RESEARCH REPORT
PUBLIC HEALTH/WORKER SAFETY

Title: Critical appraisal of evidence that low-dose, long term (growth promotion) antimicrobial use augments public health risks from antimicrobial resistant organisms – **NPB #11-024**

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Industry Summary:

Antimicrobials are important tools for ensuring the health, welfare and productivity of pigs raised for food. Banning of antimicrobial growth promotant use in Denmark, and subsequently more widely in the EU, has prompted many entities to seek more restricted availability of antimicrobials for food animals in the USA. The primary target for restriction is the use of low dose, long term administration of antimicrobials for 'production purposes'. This project evaluated the scientific evidence that low dose, long term use of antimicrobials in pig production augments public health risks from antimicrobial resistance. The appraisal focused on evidence cited in 4 key documents calling for regulatory restriction of low dose antimicrobial use (FDA Guidance for Industry #209; Preservation of Antimicrobials for Medical Treatment Act; Pew Commission Report on Industrial Farm Animal Production report; Keep Antibiotics Working Group annotated bibliography). A total of 154 papers cited by these sources were categorized as 1) descriptive (n = 48); 2) analytical (n = 37); or 3) reviews (n = 69). Two evaluation tools (one for original studies, one for reviews) were developed for systematically evaluating individual papers. Only 12 of the cited papers presented primary research data relevant to the specific purpose of this study (comparing the impact of low dose antimicrobial use to other modes of antimicrobial use in food animals). Further efforts were made to identify relevant primary research data relevant to the question. It is concluded that the vast majority the papers cited by these sources contain negligible primary research evidence to support the contention that low dose antimicrobial use exerts more selection pressure for resistant bacteria than therapeutic uses. Currently available evidence is inadequate to provide any meaningful conclusion regarding the relative effects of different modes of antimicrobial use on the emergence of antimicrobial resistance in pathogens or commensals in commercial swine populations. The precautionary principal underlying regulatory changes in Europe remains the centerpiece of arguments for similar regulation in the USA. We propose the most prudent option would be elimination, based on the precautionary principle, of use of antimicrobials for production purposes in feed of finishing pigs, but preservation of all existing therapeutic, metaphylactic and prophylactic options in weaned pigs. However, given the likely implementation of proposed changes, industry needs to focus on the expected negative impact on pig health, particularly related to enteric disease in weaned pigs, and the likely constraint of manpower of food animal veterinarians that will be necessary to provide greater veterinary oversight of all antimicrobial use in food producing animals.

Keywords: Antimicrobial, resistance, growth promotion, public health

Scientific Abstract

The purpose of this project was to critically appraise evidence related to the relationship between antimicrobial use (AMU) in food animals and antimicrobial resistance (AMR) cited in 4 key documents that support greater regulatory restriction of low dose antimicrobial use in the USA (FDA Guidance for Industry #209[a]; Preservation of Antimicrobials for Medical Treatment Act; Pew Commission Report on Industrial Farm Animal Production report; Keep Antibiotics Working Group annotated bibliography). The specific goal was to 'critically appraise the literature on antimicrobial use in pork production to determine the strength of evidence that long term use of certain levels of specific antimicrobial compounds in feed contributes greater risk to public health than other food animal antimicrobial uses'. Among the 154 references cited by these sources that were deemed relevant to AMU and AMR in food animals, only 37 (24%) were original studies that included some analysis of primary data (termed 'analytical studies'). The remaining citations were either original studies that presented data on AMU or AMR but did not make comparisons among groups (termed 'descriptive studies'; N = 48; 31%), or review papers or reports that did not present original data and analysis (termed 'review studies'; n = 69; 45%). The studies were evaluated using appraisal tools (one for analytical and descriptive studies, one for reviews) developed to enable systematic evaluation of the individual papers.

Only 12 (8%) of the cited papers were analytical studies that included primary research data relevant to the specific purpose of this project (comparing the impact of low dose antimicrobial use to other modes of antimicrobial use in food animals). These papers were reviewed in depth to identify strengths and weaknesses of the papers, and assess the evidence base for the conclusions drawn. Only one analytical paper (0.6% of the 154 relevant cited papers) directly compared the effects of a low-dose and therapeutic dose of antimicrobials administered to food animals (poultry) on the prevalence of AMR. From the perspectives of causal inference or effect estimation, the 48 descriptive studies cited by the 4 key documents were collectively uninformative regarding the association of low-dose long term administration of antimicrobials to food animals with AMR. In both the analytical and descriptive studies which reported on AMU, both the measurement and recording of AMU were found to have shortcomings in many studies. For example, only 9 (6 analytical, 3 descriptive) studies provided comprehensive details of the drug, dose, route, and duration of administration of the antimicrobial(s) used.

Appraisal of review studies focused on 37 reviews that specifically referred to AMU or AMR in their summary or stated objectives. None of the 37 reviews employed systematic review methods, but were narrative reviews (29) or reports (8). Only one review included the search methods used to identify cited sources, and only one (different) review specified inclusion and exclusion criteria for the studies they cited. Only 3 of the reviews discussed validity in analyzing studies or drawing inferences in their review process, and none discussed potential limitations. Collectively, these 37 sources cited 1,869 publications (ignoring duplications) of which 1,012 (54.2%) were determined to be original studies providing original data. The review papers appraised generally reiterated a small number of familiar examples linking antimicrobial use in animals and AMR. The majority of these examples related to antimicrobials used only therapeutically in the USA (e.g., fluoroquinolones), or antimicrobials that have never been available in the USA (avoparcin, nourseothricin). Collectively, the review studies presented negligible evidence of any differential effects among specific modes of antimicrobial use in swine production in selecting for antimicrobial resistance.

[a] The original appraisal assessed the Draft for FDA guidance 209. Additional references cited subsequently in the final guidance document, but not the draft, were later evaluated in the final revision of this report.

Due to the dearth of evidence that specifically addressed our core question (comparison of low-dose/long term use of antimicrobials in feed with other modes of AMU), we expanded the scope of the project to identify other original research studies that might inform this question. This process was not comprehensive, but adopted four strategies that identified 20 original studies, which were assessed in detail. Generally, these sources reported positive associations between aggregate AMU and AMR, but also bear testimony to the biological complexity of these relationships and the challenges of researching it. Five experimental studies specifically designed to compare low-dose/long term use and therapeutic uses in pigs generally reported no difference among treatment groups, or trends of more resistance among groups receiving therapeutic regimens. We remain unable to identify any convincing body of evidence indicating that low-dose/long term administration of antimicrobials is more likely to promote AMR than use by other modes.

We noted that none of the 4 key sources cited any published quantitative assessments of the risk of human treatment failures related to specific practices of AMU in food animals. However, several quantitative risk assessments addressing low-dose/long term AMU have estimated the risks to be extremely small. Furthermore, a small number of recent studies of both pigs and poultry have found that use of antimicrobials at low doses to control endemic diseases in food animals can be associated with improvements to animal health that translate into less carcass contamination during processing. It is inferred that this could further translate into human health benefits through reduced risk of exposure to pathogens. This small body of work needs to be expanded as it suggests that some unintended and adverse human health consequences could ensue from a non-strategic prohibition of low-dose AMU in food animals.

Currently available evidence is inadequate to provide any meaningful conclusion regarding the relative effects of different modes of antimicrobial use on the emergence of antimicrobial resistance in pathogens or commensals in commercial swine populations. The precautionary principle underlying regulatory changes in Europe remains the centerpiece of arguments for similar regulation in the USA, and FDA guidance 209 will likely lead to the removal of most growth promotant claims in the USA within 3 years. Our analysis suggests that this is not an optimal regulatory intervention. Data from Denmark shows that the lowest rate of aggregate antimicrobial use in the Danish swine industry occurred in 1999 after the withdrawal of growth promotants from finishing pigs, but before their withdrawal from weaned pigs. Recognition of the particular vulnerability, and therefore particular needs, of the weaned pig in relation to infectious disease control and prevention needs to be emphasized in discussions of strategic antimicrobial use, and its regulation, in swine. We are of the opinion that the available scientific data, and history of experiences in Europe, suggest that the most strategic path forward would be elimination, based on the precautionary principle, of AMU for production purposes in finishing pigs, but preservation of all existing therapeutic, metaphylactic and prophylactic options in weaned pigs (until 10 weeks of age). This would maintain flexibility in health management during the most crucial phase of swine production, yet should substantially reduce aggregate antimicrobial use with much less impact on animal health and welfare. However, given the likely implementation of proposed changes, industry needs to focus on the expected negative impact on pig health, particularly related to enteric disease in weaned pigs, and the likely constraint of manpower of food animal veterinarians that will be necessary to provide greater veterinary oversight of all antimicrobial use in food producing animals.

Introduction

In addition to their vital role in human medicine, antimicrobials are important tools for ensuring the health, welfare and productivity of animals raised for food, including pigs. Antimicrobial resistance is a pressing concern in human medicine throughout the world, and calls for more prudent use of these compounds are essentially unanimous. Reduction of antimicrobial use in all sectors is a laudable strategy to preserve the effectiveness of these compounds. However, and particularly in food animal medicine, it is only an intermediate goal in the quest to reduce the burden of human illness attributable to resistant organisms. It is notable that a recent review of interventions employed to reduce antimicrobial use in food animals in Denmark was accompanied by neither data nor claims of measurable benefits to human health consequent to this achievement.[1]

The process of weighing policy options related to antimicrobial use in food animals should be evidence-based and arguably should include consideration of animal health and welfare, and environmental impacts, in addition to human health outcomes. Generally, it is reasonable to assume that reduction in aggregate use of antimicrobials in any arena will lessen the pressure of selection for resistant organisms. It is also plausible that different patterns of use of antimicrobials (e.g., selection of drug; route of administration; dose; duration) will exert differential selection pressures both qualitatively (organisms impacted) and quantitatively. In food animal settings, the myriad of potential options for deploying antimicrobials will presumably have diverse implications for public health (assuming all are non-zero). However, the pharmacoepidemiology of emergence and spread of antimicrobial resistance is complex and poorly understood, and particularly in relation to public health risks linked to animal food products. Optimization of antimicrobial use in food animals requires definition of practices of greatest value to maintaining animal health and well being, as well as practices that are more or less likely to select for antimicrobial resistant organisms, or resistance determinants, of public health importance.

As antimicrobial use in pork production has been debated in the USA and elsewhere, there has been a popular but unproven thesis that administration of low doses of antimicrobials in feed (variably referred to as subtherapeutic, nontherapeutic, growth promotant or production uses) is more likely to select for resistant bacteria than are therapeutic doses, and hence lead to greater risk to public health. That assumption underpins arguments to discontinue such uses with expectations of public health benefits and without substantial negative animal health impacts. Banning of antimicrobial growth promotant use in both Sweden and Denmark was associated with increased pig disease and mortality, notably post-weaning scours.[2-4] this demonstrated that even though these antimicrobials were categorized as 'growth promotants', a practical consequence of their application was disease prevention in weaned pigs. Consequently, in Denmark cost of production increased following the ban, many producers (particularly small producers) exited the industry, and substantial changes to industry occurred in the effort to combat diarrheal diseases in weaned pigs (e.g., increased weaning age and widespread use of zinc in weaned pig diets). If low dose antimicrobial uses were similarly restricted in the United States, it is to be expected that similar health and production impacts would ensue. In reviewing the example of Danish regulations, it is also notable that aggregate use of antimicrobials in pigs in that country has not noticeably declined since the banning of growth promotant usages in weaned pigs in 2000.[1] The lowest rate of use occurred in 1999, after the banning of growth promotants in finishing pigs, but before the ban in weaned pigs. Interventions that appear to have had more definitive impact on reducing aggregate antimicrobial use in pigs in Denmark are restrictions on veterinary sale of antimicrobials (in 1994) and the 'yellow card' system recently implemented, which statistically identifies, then investigates, individual farms and veterinarians determined to be using antimicrobials at the highest rates.[1,5]

The FDA's Guidance for Industry #209 [6] states that some uses of low-level antimicrobials administered in feed are "injudicious". The Preservation of Antibiotics for Medical Treatment Act [7], which calls for the discontinuation of all nontherapeutic or routine uses of certain classes of antimicrobials in animal agriculture, has been introduced in Congress for the past several sessions. Many reports in the popular media have likened

the use of feed additive antimicrobials to sprinkling antibiotics on breakfast cereal.[8,9] Low dose use has become a specific target for advocacy groups, legislators and regulators. The purpose of this review is to identify and appraise available evidence for the role of low-dose/long term (growth promotant) usage of antimicrobials in the emergence of resistant organisms and the potential impact on public health. The appraisal is focused on evidence cited in 4 key source documents calling for regulatory restriction of low dose antimicrobial use food animals in the USA: the FDA draft Guidance for Industry #209 (FDA-209);[6] the Preservation of Antimicrobials for Medical Treatment (PAMTA) Act;[7] the Pew Commission on Industrial Farm Animal Production (PEW) report;[10] and the Keep Antibiotics Working Group (KAW) annotated bibliography.[11]

Objectives

1. Critically appraise the literature on antimicrobial use in pork production to determine the strength of evidence that long term use of specific levels of specific antimicrobial compounds in feed contributes greater risk to public health than other food animal antimicrobial uses.

2. Define the implications for the industry to guide industry policy and programming on appropriate antimicrobial usage.

Materials and Methods

The following steps outline the process followed in the appraisal, which commenced in June, 2011:

- Obtain the 4 key documents (FDA-209; PAMTA; PEW; KAW) in PDF or other electronic format and compile the reference list for each document.

- Screen the references cited in each source document, and identify all references with any relevance to antimicrobial usage and/or resistance. Titles were read and a conservative process of exclusion was used. For example, some of the key documents cited numerous references related to animal welfare or environmental impacts of agriculture other than antimicrobial resistance, and these were excluded from further consideration. At this preliminary screening, only articles with titles indicating subject matter clearly unrelated to antimicrobial use or resistance were eliminated.

- Original documents of all retained citations were procured via several mechanisms, including electronic searches via PubMed by authors' last names and/or key words from the cited titles. If not accessible via PubMed, further searches were conducted using Google Scholar or Google with relevant search strings. In some cases, lead authors of cited papers were contacted personally to obtain full text copies of the original papers.

- References were compiled in a RefWorks database, ensuring that specific fields were included (minimally: Authors, Journal/Book, Year, Title of Article, and/or URL). Completeness of the RefWorks database was verified by cross checking with the original reference lists of the 4 key documents.

- References were assessed for relevance to the appraisal objectives, and irrelevant references were identified and excluded. Six initial screening questions were used to obtain studies to be included for detailed appraisal.
    a. Does the article include a summary?
    b. Does the summary indicate observations on antimicrobial use?
    c. Does the summary indicate observations on antimicrobial resistance?
    d. Is/are the objective(s) clearly stated?

e. Do the objectives include evaluation of antimicrobial use?
f. Do the objectives include evaluation of antimicrobial resistance?

All studies in which either the summary/abstract or stated objectives indicated content related to antimicrobial use or resistance were retained for detailed appraisal. This final reference list was established in a RefWorks folder.

- Originally, we envisioned applying a single appraisal approach to all studies. However, the nature of the studies varied widely, and each selected study was categorized into one of three study types

  a. *Descriptive*: study included data describing antimicrobial use or resistance without comparison groups
  b. *Analytical*: study included data on antimicrobial use or resistance and included at least one comparative analysis
  c. *Review*: study summarized previous work on antimicrobial use or resistance but did not present original data

  Separate appraisal tools for descriptive and analytical studies (Appendix 1) and for review studies (Appendix 2) were developed. The design of the tools was based published methods for systematic appraisal of literature.[12,13] The specific questions were refined iteratively by the research team.

- Each study was then appraised using the applicable tool. To facilitate data entry for recording attributes of each publication, forms were developed in a Microsoft Access database. Most questions are self-explanatory and required only a Yes/No answer (Yes being indicated by a check mark, and no check mark indicating No). For multiple choice questions, drop down menus were used to select between options. For some questions more than one answer may have been possible. For example, if a study included multiple subpopulations and the information was more complete on one subpopulation, or different methods may have been applied in different sections of a study. In these cases, assessment of the study was based on the highest quality or most informative subpopulation. For example, when appraising antimicrobial resistance testing, if multiple methods were employed in one study the more refined method (e.g. MIC vs. disk diffusion) was recorded.

- Appraisal of review studies included documentation of the number of papers cited, and the number of citations that were original studies. This was conducted by examining abstracts of all citations. If citations were book chapters, these were deemed not to be original studies and were not examined further. Some references cited in reviews could not be located and were recorded as "cannot find".

- Based on the data obtained using the appraisal tool, a subset of 12 studies was selected for more detailed evaluation. Selected studies were those that specifically addressed the use of antimicrobials in food animals in relation to resistance and implicated antimicrobial use in animals in the development of resistance based on direct evidence. A panel of three veterinarians (principal investigator; Research Assistant; Masters of Public Health resident) critically read the 12 key studies to collectively assess the design, results and conclusions of these studies.

- To screen subsequent relevant literature not identified in the key source documents we used the Web of Knowledge citation index (http://wokinfo.com) to identify studies that had cited any of 11 of the final key publications (the 12th study was initially not included for detailed appraisal at the time this was conducted). Two approaches were used to screen the 967 studies identified. Firstly, a random sample of 50 citations was evaluated to estimate the proportion of studies that might yield original data comparing antibiotic resistance

in relation to recorded antimicrobial use in food animals. Secondly we used 'pig' as a keyword to search the citation database, thereby identifying 160 publications which were then screened for analytical studies comparing antimicrobial resistance (AMR) and antimicrobial use (AMU) in swine. References among the 967 papers that were not selected by these processes were not considered further.

- Based on the assessments made using the review study appraisal tool, we identified a subset of 7 review papers with conclusions stating some relationship between modes of antimicrobial use and resistance. All 7 reviews were examined in detail to evaluate the evidence they cited to support the inferences drawn in regarding this relationships. References cited to support statements linking antimicrobial use and resistance were identified and assessed for original data on this relationship.

