significantly inhibited by Zn and Cu.

**Figure 2:** Expression of *nikB* had a significant reduction in response to Zn and Cu. The expression of *traB* had a 10-fold reduction in response to Zn and Cu.

Conclusion
Our results show that Zn and Cu significantly inhibit conjugation of *bla*~CMY-2~ carrying plasmid presumably by interfering expression of conjugal genes.

References


Wastewater is a reservoir for clinically relevant carbapenemase and 16S rRNA methylase producing Enterobacteriaceae.

Magdalena Nüesch-Inderbinen1, Katrin Zurfluh1, Claudia Bagutti2, Peter Brodmann2, Monica Alt2, Jürg Schulze2, Séamus Fanning3, Roger Stephan1

1 Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Switzerland
2 State Laboratory of Basel-Stadt, Kannenfeldstrasse 2, 4056 Basel, Switzerland
3 UCD-Centre for Food Safety, School of Public Health, Physiotherapy & Sports Science, University College Dublin, Belfield, Dublin 4, Ireland

Background

- Carbapenemase producing (CP) Enterobacteriaceae (CPE) are a major concern for public health worldwide.
- High-level resistance to aminoglycosides due to the production of plasmid-encoded 16S rRNA methylase is emerging in Enterobacteriaceae and may be associated with the production of carbapenemases.
- One of the most pressing challenges is to determine reservoirs and transmission pathways of CPE and other MDR bacteria in order to reduce the risk to public health.
- The aim of this study was to evaluate the presence of CPE and 16S rRNA-methylase producing (MP) bacteria (MPB) in wastewater (WW).

Methods

- Sampling of each site on a total of seven occasions.
- Harvesting of bacteria from 50 ml WW by 0.45μm membrane filtration and incubation of filters in EE broth for enrichment.
- Selection of MPB on LB agar containing amikacin (200 mg/L), vancomycin (10 mg/L) and amphotericin B (5 mg/L).
- Species identification of isolates using MALDI-TOF–MS or rpoB sequencing.
- Phylogenetic and multilocus sequence typing of E. coli or K. pneumoniae.
- Screening by PCR for blaKPC, blaNDM, blaVIM, blaXM-69, blaTEM, blaSHV, blaCTX-M, armA, rmtA, rmtB, rmtC and rmtD.
- Antimicrobial susceptibility testing by disk diffusion or Etest.

Results

- A total of 49 isolates were retrieved on seven sampling occasions.
- CPE were recovered from all WW types.
- MPB were recovered from HWW, TPI and TPE.
- HWW contained a higher number of CPE isolates compared to MWW.
- HWW contained OXA-48 producing Citrobacter spp. over a period of several months.
- NDM-5 and OXA-48 producers were found in the community and in the aquatic environment.
- Isolates persisted within WW and survived the WW treatment process.
- WW was identified as a reservoir for viable CPE and MP clones harbouring clinically relevant carbapenemases.

Conclusions

- Sewage disposal networks contain carbapenemase and aminoglycoside resistant Enterobacteriaceae.
- WW is a reservoir of viable CPE and MP clones harbouring clinically relevant carbapenemases.
- There is a difference between municipal and hospital wastewater.
- Hospital wastewater contains markedly more resistant pathogens.
- Resistant clones are transmitted via wastewater treatment plants to the environment.
- Environmental pollution with OXA-48 producing bacteria is an emerging problem.
- MWW analysis may represent a surveillance and screening tool for antimicrobial resistant Enterobacteriaceae in the population.

Table 1: Carbapenemase and 16S rRNA methylase producing Enterobacteriaceae within the sewage network.