- A key paper [14] not cited among the original 4 sources, but known by the principal investigator to be an intensive study of antimicrobial use and resistance in commercial swine production, was used to identify other relevant but uncited information. Using the Web of Knowledge citation index, we located 55 papers that cited Dunlop et al (1998). The abstracts/objectives of these papers were screened for content of original data pertaining to AMU and AMR in food animals, and the relevant appraisal tool for descriptive and analytical studies was applied to 17 original studies. Five of these papers included original data on AMR in relation to AMU, and were reviewed in detail by the same process used for papers cited in the FDA-209, PAMTA, PEW, and KAW documents.

After completion of the appraisal, an expert panel of veterinary epidemiologists[b] with experience in the field of antimicrobial resistance convened at the University of Minnesota to critique the appraisal. Input from the expert panel, including some additional references, was incorporated to finalize the report. Publication of the final FDA guidance 209 document occurred late in the appraisal process. The final document included 14 references not listed in the draft document, and these references were also evaluated.

Results and Discussion

Initial screening of the 4 key documents (FDA-209; PAMTA; PEW; KAW) yielded 154 papers that were identified as likely to provide some information pertinent to the relationship between antimicrobial use in food animals and antimicrobial resistance. These were further classified as Analytical (n = 37), Descriptive (n = 48), or Review (69) studies.

Analytical Studies (Appendix 3)

Further evaluation of the summaries (abstracts) and introductions of each paper was conducted to confirm that either the summary or the stated objectives referred to either antimicrobial use or antimicrobial resistance. Among the 37 analytical studies, 16 were excluded from further evaluation because neither antimicrobial use nor antimicrobial resistance were mentioned in either the abstract or stated objectives of the study (Table 1)

Table 1: Attributes of identified analytical studies (n = 37) for selected screening criteria based on information related to antimicrobial use (AMU) or antimicrobial resistance (AMR)

[b] Julie Funk, Bo Norby (Michigan State University), Scott McEwen (Ontario Veterinary College), Leigh Rosengren (Rosengren Epidemiology Consulting, Canada), Morgan Scott (Kansas State University), Randall Singer (University of Minnesota), Wondwossen Gebreyes (The Ohio State University – written comments only)

	Yes	No
Abstract present	21	16
Clear Objective stated	21	16
Abstract refers to AMU	13	24
Abstract refers to AMR	19	18
Objective refers to AMU	11	26
Objective refers to AMR	19	18
Abstract refers to AMU and AMR	11	26
Objective refers to AMU and AMR	8	29
AMU or AMR stated in abstract or objectives	21	16

Of the 21 analytical studies considered further, 5 involved human subjects only, 10 had animal subjects, and 6 included information on both human and animal subjects. Only 5 of the studies were conducted on swine and just 9 studies provided any inclusion/exclusion criteria for selection of study subjects. The vast majority of studies (19) were conducted in clinical or commercial settings (e.g., hospital or farm), and only 2 in an experimental setting. Fourteen studies presented information on antimicrobial use that was obtained by either actual measurement of antimicrobial use (9 studies), by survey (4) or was not specified. Of these studies, reporting of the specific antibiotic used and the dose, route, and duration used were variable and only 7 studies provided information on all these basic aspects of AMU (Table 2)

Table 2: Numbers of studies reporting details of antimicrobial compound, dose, route, and duration of administration among 14 analytical studies that measured antimicrobial use (AMU)

AMU measured	Compound specified	Dose stated	Route stated	Duration stated	All stated
Recorded (n = 9)	9	6	7	7	6
Survey (n = 4)	2	0	0	1	0
Unknown (n = 1)	1	1	1	1	1
Total (n = 14)	**12**	**7**	**8**	**9**	**7**

Of the 21 analytical studies that reported AMR or AMU, 19 reported measurement of antimicrobial resistance. Of these 19 studies, all reported the method used to determine resistance (14 reported MIC methods, 4 zone diffusion methods, and one a genotyping method), and 11 reported the breakpoints used to define resistance (all using MIC methods).

Application of statistical methods was also assessed for these 21 studies. Three studies did not include statistical analysis and a further study did not specify the statistical method used. Eight studies used only univariate analysis (12 used multivariate approaches) and 11 studies failed to include confidence intervals for reported sample statistics.

Of the 19 studies that measured antimicrobial resistance, only 12 studies also measured or provided some information on AMU, and were thus selected for detailed review. Table 3 summarizes aspects of the discussion and inference for studies that did not (n = 9) and did (n = 12) included observations on both AMU and AMR.

Table 3: Number of analytical studies that discussed the limitations and representativeness of their studies, discussed the relationship between AMU and AMR, and implicated AMU in AMR

Compared AMU and AMR	Discussed Limitations	Discussed Representativeness	Discussed AMU and AMR	Implicated AMU in AMR
No (n = 9)	4	8	8	7
Yes (n = 12)	5	8	11	10
Total (n = 21)	**9**	**16**	**19**	**17**

Most studies (16 of 21) included some discussion of the representativeness of their studies in relation to wider populations, but only a minority of studies (9) included specific discussion of the limitations of their approach. Most studies (17 of 20) implicated antimicrobial use in the emergence of antimicrobial resistance in their discussion or conclusions, although in 9 studies the inference was indirect (i.e. not based on the data collected in their respective study). Indeed, most studies (7 of 9) that did not compare AMU and AMR in any analysis still implicated antimicrobial use in the emergence of resistance in their discussion or conclusions (Table 3). The nine studies that did not measure both antimicrobial use and antimicrobial resistance were eliminated from further assessment as this precluded the possibility that they provided primary evidence regarding this relationship.

General quality indicators for 12 analytical studies that reported both AMU and AMR

Of the 12 studies which reported observations on both AMU and AMR, 3 were conducted in human subjects, 7 in animal subjects, and one study included both animal and human subjects. Only 2 studies involved swine. General quality indicators for the publications are summarized by subject group in Table 4. Studies often failed to provide any details on the age, housing and nutrition of study subjects, which is arguably important for interpretation of the results in the context of animal production. Only 2 studies involved any replication of their observations.

Table 4: Number of studies (n = 12) reporting details on age, housing and nutrition of experimental subjects, the presence of a control group, and replication of observations

Subjects	Age	Housing	Nutrition	Control group	Replication
Animal (n = 7)	5	3	3	5	2
Human (n = 3)	2	0	0	1	0
Both(n = 2)	1	1	1	1	0
Total (n = 12)	**8**	**4**	**4**	**7**	**2**

Of the 12 studies, only 6 (5 being animal studies) provided comprehensive descriptions of antimicrobial administration regarding the compound, dose, route, and duration of administration (Table 5).

Table 5: Number of studies (n = 12) reporting details of antimicrobial administration (antimicrobial compound, dose, route, and duration of administration)

Subjects	Compound specified	Dose stated	Route stated	Duration stated	All stated
Animal (n = 7)	7	5	5	6	5
Both (n = 2)	2	1	1	1	1
Human (n = 3)	1	0	0	0	0
Total	**10**	**6**	**6**	**7**	**6**

Regarding measurement of antimicrobial resistance, 9 of the 12 studies used MIC methods, 2 used zone diffusion methods, and one used genotyping (i.e. detection of resistance genes). Eight of the 9 studies using MIC methods stated breakpoints used to define resistance. All but 2 studies employed statistical analysis, although one did not report the method used. Five studies used only univariate analysis, and 5 studies failed to include confidence levels for reported sample statistics. Only one of the 12 studies included a comparison of antimicrobial doses in the experimental design.[15]

Specific comments on analytical studies reporting both AMU and AMR

The following comments outline the evidence presented in the respective studies.

Akwar et al. (2007):[16] This cross-sectional observational study aimed 1) to estimate the prevalence of antimicrobial resistance among fecal *E. coli* from farm residents and identify risk factors associated with this resistance; and 2) to assess the effects of in-feed medication of swine rations on antimicrobial resistance among fecal *E. coli* from farm residents. The overall prevalence of resistance to *E. coli* among residents of farms that medicated swine rations was numerically (5.55% vs. 2.43%) but not significantly different ($p = 0.06$) from prevalence in residents of farms not medicating their swine rations. Pairwise comparisons showed some significant differences for individual antimicrobials, with the biggest difference seen with tetracycline resistance. Resistance was also observed to 3 antimicrobials (chloramphenicol, nitrofurantoin, streptomycin) that had been previously used but are no longer approved for use in food animals in Canada. Multivariable models were used to explore associations between antimicrobial use patterns and resistance to various antimicrobials. In several multivariable models, resistance in *E. coli* was associated with the time spent in pig barns, consumption of antimicrobials by residents, and treating of sick pigs within 3 months prior to the study. Use of tetracycline in weaner pigs, gentamicin in piglets, and tylosin in finisher pigs were positively associated with resistance in several models, though gentamicin use in piglets was presumably not in feed. It was concluded that results of this study suggest medication of swine rations and other management factors lead to increased antimicrobial resistance among fecal *E. coli* in farm residents. This cross-sectional observational study of purposively selected farms should be viewed as an hypothesis generating study, rather than an hypothesis testing study. Antimicrobial use was based on survey responses rather than measurement of use, and details on dose and duration of administration were not included. Therefore, no information was provided on the relative impacts on resistance of therapeutic versus low-dose/long-term antimicrobial use in animals.

Berrang et al (2007):[17] This was an experimental study of subtherapeutic tylosin phosphate (20g/ton of feed) in broilers and macrolide resistance in *Campylobacter spp.* The study was conducted in an experimental setting simulating industry. The key outcome was the presence of erythromycin resistant *Campylobacter* on carcasses, which is of public health relevance because erythromycin is a common first choice treatment for human campylobacteriosis. The study comprised 3 replicate trials of 35 birds per group, and birds were exposed at 14 days old by mixing with seeder birds inoculated with 10^7 macrolide susceptible *C. jejuni*. The treated and control birds were processed at 42 days old and 10 whole carcass rinses were collected per group. *Campylobacter* were enumerated using agar containing erythromycin, and MIC were determined for all isolates. Tylosin exposure did not affect *Campylobacter* numbers on carcasses on fresh carcasses, but broilers fed tylosin had less carcass contamination after chilling. All *Campylobacter* from carcasses of birds fed tylosin phosphate were resistant to erythromycin, while none from control birds were resistant. The study indicates that subtherapeutic use is associated with AMR, but involved only one treatment dose, therefore no information is provided on the relative impacts on resistance of therapeutic versus low-dose/long-term antimicrobial use.

Endtz et al. (1991):[18] This was a longitudinal study of fluoroquinolones (FQ) resistance in *Campylobacter* in the Netherlands. There was no measurement of FQ use, but the time frame of the study spanned the period when FQ use was introduced in both the human and animal populations, and FQ use increased in both humans and animals over this period. The inclusion of this study (and two similar ecological studies below) as 'analytical' studies, despite the absence of actual measurement of AMU, reflects the inclusive bias of our triage process at all levels. Prevalence of resistance increased from 0% to 11% (human) and to 14% (poultry products) in *C. jejuni* and *C. coli*, with resistance more prevalent in *C. coli* than *C. jejuni*. This paper is one of a group of similar and frequently cited papers that associate FQ use in poultry with resistance in human *Campylobacter* isolates.[19,20] However, as FQs are used only therapeutically in food animals, and not by low-dose/long-term administration, the findings do not have direct relevance to growth promotant usage and provide no information about the relative impacts on resistance of therapeutic versus low-dose/long-term antimicrobial use in animals.

Funk et al. (2006):[21] This randomized controlled field trial examined the effect of subtherapeutic chlortetracycline use in swine feed on resistance in both *Salmonella* and overall aerobic gram negative intestinal flora. The study involved a convenience sample of 3 commercial swine farms (with 23 barns). Treatments [50g/ton CTC for the entire finisher period (10 – 24 weeks old), vs. control] were randomly allocated to barns started each week within the farms. Two farms used no other antimicrobials, while one farm used additional preventive and treatment protocols. Fecal sampling (96 pigs per barn) was conducted one time per barn within one month of expected market age. All samples underwent enrichment culture for *Salmonella* and a subset of 48 samples underwent aerobic culture for gram negative organisms. Multilevel modeling with MLWin (University of Bristol, Centre for Multilevel Modeling) was used to assess treatment effects and the variance structures, with separate models for each antimicrobial resistance and 4 models to examine relative odds of multiple resistance. *Salmonella* prevalence was low (only 15 isolates recovered) and with no significant difference between treatments. Among over 76,000 isolates of gram negative flora analyzed, 3 major phenotypes (pan-susceptible; tetracycline resistance only; tetracycline and ampicillin resistance) accounted for 97% of isolates. Odds of resistance to tetracycline (OR = 7.2), ampicillin (OR = 1.35) and ceftriaxone (OR = 2.4) were significantly increased in treated groups, with the last association highlighted as a concern due to apparent cross-selection of resistance to a third generation cephalosporin. It should be noted that, for the less epidemiologically literate readers, the reporting of odds ratios when AMR occurs at high prevalence can appear to overstate the apparent relative risk (or prevalence ratio) when comparing groups. For example, the impressive odds ratio for tetracycline (7.2) in this study derives from a prevalence of resistance of 97.5% for the exposed group and 84.3% for the unexposed group, yielding a prevalence ratio of only 1.16. Similarly, the OR for ceftriaxone (2.4) closely estimates the prevalence ratio (2.3) but the absolute difference in prevalence of ceftriaxone resistance between the groups (0.7% vs. 0.3%) is very small. The discussion highlighted the scarcity of controlled field studies undertaken to investigate the relationship between AMU and AMR in animal populations. As the study involved only one treatment dose, no information is provided on the relative impacts on resistance of therapeutic versus low-dose/long-term antimicrobial use in animals.

Kieke et al (2006):[22] This observational study was conducted to assess the association between poultry exposure and streptogramin resistant *E. faecium* in humans. Samples were obtained from 567 newly admitted hospital patients and 100 healthy vegetarians, and participants were interviewed regarding poultry exposure. Carcass washes from conventional (160) and antibiotic free (26) birds were sampled after selection by convenience. Constitutive quinupristin-dalfopristin resistance was absent in human *E. faecium*, but 56% of conventional poultry isolates were quinupristin-dalfopristin resistant. Inducible quinupristin-dalfopristin resistance was more common in samples from patients than in those from vegetarians, and in washes of conventional compared with antibiotic-free poultry. Higher poultry

consumption was associated with inducible quinupristin-dalfopristin resistance, but no common human and poultry isolates were identified based on PFGE typing. Exposure information was obtained by telephone interviews (within 62 days for patients) and antimicrobial use from medical records. The study did not have a healthy meat-eating control group, virginiamycin use in poultry was assumed but not confirmed, and there was no process to validate the human exposure information. The authors "suggest virginiamycin use in poultry contributes to human carriage of isolates that contain streptogramin resistance genes". While indirectly implicating growth promotant usage in emergence of inducible Q/D resistance, no information is provided on the relative impacts on resistance of therapeutic versus low-dose/long-term antimicrobial use in animals.

Ladely et al. (2007):[15] This replicated experimental study of macrolide-resistance in *Campylobacter* in broilers was the only analytical study that directly compared subtherapeutic and therapeutic treatment regimes. The stated objective was to evaluate the effect of administering therapeutic and subtherapeutic concentrations of tylosin on the erythromycin susceptibility of *C. jejuni* and *C. coli* isolated from the ceca of treated broilers. The study involved 3 replicates (175 birds per replicate) of 7 treatments of 25 birds (175 birds total) in a 2x3 +1 factorial design. One treatment group was an unexposed control (i.e. non-infected, not tylosin), and the remaining groups were exposed to either *C. jejuni* or *C. coli* with antimicrobial exposures of 1) tylosin in feed at 22 ppm for 4 weeks (from 2 weeks of age); 2) tylosin in water at 529 ppm for 5 days; or 3) no tylosin exposure. No macrolide-resistant strains were recovered from broilers that had not been administered tylosin, but macrolide-resistant isolates of both species were recovered from broilers administered tylosin at both subtherapeutic and therapeutic concentrations. Erythromycin resistance was significantly more prevalent among isolates from birds exposed to tylosin at subtherapeutic concentrations for 4 weeks (83.3 and 56.1% for *C. coli* and *C. jejuni* respectively) than in birds exposed to therapeutic concentrations for 5 days (33.3 and 7.9%). This is the only study in which therapeutic versus subtherapeutic use was compared and the results indicated greater prevalence of resistance with the latter. It should be noted that the doses used for birds in this study differ from the approved levels for swine of tylosin in water (0.50 grams per 200 gallons of water, or 132 ppm), or in swine feed for therapeutic (100g/ton) and growth promotion (20 to 100g/ton) use.

Levy et al (1976):[23] This prospective controlled study in a commercial setting involved sampling of birds, workers, families and neighbors. Birds were exposed from 3 months of age to 100g of oxytetracycline per ton of feed, stated by the authors to be a level used for therapy or prophylaxis. The main outcome was the prevalence of tetracycline resistance in coliforms. Increased resistance was observed rapidly in birds and after some months in workers. A decline in the prevalence of resistance was observed after withdrawal of the medicated feed. The statistical methods used to analyze the data were not specified, and there was no indication of how the (likely) assumption of independence between samples was handled, given birds were group housed. Multiple resistance was observed, yet the most common resistance pattern did not include tetracycline resistance, and more complex resistance patterns were found in people. This widely cited study provided some of the earliest descriptions linking antimicrobial use in poultry and resistance in human commensals. However, the avian population studied (from 3 months old) was different from the contemporary broiler industry, and the study involved only one treatment dose. No information is provided on the relative impacts on resistance of therapeutic versus low-dose/long-term antimicrobial use in animals.

Peak et al: (2007)[24]***:*** This longitudinal study involved measurement of tetracycline resistance genes and 16S rRNA gene abundances in 8 lagoons at 5 cattle feedlots in USA over 6 months. The stated objective was to 'To holistically assess factors that affect the abundance and seasonality of released resistance genes in surface water below CAFOs, especially related to on-site CAFO operating practices' and examine how CAFO practices affect the absolute and relative resistance gene abundances downstream of CAFO operations. Tetracycline resistance varied between feedlot types and was positively associated

with herd size and 16srRNA level. Resistance was negatively associated with sunlight level and length of day and increased in autumn, particularly in the high use group. The study concluded there was a strong relationship between on-site antibiotic use and resistance gene abundance in surface water. However, the study had no replication and no quantitative data were gathered on tetracycline use for any feedlot, even though the study was prospective. Little detail was provided on how feed lots were categorized by AMU, or any validation procedures to confirm exposures (interviews with operators and 'personal on-site investigations'). The frequency of sampling and number of samplings was not clear, and there is no indication that the analysis adjusted for the repeated measures within lagoons. The findings indicate the concentration of tetracycline resistance genes was correlated with apparent aggregate antimicrobial use. However, because antimicrobial use was not reliably measured, no information is provided on the relative impacts on resistance of therapeutic versus low-dose/long-term antimicrobial use in animals.

Smith J et al: (2007)[25] The stated purpose of this study was to investigate the influence of antimicrobial administration on the distribution of resistance determinants and strain types among commensal *E. coli* strains isolated from broiler chicken intestines. The study involved 3 contract farms for one producer and an experimental flock with antimicrobial treatment and control groups. The history of antimicrobial usage on the commercial flocks was known. One flock had not been treated, and the other 2 were administered antimicrobials (FQ, tetracyclines) at therapeutic levels. In the treated flocks, fecal samples (100 pooled floor samples) were collected immediately after treatment. All groups in the experimental setting were fed a commercial corn-soy meal broiler diet containing monensin (90 g/ton) and bacitracin methylene disalicylate (50 g/ton). The birds in the treatment groups were given therapeutic concentrations of antimicrobials in their drinking water at 4 weeks old as follows: sarafloxacin, 20 ppm for 5 days; enrofloxacin, 25 ppm for 3 days; and oxytetracycline, 25 mg/lb for 5 days. Cecal contents from groups of 10 birds were sampled at 3, 5, and 7 weeks of age. Evaluation of resistance included both phenotypic and genotypic methods. The results indicated complex relationships in which the age of birds influenced the observed resistance in both the commercial and experimental settings. In comparisons among birds of the same age, FQ MICs were significantly higher for isolates obtained from the enrofloxacin-treated group when the chickens were 5 and 7 weeks old than for isolates obtained from the other treatment groups. However, at 5 weeks of age, lower MICs for streptomycin were observed in *E. coli* isolates from the enrofloxacin treated birds than for other groups and the enrofloxacin-treated group also exhibited a lower prevalence of resistance to streptomycin, tetracycline, and sulfathiazole after treatment. Other observations included the likely importance of individual strains of *E. coli* in influencing observed resistance profiles, and that the prevalence of integrons was influenced by age, farm and flock in addition to antimicrobial administration. In summary, the study reported complex outcomes resulting from administration of therapeutic levels of antimicrobials in commercial and experimental settings. However, the study did not address low-dose, long term administration and no information is provided on the relative impacts on resistance of therapeutic versus low-dose/long-term antimicrobial use in animals.

Smith K et al (1999):[19] This longitudinal observational study of FQ resistant *Campylobacter* infections in the USA is similar to that conducted in the Netherlands by Endtz et al., discussed above. The study was originally assessed as a descriptive study, but was included for detailed review as the only descriptive study making any direct inference (i.e., based on original data analyzed in the study) of antimicrobial use in relation to emergence of resistance, albeit at an ecological level and without actual measurement of exposure (again indicating the inclusive bias of our triage process). The study reports increased quinolone resistance in human *Campylobacter* isolates in the USA following the approval of fluoroquinolones (FQ) for therapeutic use in poultry. The study did not measure antimicrobial use in poultry, but inferred the association based on temporal patterns of resistance before and after the drug approval for animal use, supplemented by a cross-sectional data of FQ resistant *Campylobacter* in retail

poultry meat [19]. Again, as FQs are used only therapeutically, and not by low-dose/long-term administration, the findings do not have direct relevance to growth promotant usage and provide no information about the relative impacts on resistance of therapeutic versus low-dose/long-term antimicrobial use in animals.

Unicomb et al. (2006):[26] This Australian period prevalence study analyzed antimicrobial resistance patterns in *C. jejuni* isolates from 585 patients across 5 states. FQ have not been approved for use in food animals in that country, and a low prevalence (2%) of resistance was reported. In multivariate models controlling for age and underlying disease, cases infected with ciprofloxacin-resistant strains did not differ from cases with ciprofloxacin sensitive infections with respect to disease severity (fever, vomiting, bloody stools, duration of diarrhea) or likelihood and length of hospitalization. No observations were made on antimicrobial use or resistance in animals, and FQ are used therapeutically in food animals in other countries. No information is provided on the relative impacts on resistance of therapeutic versus low-dose/long-term antimicrobial use in animals. (This reference was not used in citation index to identify later papers of relevance)

Van Duijkeren et al: **(2008)**[27]**:** This cross-sectional observational study of 31 farms compared the occurrence of MRSA in farms with antimicrobial use history, and concluded that 'use of standard antimicrobial medication of the pigs seemed to be a risk factor for MRSA carriage'. MRSA prevalence was determined by testing 10 nasal swabs (selected by convenience), and animals of different ages were tested on different farm types. Antimicrobial use was determined based on a questionnaire of whether the groups of pigs sampled had received antibiotics, which drugs, and when (recording only group treatments but not individual animal treatments). Six of the farms tested supplied pigs to some of the other farms, and MRSA colonization was closely related between the source and recipient farms. Statistical analysis was not performed, possibly due the lack of independence between farms (i.e. source and recipient herds were similar in MRSA status). No information on dose was provided, and the purpose of administration (i.e., therapeutic or otherwise) was not specified for most farms. Therefore, no information is provided on the relative impacts on resistance of therapeutic versus low-dose/long-term antimicrobial use.

Using the Web of Science Citation index, we identified 967 papers that cited 11 of these papers (excluding the Unicomb paper) which we entered into a RefWorks database. To assess the likely yield of original papers reporting associations between AMU and AMR in swine, we randomly sampled 50 of the 967 papers that cited the papers reviewed above. Among the 50 selected papers, 39 (78%) were original studies but only 3 (6%) were original studies that included data on either antimicrobial use or resistance in animals. Only one paper[28] provided relevant original data on the relationship between antimicrobial use and resistance in swine (discussed below under Supplementary Information), suggesting the likely yield of original data among these 967 subsequent studies would also be low. However, an estimated yield of only 2% suggested a likely yield of some 15-20 relevant publications. Therefore we then searched this RefWorks database using the keyword of 'pig', and screened 160 titles selected by this process. This yielded 16 papers that are also discussed later (see Supplementary Information) with respect to the relationship between modes of AMU and AMR.

Descriptive Studies (Appendix 5)

Studies were designated 'descriptive' rather than 'analytical' if they included data on antimicrobial use or resistance but did not have comparison groups. Twelve of the 48 studies initially included as descriptive were triaged from further consideration as further evaluation indicated neither AMU nor AMR were the subject of investigation. Among the 36 studies retained, 13 involved animal subjects (8 of swine); 6 involved human subjects; 5 involved both human and animal samples; and the remainder did not involve studies on live subjects

(e.g., reports on environmental sampling, etc.). Of 36 papers with an abstract, 29 referred to AMR and 7 to AMU. Similarly, the majority of stated objectives of these studies indicated description of AMR rather than AMU to be the goal of the study (Table 6).

Table 6: Attributes of identified descriptive studies (n = 36) for selected screening criteria based on information related to antimicrobial use (AMU) or antimicrobial resistance (AMR)

	Yes	**No**
Abstract present	35	1
Clear Objective stated	35	1
Abstract refers to AMU	7	29
Abstract refers to AMR	29	7
Objective refers to AMU	3	33
Objective refers to AMR	27	9
Abstract refers to AMU and AMR	6	30
Objective refers to AMU and AMR	3	33
AMU or AMR stated in abstract or objectives	35	1

Of the 24 studies involving human or animal subjects, only 6 indicated inclusion/exclusion criteria for selecting study subjects and no details of age (14), housing (18), and nutrition (23), were given in the majority of reports. None of the studies were conducted in an experimental setting or included replication of observations. Of 7 studies which reported antimicrobial use, none recorded use directly (3 used surveys and methods for collection were not specified in 4), and only 3 studies included details regarding the drug, dose, route and duration of administration (Table 7).

Table 7: Number of descriptive studies (n = 7) reporting details of antimicrobial use (antimicrobial compound, dose, route, and duration of administration)

AMU measured	**Compound specified**	**Dose stated**	**Route stated**	**Duration stated**	**All stated**
Recorded (n =0)	-	-	-	-	-
Survey (n = 3)	3	2	2	2	1
Unknown (n = 4)	4	2	2	2	2
Grand Total	**7**	**4**	**4**	**4**	**3**

In the 26 studies that reported observations on antimicrobial resistance, 11 used methods that estimated MICs (of which 8 specified the breakpoints used); 5 used zone diffusion methods; 8 detected resistance genes rather than phenotype; and 2 studies did not specify the method. Of the 26 studies, 13 included some statistical analysis (5 using a multivariate method), and only 5 provided confidence intervals for reported sample statistics. No study made any assessment of antimicrobial dose in relation to antimicrobial resistance, and only one study [19] made any direct inference (i.e., based on original data analyzed in the study) of antimicrobial use in relation to emergence of resistance. This study was discussed above with the analytical studies. It was not among the 7 studies that discussed limitations to their study design or inference (Table 8). It is notable that despite the absence of measurement of both AMU and AMR in these descriptive studies, 16 of the 36 implicated antimicrobial use in antimicrobial resistance in the discussion or conclusions (Table 8).

Table 8: Number of descriptive studies that discussed the limitations and representativeness of their studies, discussed the relationship between AMU and AMR, and implicated AMU in AMR

Compared AMU and AMR	Discussed Limitations	Discussed Representativeness	Discussed AMU and AMR	Implicated AMU in AMR
No (n = 15)	7	14	15	15
Yes (n = 1)	0	1	1	1
Total (n = 16)	**7**	**15**	**16**	**16**

Purely descriptive studies constitute an important sector of the scientific literature as they can identify issues that may merit more concentrated investigation, or potentially informative trends in the temporal or spatial distribution of phenomena of interest. However, in the absence of a well-defined context, citation of purposively selected descriptive studies can be misleading, and particularly for very complex issues such as the epidemiology of AMR. It is long established that resistance determinants can be prevalent in pristine environments with no record of antimicrobial exposure.[29] More recent studies documenting multiple resistance in bacteria in ancient permafrost samples[30] and the microbiome of an isolated cave[31] further emphasize the potential for misleading inference linking AMR to AMU when based solely on descriptive data on resistance. It is evident that antibiotic resistance genes are widespread in nature and can persist without selection pressures emanating from anthropogenic activities including antimicrobial use.[32] It should be noted that resistance genes found in pristine environments likely serve different purposes in their host organism that conferring resistance. However, from the perspectives of causal inference or effect estimation, the 48 descriptive studies cited by the 4 key documents are collectively uninformative regarding the association of low-dose long term administration of antimicrobials to food animals with AMR.

Review Studies (Appendix 6)

The 4 key documents cited 69 relevant papers that we identified to be review/report papers rather than original studies. Among these, the titles of the review suggested that antimicrobial resistance or use was the focus of 47 reviews, and a further 3 stated AMU and/or AMR to be the subject of the review in a summary or statement of objectives. Further assessment was limited to 37 reviews that specifically referred to AMU or AMR in a summary (33 referred to AMR, and 31 to AMU in the summary) or stated objectives (25 referred to AMR and 17 to AMU). None of the 37 reviews employed systematic review methods, but were narrative reviews (29) or reports (8). Of all 37 reviews, only 1 stated the search methods used to identify cited sources. Similarly, only one (different) review specified inclusion and exclusion criteria for the studies they cited. No reviews stated that any criteria of validity assessment were used in the selection of studies cited, or indicated any measures were taken to identify or address potential biases in selection of cited sources. Only 3 of the reviews discussed validity in analyzing studies or drawing inferences in their review process, and no reviews discussed potential limitations of their study. Collectively, these 37 sources cited 1,869 publications (ignoring duplications) of which 1,012 (54.2%) were determined to be studies providing original data. That is, almost half the sources cited by the review papers were other reviews and reports, rather than primary sources. The proportion of primary sources varied among individual reviews from 0% to 83%.

Information presented on resistance for specific antimicrobial-bacterial pairings

Twenty five reviews discussed specific antimicrobial compounds or classes linked to resistance in specific bacteria. The most commonly discussed drug class was fluoroquinolones (17 reviews), predominantly in relation of *Campylobacter* (12 of 17), but also in other bacterial species (Table 9). Evidence from several countries supports a role of use of FQ in poultry in the emergence of FQ resistant *Campylobacter* in humans.[33] The regular citing of this relationship among review papers reflects that it is perhaps the best documented

circumstance linking a specific antimicrobial used in food animals to occurrence of resistance in an important zoonotic pathogen, although the degree of impact on public health has been questioned.[20] However, in the USA and elsewhere, FQ are used for therapeutic purposes in animals and not by low-dose, long-term administration and this scenario is not directly relevant to concerns regarding low-dose, long term AMU.

Table 9: Specific organism-antimicrobial pairs cited in 25 reviews of the relationship between antimicrobial use in animals and antimicrobial resistance

Enterococci	**20**
Vancomycin	13
Quinupristin/dalfopristin	7
Salmonella	**16**
Multiple drug resistance	10
Fluoroquinolones	3
Ceftriaxone	3
Campylobacter	**13**
Fluoroquinolones	12
Macrolides, Tetracyclines	1
E. coli/coliforms	**11**
Nourseothricin	4
Tetracyclines	3
Fluoroquinolones	2
Extended spectrum beta lactams	1
Gentamicin, apramycin	1
STEC	
Multiple drug resistance	1
Streptococci	
Tylosin	1

Enterococci (particularly *E. faecium*) were the most commonly discussed organisms (20 of 25 reviews) in reviews addressing specific antimicrobial-bacteria relationships, mostly in regard to vancomycin resistance linked to the use of avoparcin as a growth promotant (13 reviews), but also for quinupristin/dalfopristin resistance linked to use of virginiamycin in animals (Table 9). The experience with avoparcin use as a growth promotant in Europe is perhaps the best established example demonstrating that low-dose/long term AMU in animals can be associated with increased prevalence of resistant commensal organisms in animals, farm workers and people in the general community. However, relative to the example of FQ resistant *Campylobacter*, there is even less convincing evidence that this phenomenon led to any significant human illness.[34] Prior to the banning of avoparcin use in Europe, carriage of vancomycin resistant enterococci (VRE), particularly *E. faecium*, was common among healthy humans and livestock, but human clinical disease was rare. In contrast in the USA, where glycopeptides such as avoparcin have never been approved for use in animals, carriage of VRE by healthy people in the community is rare, yet clinical disease associated with VRE has been prevalent since the early 1990s, particularly in intensive care patients.[35]

The divergent patterns of glycopeptide use in the USA (common use in humans but no use in animals) versus Europe (common use in animals, sparing use in humans) has been posited as one explanation of the epidemiological differences in human clinical disease.[36] Cursorily this observation points glycopeptide use in

humans, rather than in animals, being associated with human clinical VRE infections. Furthermore, while the prevalence of VRE among animals and healthy community members in Europe decreased after the banning of avoparcin in 1996, the prevalence of nosocomial ampicillin and vancomycin resistant enterococcal infections subsequently increased.[34,37] The European Antimicrobial Resistance Surveillance System reported the proportion of glycopeptide-resistant *E. faecium* isolates from blood increased dramatically after 2000 in several European countries. However, the emergence of a particularly virulent subtype of *E. faecium* across 3 continents has been proposed to have had more influence on the epidemiology of VRE infections than antimicrobial use patterns. This virulent strain (clonal complex 17) appears to be a hospital-adapted lineage that is resistant to ampicillin and fluoroquinolones, and typically contains virulence factors that have been absent from all strains tested from animals and asymptomatic human carriers.[38,39] In summary, on one hand the avoparcin experience in Europe illustrates the concept that low-dose, long term administration of antimicrobials can lead to increased prevalence of resistant commensal organisms in animals and also associated human populations. On the other hand, it also demonstrates a scenario in which growth promotant use in animals animal, despite selection of resistant commensal organisms, was uncoupled from (or even negatively associated with) the public health burden of resistant infections caused by the respective bacterial species (i.e., history of VRE infections in USA vs. Europe, and in Europe before and after the banning of avoparcin).