<table>
<thead>
<tr>
<th>Group</th>
<th>Species</th>
<th>Isolates [n]</th>
<th>Susceptibility (%)</th>
<th>ST (Sheng et al.)</th>
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<tr>
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<tr>
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<td>Enterobacter aerogenes</td>
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<td></td>
<td>G285</td>
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</tbody>
</table>

Related literature

Detection of pathogens and microbiological toxins in water, food and feed using immunomagnetic nanoprobes

Christian Neuendorf¹, Julia Heesel¹, Greta Nölke¹, Stefan Achtsnicht², Hans-Joachim Krause² and Florian Schröper¹

¹ Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Forckenbeckstr. 6, Aachen, Germany
² Institute of Complex Systems (ICS-B), Forschungszentrum Jülich GmbH, Jülich, Germany
contact: florian.schroeper@ime.fraunhofer.de

Introduction

In Western countries quality standards for drinking water, food and feed are in general very strict, mainly ensured due to routinely based controls. However microbiological contaminations cannot be fully excluded. Regularly food products are affected by recalls due to contaminations with bacteria, fungi or microbial toxins leading to considerable economic losses and health risks. Established routine analyses are based on cultivation techniques in specially equipped analytical labs. Although very sensitive, they are time consuming. Cultivation of Legionella, e.g., requires 10 days of incubation until reliable results are gained. A disadvantage is that no information is obtained regarding toxic substances. Here we present a novel approach based on magnetic nanoprobes for fast on-site analysis of water, food and feed. Microbial contaminations as well as certain toxins can be identified in less than one hour.

Material and Methods

Magnetic immunodetection using nanoprobes

- Immunofiltration column is coated with pathogen/toxin specific antibodies
- Sample is applied to column and passes by gravity flow
- Antigen is enriched within matrix
- Target specific biotinylated antibodies are applied to column
- Streptavidin coated magnetic nanoprobes are flushed through column by gravity flow
- Magnetic beads accumulate within matrix, dependent on pre-enriched antigen
- Amount of magnetic nanoprobes determined with handheld readout device

Detection of magnetic nanoprobes

- Frequency Mixing Magnetic Detection (FMMD)
- Detection head with driving coil (low frequency) and excitation coil (high frequency)
- Set of detection coils for measuring nonlinear magnetic response of magnetic beads

Separative approach

- Nanoprobe functionalized with target specific antibodies are applied to analyze sample
- Nanoprobe capture target pathogens/toxins
- Magnetic separation and subsequent resuspension in PBS allows enrichment of target from impure samples and large volumes
- Application of separated nanoprobe to detection column
- Readout with magnetic reader and detection of contaminations

Results

1. Magnetic immunodetection of cholera toxin

Drinking water contaminations commonly occur in cases of major catastrophic events such as flooding or earthquakes.

- Spreading of epidemics such as cholera are a major threat
- Magnetic immunodetection allows highly sensitive detection of cholera toxin in water samples in less than one hour
- Samples are passed through immunofiltration columns and bound cholera toxin subunit B (CTB) is detected via applied nanoprobes
- Detection limit of 89 pg/mL

2. Separative detection of Legionella pneumophila

Even in countries like Germany, local microbial contaminations in drinking water are frequently observed. A big issue are increased levels of Legionella in local piping systems.

- Lab-based analysis requires 10 days for incubation of cultures
- Magnetic immunodetection allows detection of harmful concentrations in less than one hour
- Exemplary separative magnetic immunodetection (SMID) from 3 mL samples allows detection of 5.2x10⁸ CFU/mL
- Separative approach enables to enrich bacteria from larger sample volumes
- High potential for sensitivity increase due to enrichment from large sample volumes

3. Nanoprobe-based mold detection in food

Microbial contaminations in food can be a significant health risk. Fungal infestation of fruits and vegetables is a frequent problem, especially due to released mycotoxins. During food manufacturing processes early stages of infestation cannot be detected.

- SMID enables detection of early stage fungal infestation in fruits
- Separative approach enables easy extraction of antigen (fungal cell wall protein) from homogenized fruits
- Strawberries were inoculated with Aspergillus parasiticus spores and incubated at 28°C for 3 days
- Antibody functionalized nanoprobes were added to homogenized fruits
- Magnetic separation and subsequent detection in immunofiltration columns
- Signal increase due to fungal growth detectable after 17 h while mold becomes visible not until 48 h

Conclusion

Magnetic immunodetection enables fast and reliable on-site identification of microbial contaminations in water, food and feed.

- High sensitive detection in less than one hour
- SMID enables extraction and enrichment of targets from large and impure samples
- Enables detection of microbial contamination at an early stage
- Detection approach easily adaptable to further pathogens and toxins
Due to the widespread use of antibiotics in human and veterinary medicine, resistances have spread in the environment. However, knowledge with respect to antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in water used for drinking water production is limited.

METHODS

Culture methods
Selective CHROM agar plates were used to prove the presence of:
- Vancomycin resistant enterococci (VRE)
- Methicillin resistant Staphylococcus aureus (MRSA)
- Extended spectrum beta-lactamase (ESBL)-producing coliform bacteria

Molecular biological analysis
DNA extractions were performed from concentrated water samples using the FastDNA® SPIN Kit for Soil following the manufacturer's instructions. Samples were quantitatively analyzed for selected ARGs and bacterial 16S rDNA sequences using real-time PCR.

RESULTS

Clinically relevant antibiotic resistant bacteria
- The use of culture methods developed for clinical purpose for the analysis of environmental samples proved to be difficult due to insufficient specificity. Optimization of culture conditions and implementation of further verification tests like MALDI-TOF-MS are required.
- Analysis demonstrated the presence of VRE and ESBL bacteria in surface water.
- Soil passage led to a significant reduction in CFU/100 mL.

Antibiotic resistance genes
- Screening of 93 ESBL E. coli isolates showed the presence of different β-lactamase genes:
  - Screening of 93 ESBL E. coli isolates showed the presence of different β-lactamase genes:

<table>
<thead>
<tr>
<th>Category</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Often detected</td>
<td>Sulfonamid resistance gene sul1</td>
</tr>
<tr>
<td></td>
<td>Macrolide resistance gene ermB</td>
</tr>
<tr>
<td></td>
<td>β-lactamase gene blaTEM</td>
</tr>
<tr>
<td>Intermedian detected</td>
<td>β-lactamase gene blaCTX-M-32</td>
</tr>
<tr>
<td>Rarely detected</td>
<td>β-lactamase gene blaIMP-1</td>
</tr>
<tr>
<td></td>
<td>Methicillin resistance gene mecA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Detection frequency of different ARGs varies:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Often detected</td>
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<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Intermedian detected</td>
</tr>
<tr>
<td>Rarely detected</td>
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</tbody>
</table>

- PCR analysis of raw water showed that ARGs are more often detected in surface water as compared to groundwater.
- ARGs could be detected frequently - even after soil passage.

CONCLUSIONS

The results will help to identify and to avoid the risk of spreading multi resistant bacteria via the water pathway. Elimination of ARGs in waterworks depends on the type and conditions of treatment technology. According to the UN "sustainable development goals", mitigation of resistances spreading and improvement of raw water quality are encouraged.
Antibiotic resistant bacteria are a burden for the healthcare system and the current antibiotic resistance crisis demands not only for novel antimicrobial compounds but also for countermeasures that eliminate the dissemination of resistant bacteria and the spread of resistance genes. Wastewater as a source of antibiotic resistant bacteria has been discussed repeatedly and it is crucial to monitor the potential sources of antibiotic resistant bacteria and to observe the release into the environment. The aim of this study is to investigate the dissemination paths of antibiotic resistant bacteria in non-treated and treated wastewater and the surface water from a clinical/urban and from a rural catchment system. The study followed the dissemination of resistant bacteria from the wastewater through the wastewater treatment plant into the receiving surface waters of both systems. The bacteria were cultivated on selective agars (ESBL, MRSA, VRE) and characterized by antibiotic testing, real-time PCR targeting carbapenemase genes and typing. Multi-drug resistant bacteria producing extended spectrum beta-lactamas (ESBL) were isolated from all sample sites. Extensively drug-resistant strains (XDR) with susceptibility to only one or two antibiotics (colistin or tigecycline or amikacin) were isolated mainly from the clinical/urban system. These strains were carbapenemase producers. Molecular typing revealed a prevalence of human and healthcare associated drug-resistant isolates in the clinical/urban system, whereas in the rural system livestock associated lineages were commonly found. Several high-risk clones, i.e. multi-resistant clones with world-wide dissemination, of Pseudomonas aeruginosa (ST23S) and Klebsiella pneumoniae (ST11 and ST147) were identified in the clinical waste water samples and were also detected in the effluents of the waste water treatment plant. In conclusion, clinical wastewaters were charged with a high load of multidrug resistant bacteria. Although most of these bacteria were eliminated during wastewater treatment, dissemination into surface waters is possible.