Virginiamycin had been used as a growth promotant in animals for decades (e.g., since 1974 in the USA) without concern for resistance, because products of the streptogramin class were not historically available in human medicine. Around 2000 (1999 in the USA) quinupristin/ dalfopristin (Synercid), an injectable streptogramin, was approved in many countries to treat infections associated with vancomycin-resistant *Enterococcus faecium* bacteremia. This immediately focused attention upon potential public health implications of virginiamycin use in food animals. Similar to avoparcin and VRE, as highlighted in these review papers, several studies have reported high prevalence of streptogramin resistance in animal enterococcal isolates. However, unlike avoparcin, there is as yet little evidence that this has been associated with a high prevalence of resistance in human isolates. A study conducted by the CDC found little resistance to quinupristin-dalfopristin (Q/D) among enterococci isolated from people in the United States through mid-1999, despite decades of virginiamycin use in farm animals.[40] A large study of more than 6,000 enterococcal isolates from animals in the USA concluded that Q/D resistance among enterococci from animal sources in the USA may be less prevalent than in earlier reports with more limited sampling. They also observed that genes mediating resistance to Q/D were not widespread despite the long history of virginiamycin use for growth promotion in animals.[41] The putative association between virginiamycin use in animals and treatment failures of humans with Q/D resistant enterococcal infections to date has minimal evidentiary support. Discussions of this relationship in the review studies cited by the key sources were largely speculative or hypothetical. Furthermore, the potential importance of this putative relationship is likely declining because Synercid use is relatively rare due to its practical limitations for therapy, including the need for central venous administration, metabolic interactions, an adverse-effect profile, and a lack of activity against E. *faecalis.*[42]

Sixteen reviews also discussed concerns about antimicrobial resistance in *Salmonella* both generally and for specific subpopulations such as *S.* Typhimurium DT104 and multiple resistant *S.* Newport. The majority discussed concerns with multiple drug resistance, and some cited specific concerns related to FQ and ceftriaxone resistance. Again, these specific drugs are used therapeutically rather than by low-dose/long-term administration. No concrete evidence was identified among these reviews to implicate growth promotant usage with emergence of specific patterns of antimicrobial resistance in *Salmonella*.

Resistance in *E. coli* or coliforms was discussed in 11 reviews in relation to both therapeutic use (fluoroquinolones, extended spectrum beta lactams, tetracyclines, gentamicin, apramycin) and growth promotant use (nourseothricin, tetracyclines). The growth promotant nourseothricin was linked to the emergence of streptothricin resistant coliforms in animals and humans in Eastern Europe. There was also some evidence of possible clinical implications in humans as 1% of isolates from urinary tract infections in the region

were resistant to nourseothricin.[43] However, as with avoparcin, nourseothricin has never been available for use in food animals in the USA.

Information presented regarding antimicrobial dose and AMR

Five reviews (including one report) addressed the issue of antimicrobial dose and the emergence of resistance. However, the conclusions of 7 reviews (2 of which did not address dose specifically) included statements related to the effect of antimicrobial dose, or low-dose usage, on resistance. As the subject of dose and duration of administration is the central purpose of this appraisal, some comments on the content of those 7 reviews are given below.

Goforth and Goforth (2000):[44] This review was authored by lawyers and appears to be targeted more to a legal than a scientific readership. The documentation of many statements is minimal and illustrative (i.e., examples of sources that have stated the concept discussed) rather than analytical (i.e., summarizing of diverse scientific data or opinions). Only 18 of 58 cited sources were primary sources. The review is characterized by general statements only loosely supported by specific sources. For example, it is stated (with no sources cited) that use of subtherapeutic antibiotics in food animals *"is having devastating and potentially irreversible effects on the viability of antibiotics as agents to effectively treat diseases in human beings."* Although the focus of the review was 'subtherapeutic' antimicrobial use in animals, it included no substantive consideration of the relative impacts on AMR of low-dose, long term AMU in food animals versus other modes of use.

Aarestrup and Seyfarth (2002):[1,45] This is a short review summarizing the Danish experience with banning of growth promotant antimicrobials. The salient observation is that some reduction in prevalence of some resistant bacteria was documented through the DANMAP surveillance system. No information is provided on public health outcomes, nor information on the relative impacts on resistance of therapeutic versus subtherapeutic uses.

Catry et al. (2003):[46] This paper takes a detailed theoretical approach towards understanding the relationship between antimicrobial use and resistance. The authors examine relationships between dose, treatment interval, duration of antimicrobial treatment and formulation and the emergence of antimicrobial resistance in individual patients. Salient points include that dose, as a risk factor, can be interpreted as the total amount of active substance used in a population during a certain time period, and regional and international surveys have shown that resistance in a population is directly related to the amount (aggregate) of antimicrobial drugs used. The paper also discusses the theory of a 'selection window' for resistance, whose characteristics are defined by the therapeutic regimens (drug, dose, interval, etc.), but also concludes that '*more in vivo experiments and clinical data are required to assess the practical value of this … theory*'. The authors cite other review papers (74 of 155 cited papers were primary sources) in positing that prolonged oral administration of low dosages of antimicrobial drugs may lead to selection windows in the digestive tract in which resistance development is promoted. No information is provided on primary studies on the relative impacts on AMR of therapeutic versus low-dose/long term AMU in animal populations. The authors considered that, as in Europe, the argument for removal of antimicrobial growth promotants is founded on the precautionary principle.

***Khachatourians* (1998):**[47] This is a general review of antimicrobial use and resistance in food animals. A negative perspective is evident throughout regarding 'animal husbandry' uses of antimicrobials, but no information is provided on primary studies of the relative impacts on resistance of therapeutic versus low-dose/long term AMU in animal populations.

Shea (2004): [48] The review concludes that 'nontherapeutic uses contribute to resistance and create health dangers for humans', but no information is provided on primary studies of the relative impacts on resistance of therapeutic versus low-dose/long term AMU in animal populations.

Smolinski et al (1998): [49] This is a broad review of microbial threats to health that has only cursory discussion of antimicrobial use in animals and posits that 'the use of antimicrobials for growth production has abetted the rise in infectious diseases by contributing to antimicrobial resistance'. No information is provided on primary studies of the relative impacts on resistance of therapeutic versus low-dose/long term AMU in animal populations.

Levy (1987): [50] The author reviews previous studies from his group that demonstrated that low dose oxytetracycline administration in feed to poultry led to increased resistance in *E. coli* in the birds and human handlers, and that prevalence of resistance declined following the removal of the compound. The review illustrates other examples of antimicrobial use in food animals linked to emergence of resistance but does not provide information on primary studies of the relative impacts on resistance of therapeutic versus low-dose/long term AMU in animal populations.

Summary of cited review papers

The review papers cited by the key sources generally reiterate the most storied examples linking AMU in animals and AMR. However, both individually and collectively, they fail to identify specific studies that explicitly contrast the impact of low-dose, long duration (growth promotion) administration relative to other modes of AMU in food animals. The narrative nature of all these reviews; their almost universal failure to specify methods for procuring the sources cited; and the overall paucity of discussion of study validity or potential biases together undermine the standing of this body of references as offering an authoritative response to this specific question. Collectively, these review studies present negligible evidence of any differential effects among specific modes of antimicrobial use in swine production in selecting for antimicrobial resistance. Consequently, they also offer no substantive evidence that cessation of low-dose, long term administration of antimicrobials in food animal production would have outcomes that might differ predictably from those resulting from aggregate reduction of antimicrobial use by any mechanism. They present no evidence that measures to reduce low-dose, long term administration of antimicrobials *that do not reduce aggregate antimicrobial use* would have any measurable benefit with respect to prevalence of antimicrobial resistant organisms in food animal reservoirs and, by extension, to public health.

The apparent growth in alternative production systems over the last decade has led to a number of studies comparing 'conventional' and 'alternative' systems with respect to zoonotic pathogens and AMR. A relatively recent systematic review and meta-analysis compared the prevalence of selected pathogens, and associated antimicrobial resistance, in organic and conventional food animal systems. The authors concluded that limitations of study design diminished the value of many studies and the feasibility of performing a meaningful analysis.[51] The authors stated "most studies included in this review were observational cross-sectional studies, which can only provide evidence for an association and cannot establish causation due to a lack of temporal evidence. … Furthermore, many of the studies were based on a small sample size, and their purpose is most suitable for generating rather than testing hypotheses. They are a valuable source of initial evidence on new and emerging topics but do not provide high-quality evidence for systematic reviews or meta-analyses." These same limitations are prominent among the literature cited by the key FDA-209, PAMTA, PEW and KAW that we have examined in this appraisal.

Supplementary information

The original scope of this appraisal was defined to be the references cited by FDA-209, PAMTA, PEW and KAW. Our original assumption was that these sources, which articulate arguments for removal of growth promotant usage in food animals based on public health concerns related to AMR, would have collectively identified the most convincing evidence to support such regulatory interventions. However, our appraisal process found that none of the cited papers presented specific evidence to support the contention that growth promotant usage in swine presented greater risk for selection of dissemination of antimicrobial resistance than other modes of AMU, or could be linked to any defined or measurable public health outcome. Furthermore, only one paper[15] cited by the key sources presented evidence to support this contention in any food animal species (poultry). This led us to question the core assumption about the comprehensiveness of the key sources, particularly as we were aware of some relevant publications that none of the key sources had cited.

Consequently, we expanded the scope of the study to identify other peer reviewed publications that might specifically inform our core question, and particularly in relation to swine production. Due to resource limitations, this was not an exhaustive or systematic review. The process involved identifying further research via the following steps:

- Citation index search based on 11 analytical studies cited by the key sources that compared AMU and AMR (yielding 160 papers involving swine, and 10 for detailed review)
- Citation index search based on one pivotal paper not cited by the key sources (yielding 55 studies, 17 that were critically appraised and 5 for detailed review)
- Update of reference list in the final FDA guidance compared to the draft document (14 additional references including 5 original studies, none for detailed review)
- Risk assessments of low dose AMU in food animals in relation to public health and animal health that were not cited by the key sources (7 studies)
- Additional references suggested by the expert review panel (6 papers, 4 for detailed review)

Publications citing 11 analytical studies comparing AMU and AMR

967 papers that by October 2011 had cited any of 11 papers reviewed in detail in the analytical papers section were selected using ‘pig’ as a keyword in the RefWorks database. Abstracts of the 160 papers identified were screened and papers that indicated any comparison of antimicrobial treatment regimens with respect to AMR were reviewed in detail.

> ***Mathew et al. (1999):***[52] This observational study was designed to investigate the effects of pig age and level of antibiotic use on antibiotic resistance patterns in *E. coli*. Farms were classified as low antibiotic use if subtherapeutic feed-based antibiotics were not used or if only subtherapeutic concentrations of tetracyclines were used for brief periods, or high use (7 farms) which routinely used subtherapeutic feed-based antibiotics and/or injectable antibiotics. However, detailed measurement of AMU was not conducted and therapeutic and non-therapeutic uses were not directly compared. A much broader range of compounds was used on high use farms, and resistance generally was more prevalent in high use than low use farms. However, pig age was seen to have a strong influence on the prevalence of resistance in both groups. The study supports the concept that aggregate AMU is likely associated with greater resistance, but no comparison of modes of administration was conducted
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> ***Wagner et al. (2008):***[53] This experimental study in feeder pigs reported the impact of five different antimicrobial regimes in a 2x2+1 design. Two antimicrobials were administered as both pulse doses (chlortetracycline at 400ppm; tylosin at 100 g/ton), respectively and low-level continuous

administration in feed (chlortetracycline at 100 g/ton until 2 weeks before market; tylosin 40 g/ton until market age). The fifth group was not exposed to antimicrobials. Pulse doses were fed for 1 week, followed by 3 weeks of no antimicrobial, another week of antimicrobial, then 12 weeks without antimicrobials. Outcomes were the prevalence in fecal samples of *Salmonella*, and of antimicrobial resistance in both *Salmonella* spp. and generic *E. coli*. *Salmonella* prevalence in feces was higher at the time of placement of pigs compared to samples taken when the animals were close to market weight. Differences in resistance of *Salmonella* were not observed among treatment groups. Only resistance to cephalothin increased in *E. coli* under the pulse treatment with chlortetracycline. The authors concluded that the dosing regimens examined in this study did not influence the prevalence of antimicrobial resistance in *Salmonella* spp. or *E. coli*. However, high prevalence of resistance (>905) was observed in all groups for tetracyclines, sulfamethoxazole, and streptomycin.

Bibbal et al. (2007):[54] This experimental study in 7 week old pigs (without previous antimicrobial exposure) compared three ampicillin dosage regimens on ampicillin resistance among *Enterobacteriaceae* in swine feces. The exposure regimens used a single exposure dose (20mg/kg body weight) delivered either by injection or orally via water, with and without food deprivation. Treated animals excreted significantly higher percentages of resistant Enterobacteriaceae than the control group, but no significant differences were observed among the three treatment regimens.
However, oral administration in fed pigs led to higher excretion of blaTEM genes than intramuscular administration . All doses were therapeutic and selected for resistance but no low-dose exposures were evaluated.

Harada et al. (2008): [55] This observational study was similar to that of Mathew et al (1999), in that it compared resistance patterns in *E. coli* in relation to prior antimicrobial exposure on farms. Observations generally indicated that resistance was more prevalent in pigs exposed to the respective antimicrobials on the farm, and some patterns of cross-selection for resistance were seen. However, the study recorded only therapeutic uses of antimicrobials. The authors also reported that all the observed resistance patterns also occurred in *E. coli* isolates from pigs that were not exposed to antimicrobial therapy during at least 6 months. The authors speculated that resistance observed in the absence of recent therapeutic selective pressure might be associated with historic use or other factors such as subtherapeutic antimicrobial use, but no data were presented to support this.

Juntunen et al. (2010): [56] This field study at a single swine farm compared antimicrobial resistance in *Campylobacter coli* in feces from untreated sows and piglets (n = 57), weaned pigs treated with tylosin (n = 68) and pigs of the treated group 3–5 weeks after withdrawal of tylosin (n = 15). Weaned pigs were later sampled after tylosin had not been administered for 7 months at the farm. Resistance to at least one antimicrobial agent was more common among isolates from the treated pigs than in those from the untreated animals. Resistance to ciprofloxacin, erythromycin, nalidixic acid and streptomycin was higher among isolates from the treated pigs than untreated animals. Resistance against at least one antimicrobial was significantly lower when tylosin had not been administered for 7 months. The study did not include any age matched controls and the dosage used (140g/metric tonne) exceeds the approved level for therapeutic use of tylosin in the USA. No comparison with low-dose administration was included.

Literak et al. (2010):[57] This observational study reported susceptibility patterns to 12 antimicrobial agents in *E. coli* isolates from 2 swine farms in the Czech Republic. The farms had different antibiotic exposure histories: Farm A was a breeding stock supplier that used no antimicrobials for prophylaxis or growth promotion; According to the records, for 2 years prior to testing amoxicillin was the only antimicrobial used on the farm to treat 276 pigs (total use of 1090 g of amoxicillin). On Farm B, in the 2 years before sampling pigs were administered amoxicillin, tetracyclines, lincomycin-spectinomycin,

tulathromycin, ceftiofur, ampicillin, florfenicol (amounts specified for each) as well as unspecified amounts of penicillin and streptomycin. The authors summarize that 'Irrespective of the antimicrobial policy, the prevalence
of resistant pig *E. coli* isolates was high in both farms'. Resistance to tetracyclines was most prevalent on both farms, and a high prevalence of resistance was also seen to sulfonamides, despite absence of sulfonamide use on either farm.

Tadesse et al. (2011): [58] This observational study reported prevalence and antimicrobial resistance profiles of *Campylobacter* spp. on farm at following slaughter in 34 cohorts of pigs raised in conventional and antimicrobial-free (ABF) farms in 4 US states (in 2 regions). ABF was defined as farms not using any antimicrobials postweaning; conventional farms were those using antimicrobials routinely for prophylactic and therapeutic purposes. At the farm level, a logistic regression model was used to compare *Campylobacter* prevalence and prevalence of resistance to 6 antimicrobial agents among farm types and regions. At post slaughter level, the analysis additionally included assessment of prevalence between systems within regions. In the on-farm samples, resistance was most common to tetracyclines (65%; no difference between ABF and conventional) and erythromycin [47%; higher in conventional (63%) than ABF (37%)], and no significant differences were found for the other 4 antimicrobials. Much fewer isolates were recovered during processing, making the data more uncertain, but resistance to chloramphenicol and nalidixic acid was more prevalent in preevisceration samples from ABF than conventional carcasses. FQ resistant isolates were also detected in samples from both ABF and conventional cohorts. AMU was not measured on the conventional farms, nor clearly validated for the ABF farms, and the study does not provide evidence comparing low-dose, long term AMU with other modes of use.

Thakur and Gebreyes (2005): [59] Similar to the study of Tadesse et al (above), the objective of this observational study was to compare the prevalence and antimicrobial resistance of *Campylobacter* species in swine reared in conventional and (ABF) production systems. Information on AMU was collected from swine producers. In in both nursery and finishing stages of the conventional system, oxytetracycline (400 g/ton) and tylosin (40g/ton) were added to the feed, and penicillin and ceftiofur administered by injection. In the ABF system, no antimicrobials were used after weaning. Samples were collected from 21 groups of pigs over 2 years, but the number of farm sites included in each group is not clear. *Campylobacter coli* constituted 99% of the isolates detected. Isolates were tested with the agar dilution method for susceptibility to six antimicrobials, and resistance was most prevalent to tetracycline (66%) and erythromycin (54%). Frequency of resistance to these two antimicrobials was significantly higher among conventional herds (83.4% for tetracycline and 77% for erythromycin) than among ABF herds (56.2% for tetracycline and 34.5% for erythromycin). Multidrug-resistant *C. coli* strains were detected in both the conventional (7%) and ABF (4%) herds. In addition to the higher prevalence of resistance observed in the conventional farms, the authors noted the high prevalence of antimicrobial-resistant *C. coli* in both conventional and ABF pig production systems, pointing to the apparent ability of antimicrobial-resistant *Campylobacter* to persist in swine farm environments regardless of levels of antimicrobial use. Antimicrobial use was not directly measured on the conventional farms, where both therapeutic and growth promotant use occurred, therefore no information is available on the relative impacts of low-dose/long term AMU versus other modes of administration.