Drug resistance in isolated bacteria

Table 2: Resistant bacteria isolated from the clinical/urban and rural system. Numbers of isolates and percentages of total isolates are shown for bacteria that are resistant to cefotaxime and/or ceftazidime as well as MDR bacteria (classified as 3MRGN, 4MRGN), colistin resistant 4MRGN with positive carbapenemase real-time PCR (Carba+) and XDR bacteria.

<table>
<thead>
<tr>
<th></th>
<th>Clinical/urban</th>
<th>Rural</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotaxime/ceftazidime</td>
<td>473 (95.18%)</td>
<td>562 (95.94%)</td>
</tr>
</tbody>
</table>
| 3MRGN                          | 119 (29.05%)   | 98 (15.49%)  
| 4MRGN                          | 135 (30.42%)   | 4 (0.70%)    
| 4MRGN, colistin, Carba+        | 46 (9.68%)     | 1 (0.18%)    
| XDR                            | 26 (5.57%)     | 0 (0.00%)    
| Total no. of isolates          | 475            | 568          |

Conclusions

- Multi-drug resistant bacteria producing extended spectrum beta-lactamas (ESBL) were isolated from all sample sites.
- Extensively drug-resistant strains (XDR) were isolated mainly from the clinical/urban system. These strains were carbapenemase producers.
- Molecular typing revealed a prevalence of human and healthcare associated drug-resistant isolates in the clinical/urban system, whereas in the rural system livestock associated lineages were commonly found.
- In conclusion, clinical wastewaters were charged with a high load of multidrug resistant bacteria, which indicates an appreciable risk of dissemination of highly resistant bacteria into the aquatic environment.

This work was funded by the BMFB (Federal Ministry of Education and Research of Germany) funding measure “HyReKA” which is part of “Risk management of new pollutants and pathogens in the water cycle (RISKWa)” in the funding priority “Sustainable Water Management (NuWaM)” [grant number FKZ 02WIS1377] to CS, MP and GB.
In conclusion, we could show the existence of a sub-MIC selective window between MSC and MIC. The experiments indicate a selective effect for piperacillin at 1 mg/L, a concentration that was found in some wastewater samples in the HyReKA project by a cooperating group (pers. communication, A. Voigt & H. Färber). Although 1 mg/L is below the MIC of piperacillin, it may nevertheless favor the growth of the resistant strain.

**Minimal selective concentration**: The traditional setpoint is the minimum inhibitory concentration (MIC), defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism (Andrews, 2002). However, subinhibitory concentrations of an antibiotic that do not inhibit growth will often lower the growth rate of a susceptible strain because the cell has to manage stress responses in order to repair or prevent damage done by the antibiotic. On the other hand, the resistant strain often has to propagate large plasmids, which will also slow down growth. The MSC (minimal selective concentration) is defined as the concentration at which a resistant bacterium will start to outgrow a susceptible bacterium in competition experiments (Gullberg et al., 2011). At the MSC, the decrease in growth rate caused by the fitness cost of carrying a resistance plasmid is balanced by the decrease in growth rate exerted by subinhibitory concentrations of the antibiotic for the susceptible strain. Antibiotic concentrations between the MSC and the MIC should be avoided to prevent proliferation of resistant bacteria.

**The system**: We established a test system consisting of a natural resistance plasmid isolated from bacteria selected from clinical wastewater and fluorescently marked bacteria. The plasmid contains several resistance genes. The fluorescent bacteria harbor either GFP or mCherry as a fluorophore in their genomes. With exception of the different fluorophores, the competing bacteria are identical. By turns one of the fluorescent strains is transformed with the resistance plasmid and tested in competition experiments with the other strain.

**First results**: In a first approach we could show the minimal selective concentration for piperacillin is between 0,5 mg/L and 1 mg/L for the resistant plasmid p652 in *Acinetobacter baylyi* (Fig. 3).
Motivation

The potential risk of infection to the consumers from contaminated water calls for prompt pathogen detection and intervention to mitigate health risk. Need for a rapid, specific and sensitive detection of pathogens in water presents a key challenge in modern water quality monitoring.

A German-Australian collaboration focuses on the recent progress, future research needs to advance monitoring techniques and risk mitigation strategies.

**Microbial Source Tracking**

Source tracking markers enable the identification of fecal input origins. This allows selective and targeted actions in cause of surface and source water pollution supporting the reduction of health risks in water used for drinking, bathing or irrigation.

**Detection of Viruses**

Viruses - even in very low concentrations – pose a potential health risk. The success of qPCR detection depend on water enrichment and extraction methods. For an improved workflow, various methods were compared to give high recovery rates. Future developments will focus on mobile devices allowing rapid sampling in the field.

**Microarray Technology**

Beside the quantification of single genes, microarray technology allows the simultaneous detection of a large number of different targets. These analytical methods enable a deep view into bacterial communities including potential antibiotic resistance, virulence or degradation of contaminants.

**Scientific exchange**

The GAbi project is highly focused on sharing and joint gain of scientific knowledge, the improvement of methods, qPCR standards and the analysis of environmental samples. This opens also the possibility for both Australian and German students to take part in scientific exchanges.

**Antibiotic Resistances**

In the field of hygienic parameters antibiotic resistances gained increasing attention in the past few years. For these reasons, investigations concerning antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARG) in the aquatic environment are one of the main aims of the cooperation.

**ACKNOWLEDGEMENT**

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Antibiotic-resistant bacteria are an increasing problem and pose a threat to human health, in particular when released into the environment. Wastewater from slaughterhouses is considered a hotspot for antibiotic-resistant pathogens (ARPs) and represents a potential reservoir for their spread through the in-plant and communal sewage treatment systems. For this reason, it is important to control the effluents discharged into the aquatic environment and monitor them for ARPs.

The objective of this study is to investigate the occurrence of selected clinically relevant ARPs in wastewater from a pig slaughterhouse (incl. transport trucks). Furthermore, to examine their emergence after the three phases of the in-house and its communal wastewater treatment plants (WWTP) as well as in the receiving surface water. The data will be used to design strategies for preventing the development and spread of ARPs.

Materials & Methods

- Wastewater samples (n=18) were taken from transport trucks and along the production process inside a pig slaughterhouse in Germany, with Slaughterhouse S1 having its own biological and chemical-physical WWTPs with overall daily treatment capacity of 3100 m³. Their inflows and outflows (n=20) were determined by MALDI-TOF MS and resistance was confirmed by determining MICS using the VITEK® 2 microbial ID/AST testing system.

- The phylogenetic groups of ESBL- E. coli were determined by quadruplex PCR (1). MLST of resistant E. coli was conducted according to previously described MLST procedures (2). ESBL-producing E. coli were screened by PCR (3, 4, 5) for 8-lactamase-encoding genes belonging to the blaTEM, blaSHV, and blaCTX-M families and sequenced.