Varga et al. (2009): This observational study aimed to identify potential associations between reported AMU and AMR of fecal and environmental *Salmonella* recovered from 60 Alberta finishing swine farms. The study was based upon an earlier study of *Salmonella* prevalence in which some information on AMU had been collected by survey of producers and veterinarians. AMU was categorized by drug class and route of administration (feed, injection, water), but not by dose. Analyses were restricted to 6 antimicrobials for which the prevalence of resistance at the pen level was at least 4%. Relatively

complex multivariate models were used and included adjustment for clustering. In-feed use of tylosin in finishers was associated with increased odds of resistance of *Salmonella* to ampicillin, streptomycin, and multiple antimicrobials. Injectable penicillin use in growers was associated with decreased odds of resistance of *Salmonella* to streptomycin, kanamycin, and multiple antimicrobials. In finishers, injectable penicillin use in finishers was associated with decreased odds of resistance to ampicillin and chloramphenicol. A limitation of the study was that the analysis could not address AMR by serotype due to the large diversity of serotypes among the available isolates. Previous studies have indicated that AMR can vary markedly among serotypes within farms, and hence from animals with the same antimicrobial exposure history.[65] The overall conclusion of the authors was that AMU in pig production was inconsistently associated with AMR in *Salmonella* from finishing swine, but that moderate to high variation in AMR prevalence at pen and farm levels suggested that interventions at the pen and farm levels might be beneficial in reducing the prevalence of AMR.

Wiuff et al. (2003): [60] This short term study compared FQ resistance in pigs experimentally infected with *Salmonella* prior to exposure to FQ administered both orally, and parenterally at 3 doses. FQ resistance developed rapidly and at high prevalence among the coliform flora independent of route of administration, dose or time of initiation of the treatment. Prevalence of FQ resistance among the introduced *Salmonella* was lower with the intramuscular route, and at doses 3 or 6 times the recommended dose. The authors concluded that resistance is readily selected by treatment with enrofloxacin at the recommended dose, and that small changes in the active drug concentrations can change the intensity of selection. However, the study addresses only therapeutic use, and FQ cannot be administered for growth promotion in the USA.

When delineating the original scope of this appraisal, we did not intend to pursue the initiatives described in this section of supplementary information. Our decision to do so was prompted by the paucity of informative original studies cited by the key sources, particularly with respect to swine. Our efforts to identify other relevant sources were not comprehensive (due to resource limitations), but structured with the hope of efficiently locating highly relevant original studies by 1) screening studies that cited 11 sources that we identified to be most informative among the original citations of the key documents; 2) screening studies that cited a pivotal study of AMU and AMR in swine published in 1998; 3) screening additional references cited in the final FGA Guidance 209 document; 4) reviewing studies identified by the expert review panel. Generally, these sources reported positive associations between AMU and AMR, but also bear testimony to the biological complexity of these relationships and the challenges of researching it. Five experimental studies specifically designed to compare low-dose long term administration of antimicrobials with therapeutic use [53,61-64] consistently observed an increase in AMR associated with AMU, but reported no differences among modes of AMU. We remain unable to identify any substantial body of evidence indicating that low-dose/long term administration of antimicrobials is more likely to promote AMR than use by other modes. In the absence of this evidence, strategies that aim to reduce aggregate AMU in food animals, rather than specific uses, appears the most justifiable goal.

Citation index search based on key swine papers

We were surprised that none of the key sources had cited a series of detailed studies conducted in Canada that made a relatively comprehensive effort to measure AMU on swine farms and its relationship with resistance in *E. coli*.[14,65-67] We used the capstone paper of this series ([14] in the Web of Science citation index to identify subsequent papers that might provide specific information about the relationship between AMU and resistance in swine. By this means, 55 subsequent papers were identified, and our initial screening process identified 17 primary studies which were evaluated using the appraisal tool for descriptive and analytical studies. By this process, we identified 5 papers that compared antimicrobial use and resistance in food animals, which we

reviewed in detail along with the capstone publications. Key observations and inferences are summarized below:

Dunlop et al (1998): [14,65-67] These studies presented analyses performed on data collected from 34 purposively selected swine farms in Ontario, Canada. Antimicrobial use in feed, water, and by injection was measured on all farms for 2 months and patterns of use and rates of treatment described. Twenty fecal samples from finisher hogs within 1 month of slaughter were collected on two occasions 1 month apart from each study farm. Prevalences of resistance among fecal *E. coli* and variability of resistance among and within farms were described. Logistic regression models of antimicrobial use and resistance in fecal *E. coli* of swine tested the hypothesis that the use of antimicrobials at the group and individual-animal levels was associated with prevalence of resistance to antimicrobials (carbadox, gentamicin, nitrofurantoin, spectinomycin, sulfisoxazole, tetracyclines) that were used on the farm, and also to other antimicrobials. The final models included the antimicrobial treatment covariates and farm management factors that best explained the variation in resistance to these drugs. Results indicated that antimicrobial use during swine production was associated with increased resistance to those antimicrobials among fecal *E. coli* of finisher pigs. Furthermore, medication of feed was associated with resistance in models for 7 antimicrobials, while individual animal treatment was significant in only one model. It was concluded that individual treatment with antimicrobials had significantly less impact on the promotion or maintenance of resistance than did medication of rations. However, explicit comparisons of dose and duration of exposure, as opposed to route of administration, were not reported. Furthermore, antimicrobial use was not invariably associated with increased resistance and there was no strong evidence that prevalence of *E. coli* resistance to nitrofurantoin or spectinomycin was higher on farms that used these antimicrobials. Also for the growth promotant carbadox, although resistance was associated with use, prevalence of resistance was low. Resistance was also observed on 5 farms that did not use antimicrobials (including 47% resistance to tetracyclines), and the authors inferred that factors or substances other than antimicrobial exposure may be important in maintaining antimicrobial resistance in populations of bacteria. The authors cautiously advised that, although very plausible, the cross-sectional nature of the study limits the ability to infer that the relationships were causal. Furthermore they state that they were unable to fully control for the confounding effects of individual treatments on group treatments because individual fecal samples from finisher pigs and individual antimicrobial treatment rates were aggregated to the herd level.

Alali et al. (2009): [28] This longitudinal ecological study in a prison system examined the relationship between the prevalence of resistant commensal *E. coli* in human wastewater and swine composite fecal samples arising from group-level cohorts of humans and swine and concurrent antibiotic use in the host species. Fecal and wastewater samples were collected over 36 months and related to monthly antimicrobial use data. Human antimicrobial use was based on medical records. No formal antibiotic recording system was employed. Data on feed grade antibiotic use was derived from records of diet formulations and aggregated with monthly antibiotic dispensing records to estimate aggregate use by antibiotic class. Among swine workers, odds of tetracycline resistance were increased significantly for tetracycline use at the third quartile and above of mean monthly dosage (MMD) in pigs (OR = 1.8) compared to months with no use. The odds of tetracycline resistance in swine increased significantly with chlortetracycline use in medicated feed for the upper tercile of MMD category (OR = 2.9) as compared to no use. The authors concluded that ‘the overall effects of concurrent human and swine antibiotic use on resistant *E. coli* levels were inconsistent and modest in this study.’ However, the observations support the generally accepted view that aggregate exposure to antibiotics in populations appears to be related to resistance measured at the population level, and underline the complexity of these relationships. Because of the aggregated nature of the exposure data, no information on specific patterns of use is provided.

Checkley et al. (2010): [68] This randomized controlled trial evaluated associations between antimicrobial use and the prevalence of antimicrobial resistance in fecal *E. coli* from feedlot cattle. Specifically they compared metaphylactic (injectable) and prophylactic (in feed) antimicrobial use in groups of cattle and the prevalence of antimicrobial resistant fecal *E. coli* at several points during the feeding period. Animals were randomized to 3 treatments: control (no antimicrobials); oxytetracycline in feed at 110 g/ton; and long-acting oxytetracycline injection of 20 mg/kg body weight subcutaneously. Other antimicrobial treatments were conducted according to normal procedures to treat illnesses on the feedlot. Treatments were allocated by pen with 8 pens per treatment group. MICs to 7 antimicrobials were determined on 3 generic *E. coli* isolates per animal. Increased prevalence of resistance was observed in both treatment groups compared to controls soon after arrival, but differences were short term and no differences were detected preslaughter. Oxytetracycline administration in feed was associated with an increased proportion of animals with 1 or more fecal *E. coli* isolates resistant to tetracycline and sulphamethoxazole. Resistance increased in all groups regardless of treatment between arrival and preslaughter sampling, at which time no differences in resistance among groups were found.

O'Connor et al. (2008): [69] This randomized controlled trial in a research feedlot compared changes in the prevalence of resistance to antibiotics in *E. coli* from cattle receiving oxytetracycline injected subcutaneously in addition to in-feed chlortetracycline, with *E. coli* from cattle receiving only in-feed chlortetracycline. Resistance to 19 antibiotics was examined. Injectable oxytetracycline was significantly associated increased prevalence of resistance only to chloramphenicol and sulfisoxazole. The study did not include an untreated control group, and injected and uninjected animals were housed together. Due to the lack of a control group, and the fact that all animals received the same antimicrobial in feed, the study provides no information regarding relative impacts on resistance of therapeutic versus subtherapeutic uses in animal populations.

Rosengren et al. (2007):[70] This cross-sectional observational study examined resistance of *E. coli* in grow finish pigs to 16 antimicrobials in relation to historic records of AMU on the farms. The data were derived from farm records for AMU in feed and water. Data for each exposure of suckling, nursery, grow-finish pig, or sows in the previous 12 months included the product used, number of pigs exposed, and duration of exposure. Antimicrobial use was aggregated by class and described as the exposure per pig-day (or 1,000 or 100,000 pigs days), but parenteral AMU was not measured. The statistical models were based on binomial models using generalized estimating equations to adjust for clustering. Models were constructed for 7 compounds, only one of which (tetracyclines) was used in the feed or water of the farms. Six of the seven antimicrobial resistance outcomes had significant AMU risk factors of which 3 were associations with exposure to drugs in the same antimicrobial class. In contrast, resistances to kanamycin and streptomycin were not associated with use of these classes of drugs. Two models indicated negative associations between AMU and AMR. Overall the study supports a positive association between aggregate AMU and AMR, but does not provide direct comparison of different modes of AMU by dose or purpose.

Varga et al. (2009): [71] This cross-sectional observational study employed multivariable models to examine associations between reported patterns of antimicrobial use on 90 swine farms in Western Canada and antimicrobial resistance in *E. coli* isolated from pen fecal samples. Antimicrobial use was assessed with a one-time questionnaire of producers. Analysis was restricted to 7 antimicrobials to which resistance was detected in at least 5% of samples, and was reported only for the 78 farrow-to-finish farms in the study. AMU variables were binary in the analysis, representing reported group or specific AMU via feed and water, or individual AMU via injection, in various phases of pig production. Specific data on dose or duration of administration were not included. Similarly outcome variables were also binary. Associations between reported AMU and resistance to individual antimicrobials were examined with independent logistic regression models for each antimicrobial. Two models also explored

multiple resistance outcomes. Relatively complex multivariate modeling was use to attempt to account for repeated observations and the hierarchical structure of the data. As the study modeled a large number of exposures and outcomes, the results are complex, but overall suggested that AMU on these farms, and particularly in-feed AMU, was positively associated with resistance in *E. coli* isolates. In feed use was also positively associated with AMR in both models examining multiple resistance. They also found use of an injectable trimethoprim–sulfonamide in nursery pigs and injectable oxytetracycline in growers was negatively associated with resistance, an observation the authors considered as inconsistent with most theory and evidence regarding antimicrobial resistance. A limitation of the study, indicated by the authors, was that no information was obtained about the rationale for AMU (i.e., therapeutic or growth promotion), dose or duration of treatment, changes in AMU over time, or dose regimens. The study supports that aggregate AMU is associated with resistance, particularly in feed administration, but does not explicitly examine dose and duration relationships due to limited measurement of AMU.

Risk assessments of low dose AMU in food animals on public health and animal health

None of the 4 key documents (FDA-209, PAMTA, PEW and KAW) employed systematic review methods or described the processes by which they selected their sources. Although PAMTA and KAW are clearly politically (moreso than scientifically) oriented sources, PEW and FDA-209 are more likely to be perceived as ostensibly 'scientific' documents. For completeness, we identified several comprehensive quantitative assessments of the risk of human treatment failures related to specific practices of AMU in food animals. [72-76]. These studies have uniformly estimated the risks to be extremely (or 'vanishingly') small. Published quantitative risk assessments that address AMU in animals in relation to human health were recently reviewed in detail.[77] The studies included involved both low-dose/long term use and therapeutic use for compounds considered of 'critical importance' to human health. While some studies estimated relatively high burdens of resistant *Campylobacter* and *Salmonella* infections that might be attributable to AMU in animals, they did not assess any specific modes of AMU. McEwen (2012) pointed out that in all cases important data gaps were recognized and contributed substantial uncertainty to the model outcomes. However, we did not identify any published quantitative risk assessment that predicts substantial risk of human clinical treatment failures associated with any specific antimicrobial use in food animals.

Furthermore, some relatively recent studies present evidence that use of antimicrobials at low doses to control endemic diseases in food animals can be associated with improvements to animal health that translate into less carcass contamination during processing, and thereby may deliver human health benefits through reduced risk of exposure to pathogens.[78-80] This body of work is currently very small, but the implications are substantial and more research is indicated to confirm or refute these observations.

Updated reference list from Final FDA guidance 209

This appraisal was first conducted using the FDA draft guidance 209 document. The reference list of the final guidance 209 document included 14 papers not included in the draft version. Of these 14, 11 were original studies.[71,81-90] These studies were screened to evaluate their stated objectives and methods and major outcomes. Generally all studies presented information indicating positive associations between AMU and AMR. However, because none of the studies compared low-dose/long-term antimicrobial use with other modes of AMU they were not assessed further.

Additional references suggested by the expert review panel

The expert panel convened to review the final draft of this report identified some additional studies relevant to the core question of the relative impact of low-dose/long term use of antimicrobials on emergence of AMR.

These included a series of publications that directly compared 'therapeutic' (220 ppm for 14 days followed by 71 days without antimicrobials); 'subtherapeutic' (27.5 ppm for 85 days) of chlortetracycline in feed, and no administration, on resistance in coliforms and anaerobic bacteria in the feces or intestinal tracts of pigs.[61-64] This set of studies compared the effects of these treatments in 2 sub-populations of growing pigs that originated from unrelated breeding herds with different histories of AMU [one herd being antibiotic free (ABF) for 8 years; the other conventional herd regularly using antimicrobials, particularly chlortetracycline, in feed]. All pigs were housed together with treatments separated by empty in a new facility, and pigs were sampled at several time points before, during, and after application of the treatments. Salient observations were that resistance was less common in pigs sourced from the ABF herd before treatments; that administration of chlortetracycline at either dose increased the percentage of resistant organisms, particularly in the ABF sourced pigs; that removal of antimicrobials did not result in any immediate of marked reduction in resistance; and that patterns of resistance were complex and influenced by factors other than the use of antimicrobials in feed, including sampling factors (fecal vs. intestinal content; coliforms vs. anaerobes). Notably, in the study of fecal coliforms, it was concluded that return of pigs from a therapeutic treatment regime to antimicrobial free feed did not result in an observable reduction in AMR, and that resistance (including multiple resistance) tended to be less among isolates from the subtherapeutic treatment group compared to the therapeutic group.[64] It is noted that these studies were conducted on relatively small populations of pigs (36-40) and that the joint housing of treatment groups likely led to some transmission of organism among the groups. Acknowledging these limitations, the data did not indicate that the low-dose, longer term use led to more resistance than short term therapeutic use

Summary and Conclusions

Availability of antimicrobials for use in human medicine, veterinary medicine and food animal production is a privilege rather than a right. Given the current high level of scrutiny of antimicrobial use in all arenas, it is remarkable to think that in the 1950s antimicrobials became available for over-the-counter sale and use in food animal populations in many developed countries. Moreover, low-dose/long duration feeding of antimicrobials was widely adopted in food animal production after it was recognized that this could enhance growth rate and feed efficiency in poultry and swine. At that time, the phenomenon of antimicrobial resistance emerging consequent to antimicrobial use was already well established, albeit of less immediate clinical consequence. It is also notable that unrestricted over-the-counter sale of antimicrobials remains the norm for both humans and animals in many developing countries. Few would argue that this is wise, and the likelihood of imprudent use is inevitably a function of availability. However, it has been difficult to establish that antimicrobial use in food animals has been detrimental in terms of the incidence of clinical treatment failures in human infections. An extensive North American review in 2006 concluded that "the extent to which antibiotic use in food animals produces clinically important antibiotic resistant infections in humans is unknown".[91] This enduring uncertainty over the public health consequences of antimicrobial use in animals has frustrated groups who have avidly and conscientiously pursued greater regulation of antimicrobial use in food animals in the USA.

The vast majority the papers cited by these key sources (FDA-209, PAMTA, PEW, KAW) contain no primary data to support the contention that low-dose/long term antimicrobial use exerts more selection pressure for resistant bacteria than therapeutic uses, or augments public health risks from antimicrobial resistant organisms. Generally, considerable shortcomings in design or reporting of these studies of AMU and AMR were prevalent. Prominent examples were the failure to comprehensively describe AMU in terms of drug, dose, duration and route of administration; failure to provide even minimal details on selection of subjects and age, housing and nutrition of research subjects; and superficial description of statistical methods and failure to even report confidence intervals in many studies. Among all the citations of the 4 core documents, only one study directly compared the effects of modes of AMU in a food animal species (poultry) on AMR in a zoonotic pathogen.[15] Specifically with respect to swine, we identified more analytical studies addressing the relationship between

AMU and AMR in swine that were overlooked by authors of the core documents (see Supplementary Information), than had been cited by them. However, these latter sources collectively bear testimony more to the complexity of the biological systems involved, than to any evident difference in AMR related to specific modes of AMU. The dearth of randomized controlled trials in the relevant literature is striking, and contributes to the enduring difficulty in drawing credible and robust inferences about this question. Our ultimate assessment is that available evidence is inadequate to provide any meaningful comparison of different modes of antimicrobial resistance in relation to the emergence of antimicrobial resistance in pathogens or commensals in commercial swine populations. In the absence of evidence indicating any differential effect among modes of antimicrobial use in food animals, the conservative position should be that reducing aggregate use of antimicrobials is the most appropriate goal.

We believe that there is broad consensus that reduction in aggregate use of antimicrobials in any arena will lessen the selection pressure for resistant organisms. Many, though not all, would also accept that the process of weighing policy options related to antimicrobial use in food animals should be evidence-based and include consideration of animal health and welfare, and environmental impacts, in addition to putative human health concerns. As we traverse the spectrum of 'therapy-metaphylaxis-prophylaxis-growth promotion' uses, enthusiasm for antimicrobial use in animals will understandably decline. If one assumes that any reduction of antimicrobial use is desirable (i.e., less is better) it seems logical to eliminate uses that are 'less necessary' or less justifiable in terms of benefits to animal health and welfare. A truly strategic approach would require understanding of the relative consequences (in terms of prevalence of resistant organisms with implications for public health, and their actual clinical impact) of these different strategies for deploying antimicrobials in food animal populations. Optimization of antimicrobial use in food animals also requires better definition of practices of greatest value to maintaining animal health and well-being.

Both the Swedish and Danish bans on antimicrobial growth promotants in pigs had documented adverse consequences for pig health, most notably the increased incidence of enteric disease in weaned pigs and increased need for therapeutic antimicrobials. [2,4,92] More recently, antimicrobial use in fattening pigs in the Netherlands also increased markedly (around 50%) from 2006 to 2008 following the removal of antimicrobials for growth promotion.[c] Given the consistency of this outcome in 3 countries, the expectation should be that aggregate AMU in swine would increase in the immediate (1 – 2 years) aftermath of removal of growth promotant claims in the USA. In both Denmark and Sweden, the impact of removal of growth promotant antimicrobials on the health of finishing pigs appears to have been minimal. We should not ignore the data from Denmark showing that the lowest rate of aggregate antimicrobial use in the Danish swine industry occurred in 1999 after the withdrawal of growth promotants from finishing pigs, but before the their withdrawal from weaned pigs. [1] Recognition of the particular vulnerability, and therefore particular needs, of the weaned pig in relation to infectious disease control and prevention needs to be emphasized in discussions of strategic antimicrobial use in swine.

FDA guidance 209 will likely lead to the removal of most, if not all, growth promotant claims in the USA within 3 years for all ages of pigs. Considering the available scientific data, and the history of experiences in Europe, we propose that a more prudent path forward would have been the elimination (based on the precautionary principle), of use of antimicrobials for production purposes in feed of finishing pigs, but preservation of all existing therapeutic, metaphylactic and prophylactic options in weaned pigs (e.g., up to 10 weeks of age). This should substantially reduce aggregate antimicrobial use in the final 4 months prior to marketing when daily feed intake of pigs is highest, but would also maintain flexibility in health management during the most crucial 6-7 weeks of post-weaning life. However, as the regulatory changes already in motion are unlikely to be significantly altered, the focus of the industry and swine veterinary community needs to shift towards managing the probable effects on pig health and welfare. It is well documented that in Denmark cost of

[c] http://www.wageningenur.nl/en/Research-Results/Projects-and-programmes/MARAN-Antibiotic-usage.htm

production increased following the growth promotant ban, many producers (particularly small producers) exited the industry, and substantial changes to industry occurred in the effort to combat diarrheal diseases in weaned pigs (e.g., increased weaning age and widespread use of zinc in weaned pig diets). If low dose antimicrobial uses were similarly restricted in the United States, it is to be expected that similar health and production impacts would ensue. It is also feasible that the negative consequences could be more dramatic, given the overall younger age of weaning in the USA and less intensive veterinary oversight of many herds.

A key factor influencing the impact of expected regulatory changes on animal health will be the implementation strategies that are put in place by the FDA, particularly in relation to phasing out of over-the-counter sales and the need for veterinary oversight. The current document calls for more voluntary involvement of veterinarians in all uses of antimicrobials for food producing animals, but does not specify how this will be achieved. In Europe, all antimicrobials used for food producing animals are prescription only, and prescriptions are usually time limited to ensure frequent veterinary involvement on farms. For example, in Denmark pig farmers must sign a voluntary veterinary advisory service contract with a veterinarian stipulating that the veterinarian must visit the farm not less than 12 times a year. Veterinarians can prescribe antibiotics for no longer than 35 days for a site following a farm visit.[d] The stipulation for regular herd health visits to underpin prescription of veterinary medicines in Denmark was in place from 1994, or about 3 to 5 years prior to the removal of growth promotants from finishing or nursery pigs. Although the veterinary-client-patient relationship plays a central role in veterinary prescription drug use in the USA, it places much less stringent demands on veterinary oversight than in Europe. This present two major challenges that need to be anticipated in the USA – 1) availability of veterinarians to deal with increased incidence of disease, particularly enteric disease of weaned pigs, following restrictions on currently available products; and 2) veterinary manpower and cost to meet requirements for greater oversight and need for prescriptions.

[d] https://www.foedevarestyrelsen.dk/english/Animal/AnimalHealth/Veterinary_medicine/Pages/default.aspx

References

1. Aarestrup F. Sustainable farming: Get pigs off antibiotics. *Nature*. 2012;486(7404):465-466.

2. Vigre H, Larsen PB, Andreasen M, Christensen J, Jorsal SE. The effect of discontinued use of antimicrobial growth promoters on the risk of therapeutic antibiotic treatment in Danish farrow-to-finish pig farms. *Epidemiol Infect*. 2008;136(1):92-107.

3. Wierup M. The swedish experience of the 1986 year ban of antimicrobial growth promoters, with special reference to animal health, disease prevention, productivity, and usage of antimicrobials. *Microb Drug Resist*. 2001;7(2):183-190.

4. WHO. Impacts of antimicrobial growth promoter termination in Denmark. http://www.who.int/gfn/en/Expertsreportgrowthpromoterdenmark.pdf. Updated 2003. Accessed 6/21/2011, 2011.

5. Alban L, Dahl J, Andreasen M, Petersen JV, Sandberg M. Possible impact of the "yellow card" antimicrobial scheme on meat inspection lesions in Danish finisher pigs. *Prev Vet Med*. 2013;108(4):334-341.

6. Food and Drug Administration. The judicious use of medically important antimicrobial drugs in food-producing animals. http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM216936.pdf. Updated 6/28/2010. Accessed 6/6/2011, 2011.

7. 111th Congress. H.R. 1549 (Preservation of Antibiotics for Medical Treatment Act of 2009). . 2009;2011(6/6/2011). Accessed 6/6/2011.

8. McVeigh T. Scientists: Overuse of antibiotics in animal agriculture endangers humans. *The Guardian*. 9/9/2012. Available from: http://www.theguardian.com/science/2012/sep/19/scientists-antibiotics-animal-agriculture.

9. Gunter A. Public health warning: This meat may contain life-threatening antibiotic resistant bacteria. *Huffington Post*. May 1, 2013. Available from: http://www.huffingtonpost.com/andrew-gunther/public-health-warning_b_3188174.html.

10. Pew Charitable Trusts. Putting meat on the table: Industrial farm animal production in America. http://www.ncifap.org/bin/s/a/PCIFAPSmry.pdf. Updated 2008. Accessed 6/6/2011.

11. Keep Antibiotics Working.com. Annotated Bibliography http://www.keepantibioticsworking.com/new/indepth_keyevid.cfm. Accessed 6/9/2011.

12. Steinberg EP, Eknoyan G, Levin NW, et al. Methods used to evaluate the quality of evidence underlying the national kidney foundation-dialysis outcomes quality initiative clinical practice guidelines: Description, findings, and implications. *Am J Kidney Dis*. 2000;36(1):1-11.

13. Shamliyan TA, Kane RL, Ansari MT, et al. Development quality criteria to evaluate nontherapeutic studies of incidence, prevalence, or risk factors of chronic diseases: Pilot study of new checklists. *J Clin Epidemiol*. 2011;64(6):637-657.

14. Dunlop RH, McEwen SA, Meek AH, Clarke RC, Black WD, Friendship RM. Associations among antimicrobial drug treatments and antimicrobial resistance of fecal *Escherichia coli* of swine on 34 farrow-to-finish farms in Ontario, Canada. *Prev Vet Med*. 1998;34(4):283-305.

15. Ladely SR, Harrison MA, Fedorka-Cray PJ, Berrang ME, Englen MD, Meinersmann RJ. Development of macrolide-resistant *Campylobacter* in broilers administered subtherapeutic or therapeutic concentrations of tylosin. *J Food Prot*. 2007;70(8):1945-1951.

16. Akwar TH, Poppe C, Wilson J, et al. Risk factors for antimicrobial resistance among fecal *Escherichia coli* from residents on forty-three swine farms. *Microb Drug Resist*. 2007;13(1):69-76.

17. Berrang ME, Ladely SR, Meinersmann RJ, Fedorka-Cray PJ. Subtherapeutic tylosin phosphate in broiler feed affects *Campylobacter* on carcasses during processing. *Poult Sci*. 2007;86(6):1229-1233.

18. Endtz HP, Ruijs GJ, van Klingeren B, Jansen WH, van der Reyden T, Mouton RP. Quinolone resistance in *Campylobacter* isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. *J Antimicrob Chemother*. 1991;27(2):199-208.

19. Smith KE, Besser JM, Hedberg CW, et al. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992-1998. investigation team. *N Engl J Med*. 1999;340(20):1525-1532.

20. Wassenaar TM. Use of antimicrobial agents in veterinary medicine and implications for human health. *Crit Rev Microbiol*. 2005;31(3):155-169.

21. Funk JA, Lejeune JT, Wittum TE, Rajala-Schultz PJ. The effect of subtherapeutic chlortetracycline on antimicrobial resistance in the fecal flora of swine. *Microb Drug Resist*. 2006;12(3):210-218. http://www.liebertonline.com/doi/pdf/10.1089/mdr.2006.12.210.

22. Kieke AL, Borchardt MA, Kieke BA, et al. Use of streptogramin growth promoters in poultry and isolation of streptogramin-resistant *Enterococcus faecium* from humans. *J Infect Dis*. 2006;194(9):1200-1208.

23. Levy SB, FitzGerald GB, Macone AB. Changes in intestinal flora of farm personnel after introduction of a tetracycline-supplemented feed on a farm. *N Engl J Med*. 1976;295(11):583-588.

24. Peak N, Knapp CW, Yang RK, et al. Abundance of six tetracycline resistance genes in wastewater lagoons at cattle feedlots with different antibiotic use strategies. *Environ Microbiol*. 2007;9(1):143-151. http://onlinelibrary.wiley.com/doi/10.1111/j.1462-2920.2006.01123.x/full.

25. Smith JL, Drum DJ, Dai Y, et al. Impact of antimicrobial usage on antimicrobial resistance in commensal *Escherichia coli* strains colonizing broiler chickens. *Appl Environ Microbiol*. 2007;73(5):1404-1414.

26. Unicomb LE, Ferguson J, Stafford RJ, et al. Low-level fluoroquinolone resistance among *Campylobacter jejuni* isolates in Australia. *Clin Infect Dis*. 2006;42(10):1368-1374.

27. van Duijkeren E, Houwers DJ, Schoormans A, et al. Transmission of methicillin-resistant *Staphylococcus intermedius* between humans and animals. *Vet Microbiol*. 2008;128(1-2):213-215.

28. Alali WQ, Scott HM, Christian KL, Fajt VR, Harvey RB, Lawhorn DB. Relationship between level of antibiotic use and resistance among *Escherichia coli* isolates from integrated multi-site cohorts of humans and swine. *Prev Vet Med*. 2009;90(3-4):160-167.

29. Gardner P, Smith DH, Beer H, Moellering RC,Jr. Recovery of resistance (R) factors from a drug-free community. *Lancet*. 1969;2(7624):774-776.

30. D'Costa VM, King CE, Kalan L, et al. Antibiotic resistance is ancient. *Nature*. 2011;477(7365):457-461.

31. Bhullar K, Waglechner N, Pawlowski A, et al. Antibiotic resistance is prevalent in an isolated cave microbiome. *PLoS One*. 2012;7(4):e34953.

32. Martinez JL. Natural antibiotic resistance and contamination by antibiotic resistance determinants: The two ages in the evolution of resistance to antimicrobials. *Front Microbiol*. 2012;3:1.

33. Smith K, Bender J, Osterholm M. Antimicrobial resistance in animals and relevance to human infections. In: Nachamkin I, Blaser M, eds. *Campylobacter.* 2nd ed. Washington, DC: ASM Press; 2000.

34. Werner G, Coque TM, Hammerum AM, et al. Emergence and spread of vancomycin resistance among enterococci in europe. *Euro Surveill*. 2008;13(47):19046.

35. Nosocomial enterococci resistant to vancomycin—United states, 1989–1993. *Morbidity and Mortality Weekly Reports*. 1993;42:597-599.

36. Goossens H. Spread of vancomycin-resistant enterococci: Differences between the United States and Europe. *Infect Control Hosp Epidemiol*. 1998;19(8):546-551.

37. Arias CA, Murray BE. The rise of the *Enterococcus*: Beyond vancomycin resistance. *Nat Rev Microbiol*. 2012;10(4):266-278. .

38. Willems RJ, Homan W, Top J, et al. Variant esp gene as a marker of a distinct genetic lineage of vancomycin-resistant *Enterococcus faecium* spreading in hospitals. *Lancet*. 2001;357(9259):853-855.

39. Heikens E, van Schaik W, Leavis HL, Bonten MJ, Willems RJ. Identification of a novel genomic island specific to hospital-acquired clonal complex 17 *Enterococcus faecium* isolates. *Appl Environ Microbiol*. 2008;74(22):7094-7097.

40. McDonald LC, Rossiter S, Mackinson C, et al. Quinupristin-dalfopristin-resistant *Enterococcus faecium* on chicken and in human stool specimens. *N Engl J Med*. 2001;345(16):1155-1160.

41. Jackson CR, Fedorka-Cray PJ, Barrett JB, Hiott LM, Woodley TA. Prevalence of streptogramin resistance in enterococci from animals: Identification of vatD from animal sources in the USA. *Int J Antimicrob Agents*. 2007;30(1):60-66.

42. Cattoir V, Leclercq R. Twenty-five years of shared life with vancomycin-resistant enterococci: Is it time to divorce? *J Antimicrob Chemother*. 2013;68(4):731-42.. .

43. Hummel R, Tschape H, Witte W. Spread of plasmid-mediated nourseothricin resistance due to antibiotic use in animal husbandry. *J Basic Microbiol*. 1986;26(8):461-466.

44. Goforth RL, Goforth CR. Appropriate regulation of antibiotics in livestock feed. *Boston College Environmental Affairs Law Review*. 2000;28(1):39.

45. Aarestrup F, Seyfarth A. Effect of intervention on the occurrence of antimicrobial resistance. http://www.ncbi.nlm.nih.gov/pubmed/10822862. Updated 2000. Accessed 6/21/2011.

46. Catry B, Laevens H, Devriese LA, Opsomer G, De Kruif A. Antimicrobial resistance in livestock. *J Vet Pharmacol Ther*. 2003;26(2):81-93.

47. Khachatourians GG. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. *CMAJ*. 1998;159(9):1129-1136. http://www.cmaj.ca/content/159/9/1129.full.pdf+html.

48. Shea KM, American Academy of Pediatrics Committee on Environmental Health, American Academy of Pediatrics Committee on Infectious Diseases. Nontherapeutic use of antimicrobial agents in animal agriculture: Implications for pediatrics. *Pediatrics*. 2004;114(3):862-868. http://pediatrics.aappublications.org/content/114/3/862.full.

49. Smolinski MS, Hamburg MA, Lederberg J. *Microbial threats to health: Emergence, detection, and response*. Washington, D.C.: National Academies Press; 2003:367. Access electronic version http://books.nap.edu/books/030908864X/html/.

50. Levy SB. Antibiotic use for growth promotion in animals: Ecologic and public health consequences. *Journal of food protection (USA)*. 1987;50(7):616-620.

51. Young I, Rajic A, Wilhelm BJ, Waddell L, Parker S, McEwen SA. Comparison of the prevalence of bacterial enteropathogens, potentially zoonotic bacteria and bacterial resistance to antimicrobials in organic and conventional poultry, swine and beef production: A systematic review and meta-analysis. *Epidemiol Infect*. 2009;137(9):1217-1232.

52. Mathew AG, Saxton AM, Upchurch WG, Chattin SE. Multiple antibiotic resistance patterns of *Escherichia coli* isolates from swine farms. *Appl Environ Microbiol*. 1999;65(6):2770-2772.

53. Wagner BA, Straw BE, Fedorka-Cray PJ, Dargatz DA. Effect of antimicrobial dosage regimen on *Salmonella* and *Escherichia coli* isolates from feeder swine. *Appl Environ Microbiol*. 2008;74(6):1731-1739.

54. Bibbal D, Dupouy V, Ferre JP, et al. Impact of three ampicillin dosage regimens on selection of ampicillin resistance in enterobacteriaceae and excretion of blaTEM genes in swine feces. *Appl Environ Microbiol*. 2007;73(15):4785-4790.

55. Harada K, Asai T, Ozawa M, Kojima A, Takahashi T. Farm-level impact of therapeutic antimicrobial use on antimicrobial-resistant populations of *Escherichia coli* isolates from pigs. *Microb Drug Resist*. 2008;14(3):239-244.

56. Juntunen P, Heiska H, Olkkola S, Myllyniemi AL, Hanninen ML. Antimicrobial resistance in *Campylobacter coli* selected by tylosin treatment at a pig farm. *Vet Microbiol*. 2010;146(1-2):90-97.

57. Literak I, Dolejska M, Rybarikova J, et al. Highly variable patterns of antimicrobial resistance in commensal *Escherichia coli* isolates from pigs, sympatric rodents, and flies. *Microb Drug Resist*. 2009;15(3):229-237.