- Ciprofloxacin-resistant isolates were screened by PCR (6) for eight plasmid-mediated quinolone resistance genes qnrABCD5, qepA, qepX and aac(6’)-Ib-cr.

- The colistin-resistant strains were screened for the presence of mobile colistin resistance genes

- The most common phylogenetic groups of E. coli (n=72) were C (34.7%), B1 (29.2%) and E (23.6%), followed by F (4.2%) and A (1.4%). The abundance of extraintestinal pathogenic E. coli (B2) was low at 4.2% (n=372), whereas two such strains were isolated from the inflow of the communal WWTP. The MLST results of 11 3MDRO E. coli strains showed a diversity of STs. The most common STs were ST-641 (n=311) and ST-224 (n=211). The rest of the isolates were assigned to ST-617, ST-1431, ST-338, ST-3385, ST-542 and ST-744.

- The most common ß-lactamase genes of ESBL-producing E. coli (n=644) were CTX-M-1 (38.6%), CTX-M-9 (16.1%), CTX-M-24 (10.6%), SHV-12 (9.3%) and TEM-1 (4.2%). Of these, only one isolate of A. calcoaceticus-baumannii complex was positive for both mcr-1 and mcr-2.

- The overall rate of 3MDRO strains from the slaughterhouse was 15.8% (n=26/165); Klebsiella spp. – 36.0% (n=925), E. coli – 16.1% (n=14/87), A. calcoaceticus-baumannii complex (n=3/44) – 6.8%. Whereas the total rate of the 3MDRO strains from the communal WWTP was higher at 48.1%: Enterobacter spp. – 100% (n=2/2), K. oxytoca – 83.3% (n=10/12), K. pneumoniae – 58.3% (n=7/12), E. coli – 43.9% (n=18/41) and A. calcoaceticus-baumannii complex (n=1/11) – 9.1%.

- 6.7% of the overall strains were colistin-resistant (n=11/165), of these 1.8% were additionally 3MDRO (n=3/165). To such colistin-resistant species belonged A. calcoaceticus-baumannii complex (n=5/11), E. coli (n=4/11) and E. asburiae (n=2/11). Of these, only one isolate of A. calcoaceticus-baumannii complex was positive for both mcr-1 and mcr-2.

- All 3MDRO K. pneumoniae strains (n=10/10) were positive for PMQR. All of them carried qnrA and qnrS. Thirty-three of them in combination with qnrB and 60.0% with qnrS. Only two out of ten 3MDRO K. oxytoca strains were positive for qnrB and qnrS. 40% of ciprofloxacin-resistant E. coli isolates (n=230) carried PMQR genes. The most abundant were qnrB (n=7/12), followed by acne(6’)-Ib-cr (n=2/11), qnrS (n=2/11) and gnpQ (n=1/11). All c. freundii (n=2) and E. asburiae (n=2) were positive for qnrB and qnrS, respectively. Whereas none of the isolates from A. calcoaceticus-baumannii complex (n=3) harbored PMQR genes.

- The spa typing of MRSA strains (n=65) revealed eight known livestock-associated types: 1011 (50.8%), 9204 (32.2%), B1010 (6.2%), B12576 (3.1%) and 11793, 14224, 1899, 18926 (each 1.5%), whereas one isolate from the inflow of the communal WWTP was identified as a hospital-acquired MRSA clone 1003.

Conclusions

- The overall rate of 3MDRO strains from the communal WWTP at 48.1% was higher in comparison to those from the slaughterhouse (15.8%). 49.1% of them carried PMQR genes.

- A wide variety of bla genes was found among ESBL-producing isolates, including some clinically relevant ones. All strains were carbapenem-susceptible.

- The abundance of mcr genes among colistin-resistant strains was low at 9.1%.

- The occurrence of ExPEC was low at 4.2%.

- All isolated MRSA strains from the slaughterhouse wastewater were known livestock-associated types.

References