58. Tadesse DA, Bahnson PB, Funk JA, et al. Prevalence and antimicrobial resistance profile of *Campylobacter* spp. isolated from conventional and antimicrobial-free swine production systems from different U.S. regions. *Foodborne Pathog Dis*. 2011;8(3):367-374.

59. Thakur S, Gebreyes WA. Prevalence and antimicrobial resistance of *Campylobacter* in antimicrobial-free and conventional pig production systems. *J Food Prot*. 2005;68(11):2402-2410.

60. Wiuff C, Lykkesfeldt J, Svendsen O, Aarestrup FM. The effects of oral and intramuscular administration and dose escalation of enrofloxacin on the selection of quinolone resistance among *Salmonella* and coliforms in pigs. *Res Vet Sci*. 2003;75(3):185-193.

61. Dawson KA, Langlois BE, Stahly TS, Cromwell GL. Antibiotic resistance in anaerobic and coliform bacteria from the intestinal tract of swine fed therapeutic and subtherapeutic concentrations of chlortetracycline. *J Anim Sci*. 1984;58(1):123-131.

62. Dawson KA, Langlois BE, Stahly TS, Cromwell GL. Multiple antibiotic resistance in fecal, cecal and colonic coliforms from pigs fed therapeutic and subtherapeutic concentrations of chlortetracycline. *J Anim Sci*. 1983;57(5):1225-1234.

63. Langlois BE, Cromwell GL, Stahly TS, Dawson KA, Hays VW. Antibiotic resistance of fecal coliforms after long-term withdrawal of therapeutic and subtherapeutic antibiotic use in a swine herd. *Appl Environ Microbiol*. 1983;46(6):1433-1434.

64. Langlois BE, Dawson KA, Stahly TS, Cromwell GL. Antibiotic resistance of fecal coliforms from swine fed subtherapeutic and therapeutic levels of chlortetracycline. *J Anim Sci*. 1984;58(3):666-674.

65. Dunlop RH, McEwen SA, Meek AH, Friendship RA, Clarke RC, Black WD. Antimicrobial drug use and related management practices among Ontario swine producers. *Can Vet J*. 1998;39(2):87-96.

66. Dunlop RH, McEwen SA, Meek AH, Black WD, Clarke RC, Friendship RM. Individual and group antimicrobial usage rates on 34 farrow-to-finish swine farms in Ontario, Canada. *Prev Vet Med*. 1998;34(4):247-264.

67. Dunlop RH, McEwen SA, Meek AH, Black WD, Friendship RM, Clarke RC. Prevalences of resistance to seven antimicrobials among fecal *Escherichia coli* of swine on thirty-four farrow-to-finish farms in Ontario, Canada. *Prev Vet Med*. 1998;34(4):265-282.

68. Checkley SL, Campbell JR, Chirino-Trejo M, Janzen ED, Waldner CL. Associations between antimicrobial use and the prevalence of antimicrobial resistance in fecal *Escherichia coli* from feedlot cattle in western canada. *Can Vet J*. 2010;51(8):853-861.

69. O'Connor AM, Poppe C, McEwen SA. Changes in the prevalence of resistant *Escherichia coli* in cattle receiving subcutaneously injectable oxytetracycline in addition to in-feed chlortetracycline compared with cattle receiving only in-feed chlortetracycline. *Can J Vet Res*. 2002;66(3):145-150.

70. Rosengren LB, Waldner CL, Reid-Smith RJ, Dowling PM, Harding JC. Associations between feed and water antimicrobial use in farrow-to-finish swine herds and antimicrobial resistance of fecal *Escherichia coli* from grow-finish pigs. *Microb Drug Resist*. 2007;13(4):261-269.

71. Varga C, Rajic A, McFall ME, et al. Associations between reported on-farm antimicrobial use practices and observed antimicrobial resistance in generic fecal Escherichia coli isolated from Alberta finishing swine farms. *Prev Vet Med*. 2009;88(3):185-192.

72. Cox LA, Jr, Popken DA, Mathers JJ. Human health risk assessment of penicillin/aminopenicillin resistance in enterococci due to penicillin use in food animals. *Risk Anal*. 2009;29(6):796-805.

73. Cox LA, Jr, Popken DA. Quantifying human health risks from virginiamycin used in chickens. *Risk Anal*. 2004;24(1):271-288.

74. Hurd HS, Doores S, Hayes D, et al. Public health consequences of macrolide use in food animals: A deterministic risk assessment. *J Food Prot*. 2004;67(5):980-992.

75. Hurd HS, Malladi S. A stochastic assessment of the public health risks of the use of macrolide antibiotics in food animals. *Risk Anal*. 2008;28(3):695-710.

76. Cox LA,Jr, Popken DA. Assessing potential human health hazards and benefits from subtherapeutic antibiotics in the united states: Tetracyclines as a case study. *Risk Anal*. 2010;30(3):432-457.

77. McEwen SA. Quantitative human health risk assessments of antimicrobial use in animals and selection of resistance: A review of publicly available reports. *Rev Sci Tech*. 2012;31(1):261-276.

78. Russell SM. The effect of airsacculitis on bird weights, uniformity, fecal contamination, processing errors, and populations of campylobacter spp. and *Escherichia coli. Poult Sci*. 2003;82(8):1326-1331.

79. Singer RS, Hofacre CL. Potential impacts of antibiotic use in poultry production. *Avian Dis*. 2006;50(2):161-172.

80. Hurd HS, Brudvig J, Dickson J, et al. Swine health impact on carcass contamination and human foodborne risk. *Public Health Rep*. 2008;123(3):343-351.

81. Alali WQ, Scott HM, Harvey RB, Norby B, Lawhorn DB, Pillai SD. Longitudinal study of antimicrobial resistance among *Escherichia coli* isolates from integrated multisite cohorts of humans and swine. *Appl Environ Microbiol*. 2008;74(12):3672-3681.

82. Harvey R, Funk J, Wittum TE, Hoet AE. A metagenomic approach for determining prevalence of tetracycline resistance genes in the fecal flora of conventionally raised feedlot steers and feedlot steers raised without antimicrobials. *Am J Vet Res*. 2009;70(2):198-202.

83. Vieira AR, Houe H, Wegener HC, Lo Fo Wong DM, Emborg HD. Association between tetracycline consumption and tetracycline resistance in *Escherichia coli* from healthy Danish slaughter pigs. *Foodborne Pathog Dis*. 2009;6(1):99-109.

84. Sharma R, Munns K, Alexander T, et al. Diversity and distribution of commensal fecal *Escherichia coli* bacteria in beef cattle administered selected subtherapeutic antimicrobials in a feedlot setting. *Appl Environ Microbiol*. 2008;74(20):6178-6186.

85. Alexander TW, Inglis GD, Yanke LJ, et al. Farm-to-fork characterization of *Escherichia coli* associated with feedlot cattle with a known history of antimicrobial use. *Int J Food Microbiol*. 2010;137(1):40-48.

86. Sapkota AR, Hulet RM, Zhang G, et al. Lower prevalence of antibiotic-resistant enterococci on U.S. conventional poultry farms that transitioned to organic practices. *Environ Health Perspect*. 2011;119(11):1622-1628.

87. Mirzaagha P, Louie M, Sharma R, Yanke LJ, Topp E, McAllister TA. Distribution and characterization of ampicillin- and tetracycline-resistant *Escherichia coli* from feedlot cattle fed subtherapeutic antimicrobials. *BMC Microbiol*. 2011;11:78-2180-11-78.

88. Looft T, Johnson TA, Allen HK, et al. In-feed antibiotic effects on the swine intestinal microbiome. *Proc Natl Acad Sci U S A*. 2012;109(5):1691-1696.

89. Alexander TW, Yanke LJ, Topp E, et al. Effect of subtherapeutic administration of antibiotics on the prevalence of antibiotic-resistant *Escherichia coli* bacteria in feedlot cattle. *Appl Environ Microbiol*. 2008;74(14):4405-4416.

90. Vieira AR, Collignon P, Aarestrup FM, et al. Association between antimicrobial resistance in *Escherichia coli* isolates from food animals and blood stream isolates from humans in europe: An ecological study. *Foodborne Pathog Dis*. 2011;8(12):1295-1301.

91. Anon. Antimicrobial resistance: Implications for the food system. *Comprehensive Reviews in Food Science and Food Safety*. 2006;5(3):71-137.

92. Wierup M. The control of microbial diseases in animals: Alternatives to the use of antibiotics. *Int J Antimicrob Agents*. 2000;14(4):315-319.

APPENDIX 1: APPRAISAL TOOL FOR DESCRIPTIVE AND ANALYTICAL STUDIES

Summary/objectives

1. Does article include a summary?
2. Does the summary indicate observations on antimicrobial use?
3. Does the summary indicate observations on antimicrobial resistance?
4. Is/are the objective(s) clearly stated?
5. Does objective include evaluation of antimicrobial use?
6. Does objective include evaluation of antimicrobial resistance?

Methods

Study population

7. Are inclusion/exclusion criteria for selecting subjects described?
8. Is the study population animal or human?
9. If animal, are they swine or other animals?
10. Is the age of the subjects described?
11. Is the housing system described?
12. Is the nutrition of the animals described?
13. Was the study done in an experimental or commercial/clinical setting?

Treatments

14. Were multiple groups (treatments) included?
15. Was an untreated control group included?
16. Was the study replicated?

Antimicrobial Use

17. Was antimicrobial use reported?
18. Was the antimicrobial specified?
19. Was antimicrobial use recorded, or obtained by survey questionnaire?
20. Was the antimicrobial dose reported?
21. Was the antimicrobial route recorded?
22. Was the antimicrobial duration recorded?
23. Were antimicrobial blood (or tissue/urine/other) levels measured?

Antimicrobial Resistance

24. Was antimicrobial resistance reported?
25. Was the method of resistance testing described or referenced?
26. Was the resistance description categorical, or by MIC?
27. Was the origin of MIC breakpoints specified?

Statistical Analysis

28. Was it conducted?
29. Were specific statistical tests indicated?
30. Were multivariate methods used?
31. Were confidence intervals included?

Results

32. Does the analysis directly compare antimicrobial resistance in relation to use?
33. Is there comparison of antimicrobial dose and resistance?

Discussion

34. Were the limitations of the study discussed?
35. Was the relationship of the study population to other populations discussed?
36. Is the use of antimicrobials in food animals in relation to resistance discussed?
37. Is antimicrobial use in animals implicated in the development of resistance?
38. If so, is this with direct evidence or by inference?

APPENDIX 2: APPRAISAL TOOL FOR REVIEW STUDIES

1. Does article include a summary?
2. Does the summary indicate observations on antimicrobial use?
3. Does the summary indicate observations on antimicrobial resistance?
4. Is/are the objective(s) clearly stated?
5. Does objective include evaluation of antimicrobial use?
6. Does objective include evaluation of antimicrobial resistance?
7. Was the review narrative, systematic or a meta-analysis?
8. Were the search methods used to find evidence reported?
9. Were the criteria for inclusion/exclusion of studies reported?
10. Was study validity a specific criterion for inclusion/exclusion?
11. Did the study selection methods indicate measures to avoid bias?
12. How many studies were cited?
13. How many original studies were cited?
14. Was study validity discussed in analyzing studies or in drawing inferences in review?
15. Were methods used to combine the findings of relevant studies (to reach a conclusion) reported?
16. Did the review specifically compare studies of antimicrobial dose and emergence of resistance?
17. Does the review focus on specific antimicrobial uses linked to resistance in specific bacteria?
18. Do the conclusions address the effects of antimicrobial dose on resistance?
19. Were the limitations of the study discussed?

APPENDIX 3: ANALYTICAL STUDIES

1. Akwar, T. H., C. Poppe, J. Wilson, R. J. Reid-Smith, M. Dyck, J. Waddington, D. Shang, N. Dassie, and S. A. McEwen. 2007. "Risk Factors for Antimicrobial Resistance among Fecal Escherichia Coli from Residents on Forty-Three Swine Farms." *Microbial Drug Resistance (Larchmont, N.Y.)* 13 (1): 69-76. doi:10.1089/mdr.2006.9999.
2. Anderson, M. E. and M. D. Sobsey. 2006. "Detection and Occurrence of Antimicrobially Resistant E. Coli in Groundwater on Or Near Swine Farms in Eastern North Carolina." *Water Science and Technology : A Journal of the International Association on Water Pollution Research* 54 (3): 211-218. http://www.iwaponline.com/wst/05403/0211/054030211.pdf.
3. Aubry-Damon, H., K. Grenet, P. Sall-Ndiaye, D. Che, E. Cordeiro, M. E. Bougnoux, E. Rigaud, et al. 2004. "Antimicrobial Resistance in Commensal Flora of Pig Farmers." *Emerging Infectious Diseases* 10 (5): 873-879. http://wwwnc.cdc.gov/eid/article/10/5/03-0735_article.htm.
4. Barza, M. and K. Travers. 2002. "Excess Infections due to Antimicrobial Resistance: The "Attributable Fraction"." *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America* 34 Suppl 3: S126-30. doi:10.1086/340250.
5. Berrang, M. E., S. R. Ladely, R. J. Meinersmann, and P. J. Fedorka-Cray. 2007. "Subtherapeutic Tylosin Phosphate in Broiler Feed Affects Campylobacter on Carcasses during Processing." *Poultry Science* 86 (6): 1229-1233.
6. Cosgrove, S. E., Y. Qi, K. S. Kaye, S. Harbarth, A. W. Karchmer, and Y. Carmeli. 2005. "The Impact of Methicillin Resistance in Staphylococcus Aureus Bacteremia on Patient Outcomes: Mortality, Length of Stay, and Hospital Charges." *Infection Control and Hospital Epidemiology : The Official Journal of the Society of Hospital Epidemiologists of America* 26 (2): 166-174. doi:10.1086/502522.
7. Dritz, S. S., M. D. Tokach, R. D. Goodband, and J. L. Nelssen. 2002. "Effects of Administration of Antimicrobials in Feed on Growth Rate and Feed Efficiency of Pigs in Multisite Production Systems." *Journal of the American Veterinary Medical Association* 220 (11): 1690-1695. http://avmajournals.avma.org/doi/pdfplus/10.2460/javma.2002.220.1690.
8. Endtz, H. P., G. J. Ruijs, B. van Klingeren, W. H. Jansen, T. van der Reyden, and R. P. Mouton. 1991. "Quinolone Resistance in Campylobacter Isolated from Man and Poultry Following the Introduction of Fluoroquinolones in Veterinary Medicine." *The Journal of Antimicrobial Chemotherapy* 27 (2): 199-208.
9. Fosse, J., M. Laroche, N. Oudot, H. Seegers, and C. Magras 2011. On-farm multi-contamination of pigs by food-borne bacterial zoonotic hazards: an exploratory study. Veterinary Microbiology 147:209-213.
10. Funk, J. A., J. T. Lejeune, T. E. Wittum, and P. J. Rajala-Schultz. 2006. "The Effect of Subtherapeutic Chlortetracycline on Antimicrobial Resistance in the Fecal Flora of Swine." *Microbial Drug Resistance (Larchmont, N.Y.)* 12 (3): 210-218. doi:10.1089/mdr.2006.12.210. http://www.liebertonline.com/doi/pdf/10.1089/mdr.2006.12.210.
11. Graham, J. P., J. J. Boland, and E. Silbergeld. 2007. "Growth Promoting Antibiotics in Food Animal Production: An Economic Analysis." *Public Health Reports (Washington, D.C.: 1974)* 122 (1): 79-87.
12. Gupta, A., J. Fontana, C. Crowe, B. Bolstorff, A. Stout, S. Van Duyne, M. P. Hoekstra, et al. 2003. "Emergence of Multidrug-Resistant Salmonella Enterica Serotype Newport Infections Resistant to Expanded-Spectrum Cephalosporins in the United States." *The Journal of Infectious Diseases* 188 (11): 1707-1716. doi:10.1086/379668.
13. Helms, M., J. Simonsen, and K. Molbak. 2004. "Quinolone Resistance is Associated with Increased Risk of Invasive Illness Or Death during Infection with Salmonella Serotype Typhimurium." *The Journal of Infectious Diseases* 190 (9): 1652-1654. doi:10.1086/424570.

14. Helms, M., J. Simonsen, K. E. Olsen, and K. Molbak. 2005. "Adverse Health Events Associated with Antimicrobial Drug Resistance in Campylobacter Species: A Registry-Based Cohort Study." *The Journal of Infectious Diseases* 191 (7): 1050-1055. doi:10.1086/428453.

15. Idris, U., J. Lu, M. Maier, S. Sanchez, C. L. Hofacre, B. G. Harmon, J. J. Maurer, and M. D. Lee. 2006. "Dissemination of Fluoroquinolone-Resistant Campylobacter Spp. within an Integrated Commercial Poultry Production System." *Applied and Environmental Microbiology* 72 (5): 3441-3447. doi:10.1128/AEM.72.5.3441-3447.2006.

16. Johnson, J. R., M. R. Sannes, C. Croy, B. Johnston, C. Clabots, M. A. Kuskowski, J. Bender, K. E. Smith, P. L. Winokur, and E. A. Belongia. 2007. "Antimicrobial Drug-Resistant Escherichia Coli from Humans and Poultry Products, Minnesota and Wisconsin, 2002-2004." *Emerging Infectious Diseases* 13 (6): 838-846. http://wwwnc.cdc.gov/eid/article/13/6/pdfs/06-1576.pdf.

17. Kieke, A. L., M. A. Borchardt, B. A. Kieke, S. K. Spencer, M. F. Vandermause, K. E. Smith, S. L. Jawahir, E. A. Belongia, and Marshfield Enterococcal Study Group. 2006. "Use of Streptogramin Growth Promoters in Poultry and Isolation of Streptogramin-Resistant Enterococcus Faecium from Humans." *The Journal of Infectious Diseases* 194 (9): 1200-1208. doi:10.1086/508189.

18. Ladely, S. R., M. A. Harrison, P. J. Fedorka-Cray, M. E. Berrang, M. D. Englen, and R. J. Meinersmann. 2007. "Development of Macrolide-Resistant Campylobacter in Broilers Administered Subtherapeutic Or Therapeutic Concentrations of Tylosin." *Journal of Food Protection* 70 (8): 1945-1951.

19. Lester, C. H., N. Frimodt-Moller, T. L. Sorensen, D. L. Monnet, and A. M. Hammerum. 2006. "In Vivo Transfer of the vanA Resistance Gene from an Enterococcus Faecium Isolate of Animal Origin to an E. Faecium Isolate of Human Origin in the Intestines of Human Volunteers." *Antimicrobial Agents and Chemotherapy* 50 (2): 596-599. doi:10.1128/AAC.50.2.596-599.2006.

20. Levy, Stuart B., G. B. FitzGerald, and A. B. Macone. 1976. "Changes in Intestinal Flora of Farm Personnel After Introduction of a Tetracycline-Supplemented Feed on a Farm." *The New England Journal of Medicine* 295 (11): 583-588. doi:10.1056/NEJM197609092951103.

21. Lewis, H. C., K. Molbak, C. Reese, F. M. Aarestrup, M. Selchau, M. Sorum, and R. L. Skov. 2008. "Pigs as Source of Methicillin-Resistant Staphylococcus Aureus CC398 Infections in Humans, Denmark." *Emerging Infectious Diseases* 14 (9): 1383-1389. http://wwwnc.cdc.gov/eid/article/14/9/pdfs/07-1576.pdf.

22. Martin, L. J., M. Fyfe, K. Dore, J. A. Buxton, F. Pollari, B. Henry, D. Middleton, et al. 2004. "Increased Burden of Illness Associated with Antimicrobial-Resistant Salmonella Enterica Serotype Typhimurium Infections." *The Journal of Infectious Diseases* 189 (3): 377-384. doi:10.1086/381270.

23. Merchant, J. A., A. L. Naleway, E. R. Svendsen, K. M. Kelly, L. F. Burmeister, A. M. Stromquist, C. D. Taylor, et al. 2005. "Asthma and Farm Exposures in a Cohort of Rural Iowa Children." *Environmental Health Perspectives* 113 (3): 350-356.

24. Myers, K. P., C. W. Olsen, S. F. Setterquist, A. W. Capuano, K. J. Donham, E. L. Thacker, J. A. Merchant, and G. C. Gray. 2006. "Are Swine Workers in the United States at Increased Risk of Infection with Zoonotic Influenza Virus?" *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America* 42 (1): 14-20. doi:10.1086/498977.

25. Myers, K. P., S. F. Setterquist, A. W. Capuano, and G. C. Gray. 2007. "Infection due to 3 Avian Influenza Subtypes in United States Veterinarians." *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America* 45 (1): 4-9. doi:10.1086/518579.

26. Nelson, J. M., K. E. Smith, D. J. Vugia, T. Rabatsky-Ehr, S. D. Segler, H. D. Kassenborg, S. M. Zansky, et al. 2004. "Prolonged Diarrhea due to Ciprofloxacin-Resistant Campylobacter Infection." *The Journal of Infectious Diseases* 190 (6): 1150-1157. doi:10.1086/423282.

27. Peak, N., C. W. Knapp, R. K. Yang, M. M. Hanfelt, M. S. Smith, D. S. Aga, and D. W. Graham. 2007. "Abundance of Six Tetracycline Resistance Genes in Wastewater Lagoons at Cattle Feedlots with Different Antibiotic use Strategies." *Environmental Microbiology* 9 (1): 143-151. doi:10.1111/j.1462-2920.2006.01123.x. http://onlinelibrary.wiley.com/doi/10.1111/j.1462-2920.2006.01123.x/full.

28. Price, L. B., J. P. Graham, L. G. Lackey, A. Roess, R. Vailes, and E. Silbergeld. 2007. "Elevated Risk of Carrying Gentamicin-Resistant Escherichia Coli among U.S. Poultry Workers." *Environmental Health Perspectives* 115 (12): 1738-1742. doi:10.1289/ehp.10191.

29. Radon, K., A. Schulze, V. Ehrenstein, R. T. van Strien, G. Praml, and D. Nowak. 2007. "Environmental Exposure to Confined Animal Feeding Operations and Respiratory Health of Neighboring Residents." *Epidemiology (Cambridge, Mass.)* 18 (3): 300-308. doi:10.1097/01.ede.0000259966.62137.84.

30. Schiffman, S. S., E. A. Miller, M. S. Suggs, and B. G. Graham. 1995. "The Effect of Environmental Odors Emanating from Commercial Swine Operations on the Mood of Nearby Residents." *Brain Research Bulletin* 37 (4): 369-375.

31. Smith, J. L., D. J. Drum, Y. Dai, J. M. Kim, S. Sanchez, J. J. Maurer, C. L. Hofacre, and M. D. Lee. 2007. "Impact of Antimicrobial Usage on Antimicrobial Resistance in Commensal Escherichia Coli Strains Colonizing Broiler Chickens." *Applied and Environmental Microbiology* 73 (5): 1404-1414. doi:10.1128/AEM.01193-06.

32. Stokstad, E. L. and T. H. Jukes. 1958. "Studies of the Growth-Promoting Effect of Antibiotics in Chicks on a Purified Diet." *Antibiotics Annual* 6: 998-1002.

33. Unicomb, L. E., J. Ferguson, R. J. Stafford, R. Ashbolt, M. D. Kirk, N. G. Becker, M. S. Patel, et al. 2006. "Low-Level Fluoroquinolone Resistance among Campylobacter Jejuni Isolates in Australia." *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America* 42 (10): 1368-1374. doi:10.1086/503426.

34. van Duijkeren, E., R. Ikawaty, M. J. Broekhuizen-Stins, M. D. Jansen, E. C. Spalburg, A. J. de Neeling, J. G. Allaart, A. van Nes, J. A. Wagenaar, and A. C. Fluit. 2008b. "Transmission of Methicillin-Resistant Staphylococcus Aureus Strains between Different Kinds of Pig Farms." *Veterinary Microbiology* 126 (4): 383-389. doi:10.1016/j.vetmic.2007.07.021.

35. van Loo, I., X. Huijsdens, E. Tiemersma, A. de Neeling, N. van de Sande-Bruinsma, D. Beaujean, A. Voss, and J. Kluytmans. 2007. "Emergence of Methicillin-Resistant Staphylococcus Aureus of Animal Origin in Humans." *Emerging Infectious Diseases* 13 (12): 1834-1839.

36. Varma, J. K., K. D. Greene, J. Ovitt, T. J. Barrett, F. Medalla, and F. J. Angulo. 2005a. "Hospitalization and Antimicrobial Resistance in Salmonella Outbreaks, 1984-2002." *Emerging Infectious Diseases* 11 (6): 943-946.

37. Varma, J. K., K. Molbak, T. J. Barrett, J. L. Beebe, T. F. Jones, T. Rabatsky-Ehr, K. E. Smith, D. J. Vugia, H. G. Chang, and F. J. Angulo. 2005b. "Antimicrobial-Resistant Nontyphoidal Salmonella is Associated with Excess Bloodstream Infections and Hospitalizations." *The Journal of Infectious Diseases* 191 (4): 554-561. doi:10.1086/427263.

APPENDIX 4: DESCRIPTIVE STUDIES

1. Anon. 2003. Of birds and bacteria: "superbugs" that resist the usual antibiotic treatments are nasty, and they could be in your chicken dinner. Here's how to protect yourself. Consumer Reports. January 01, 2003. http://www.accessmylibrary.com/coms2/summary_0286-24451905_ITM.

2. Anon. 2006. E. Coli Outbreak from Spinach - Update: Oct. 6, 2006 | CDC Foodborne and Diarrheal Diseases Branch.", accessed 6/21/2011, 2011, http://www.cdc.gov/foodborne/ecolispinach/100606.htm.

3. "National Ground Water Association - LONG-TERM MONITORING OF THE OCCURRENCE OF ANTIBIOTIC RESIDUES AND ANTIBIOTIC RESISTANCE GENES IN GROUNDWATER NEAR SWINE CONFINEMENT FACILITIES DISRUPTING CHEMICALS IN WATER, OCTOBER 13-15, 2004, MINNEAPOLIS; P158-174 - Groundwateronline.", accessed 6/21/2011, 2011, http://ngwa.staging.10floor.com/gwol/042379986.aspx.

4. Bakr, W. M., M. Fawzi, and M. H. Hashish. 2004. "Detection of Coagulase Positive Staphylococci in Meat Products Sold in Alexandria using Two Different Media." *The Journal of the Egyptian Public Health Association* 79 (1-2): 31-42.

5. Barham, A. R., B. L. Barham, A. K. Johnson, D. M. Allen, J. R. Blanton Jr, and M. F. Miller. 2002. "Effects of the Transportation of Beef Cattle from the Feedyard to the Packing Plant on Prevalence Levels of Escherichia Coli O157 and Salmonella Spp." *Journal of Food Protection* 65 (2): 280-283.

6. Batt, A. L., D. D. Snow, and D. S. Aga. 2006. "Occurrence of Sulfonamide Antimicrobials in Private Water Wells in Washington County, Idaho, USA." *Chemosphere* 64 (11): 1963-1971. doi:10.1016/j.chemosphere.2006.01.029.

7. Chapin, A., A. Rule, K. Gibson, T. Buckley, and K. Schwab. 2005. "Airborne Multidrug-Resistant Bacteria Isolated from a Concentrated Swine Feeding Operation." *Environmental Health Perspectives* 113 (2): 137-142. http://ehp03.niehs.nih.gov/article/fetchArticle.action?articleURI=info%3Adoi%2F10.1289%2Fehp.7473.

8. Chee-Sanford, J. C., R. I. Aminov, I. J. Krapac, N. Garrigues-Jeanjean, and R. I. Mackie. 2001. "Occurrence and Diversity of Tetracycline Resistance Genes in Lagoons and Groundwater Underlying Two Swine Production Facilities." *Applied and Environmental Microbiology* 67 (4): 1494-1502. doi:10.1128/AEM.67.4.1494-1502.2001.

9. Dunne, E. F., P. D. Fey, P. Kludt, R. Reporter, F. Mostashari, P. Shillam, J. Wicklund, et al. 2000. "Emergence of Domestically Acquired Ceftriaxone-Resistant Salmonella Infections Associated with AmpC Beta-Lactamase." *JAMA : The Journal of the American Medical Association* 284 (24): 3151-3156.

10. Fey, P. D., T. J. Safranek, M. E. Rupp, E. F. Dunne, E. Ribot, P. C. Iwen, P. A. Bradford, F. J. Angulo, and S. H. Hinrichs. 2000. "Ceftriaxone-Resistant Salmonella Infection Acquired by a Child from Cattle." *The New England Journal of Medicine* 342 (17): 1242-1249. doi:10.1056/NEJM200004273421703.

11. Frenzen, P. D., A. Drake, F. J. Angulo, and Emerging Infections Program FoodNet Working Group. 2005. "Economic Cost of Illness due to Escherichia Coli O157 Infections in the United States." *Journal of Food Protection* 68 (12): 2623-2630.

12. Gibbs, S. G., C. F. Green, P. M. Tarwater, and P. V. Scarpino. 2004. "Airborne Antibiotic Resistant and Nonresistant Bacteria and Fungi Recovered from Two Swine Herd Confined Animal Feeding Operations." *Journal of Occupational and Environmental Hygiene* 1 (11): 699-706. http://www.tandfonline.com/doi/full/10.1080/15459620490515824.

13. Gibbs, S. G., C. F. Green, P. M. Tarwater, L. C. Mota, K. D. Mena, and P.V. Scarpino. 2006. "Isolation of Antibiotic-Resistant Bacteria from the Air Plume Downwind of a Swine Confined Or Concentrated Animal Feeding Operation." *Environmental Health Perspectives* 114 (7): 1032 <last_page> 1037. doi:10.1289/ehp.8910.

14. Graham, J. P., L. B. Price, S. L. Evans, T. K. Graczyk, and E. K. Silbergeld. 2009. "Antibiotic Resistant Enterococci and Staphylococci Isolated from Flies Collected Near Confined Poultry Feeding Operations." *The Science of the Total Environment* 407 (8): 2701-2710. doi:10.1016/j.scitotenv.2008.11.056.

15. Hanselman, B. A., S. A. Kruth, J. Rousseau, D. E. Low, B. M. Willey, A. McGeer, and J. S. Weese. 2006. "Methicillin-Resistant Staphylococcus Aureus Colonization in Veterinary Personnel." *Emerging Infectious Diseases* 12 (12): 1933-1938. http://wwwnc.cdc.gov/eid/article/12/12/06-0231_article.htm.

16. Harris, N. V., D. Thompson, D. C. Martin, and C.M. Nolan. 1986. "A Survey of Campylobacter and Other Bacterial Contaminants of Pre-Market Chicken and Retail Poultry and Meats, King County, Washington." *American Journal of Public Health* 76 (4): 401-406. http://search.ebscohost.com/login.aspx?direct=true&db=aph&AN=4686245&site=ehost-live.

17. Huijsdens, X. W., B. J. van Dijke, E. Spalburg, M. G. van Santen-Verheuvel, M. E. Heck, G. N. Pluister, A. Voss, W. J. Wannet, and A. J. de Neeling. 2006. "Community-Acquired MRSA and Pig-Farming." *Annals of Clinical Microbiology and Antimicrobials* 5: 26. doi:10.1186/1476-0711-5-26.

18. Johnson, J. R., M. A. Kuskowski, M. Menard, A. Gajewski, M. Xercavins, and J. Garau. 2006. "Similarity between Human and Chicken Escherichia Coli Isolates in Relation to Ciprofloxacin Resistance Status." *The Journal of Infectious Diseases* 194 (1): 71-78. doi:10.1086/504921. http://jid.oxfordjournals.org/content/194/1/71.full.pdf%20html.

19. Khanna, T., R. Friendship, C. Dewey, and J. S. Weese. 2008. "Methicillin Resistant Staphylococcus Aureus Colonization in Pigs and Pig Farmers." *Veterinary Microbiology* 128 (3-4): 298-303. doi:10.1016/j.vetmic.2007.10.006. http://www.sciencedirect.com/science/article/pii/S0378113507004932.

20. Kitai, S., A. Shimizu, J. Kawano, E. Sato, C. Nakano, H. Kitagawa, K. Fujio, K. Matsumura, R. Yasuda, and T. Inamoto. 2005. "Prevalence and Characterization of Staphylococcus Aureus and Enterotoxigenic Staphylococcus Aureus in Retail Raw Chicken Meat Throughout Japan." *The Journal of Veterinary Medical Science / the Japanese Society of Veterinary Science* 67 (3): 269-274.

21. Klevens, R. M., M. A. Morrison, J. Nadle, S. Petit, K. Gershman, S. Ray, L. H. Harrison, et al. 2007. "Invasive Methicillin-Resistant Staphylococcus Aureus Infections in the United States." *JAMA : The Journal of the American Medical Association* 298 (15): 1763-1771. doi:10.1001/jama.298.15.1763.

22. Koike S, I. G. Krapac, H. D. Oliver, A. C. Yannarell, J. C. Chee-Sanford, R. I. Aminov, and R. I. Mackie. 2007. "Monitoring and Source Tracking of Tetracycline Resistance Genes in Lagoons and Groundwater Adjacent to Swine Production Facilities Over a 3-Year Period." *Applied and Environmental Microbiology* 73 (15): 4813-4823. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1951052/?tool=pmcentrez.

23. Kolpin, Dana W., Edward T. Furlong, Michael T. Meyer, E. Michael Thurman, Steven D. Zaugg, Larry B. Barber, and Herbert T. Buxton. 2002. "Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999-2000: A National Reconnaissance." *Environmental Science & Technology* 36 (6): 1202 <last_page> 1211. doi:10.1021/es011055j.

24. Lyons, R. W., C. L. Samples, H. N. DeSilva, K. A. Ross, E. M. Julian, and P. J. Checko. 1980. An Epidemic of Resistant Salmonella in a Nursery. Animal-to-Human Spread. The Journal of the American Medical Association 243 (6): 546-547.

25. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. "Food-Related Illness and Death in the United States." *Emerging Infectious Diseases* 5 (5): 607-625.

26. Mellon MG, Benbrook C, Benbrook KL. "Hogging it: Estimates of Antimicrobial Abuse in Livestock." ucsusa.org., accessed 7/13, 2011, http://www.ucsusa.org/food_and_agriculture/science_and_impacts/impacts_industrial_agriculture/hogging-it-estimates-of.html.

27. Molbak, K., D. L. Baggesen, F. M. Aarestrup, J. M. Ebbesen, J. Engberg, K. Frydendahl, P. Gerner-Smidt, A. M. Petersen, and H. C. Wegener. 1999. "An Outbreak of Multidrug-Resistant, Quinolone-Resistant Salmonella Enterica Serotype Typhimurium DT104." *The New England Journal of Medicine* 341 (19): 1420-1425. doi:10.1056/NEJM199911043411902.

28. Moreno, M. A., L. Dominguez, T. Teshager, I. A. Herrero, and M. C. Porrero. 2000. "Antibiotic Resistance Monitoring: The Spanish Programme. the VAV Network. Red De Vigilancia De Resistencias Antibioticas En Bacterias De Origen Veterinario." *International Journal of Antimicrobial Agents* 14 (4): 285-290.

29. Nannapaneni, R., R. Story, K. C. Wiggins, and M. G. Johnson. 2005. "Concurrent Quantitation of Total Campylobacter and Total Ciprofloxacin-Resistant Campylobacter Loads in Rinses from Retail Raw Chicken Carcasses from 2001 to 2003 by Direct Plating at 42 Degrees C." *Applied and Environmental Microbiology* 71 (8): 4510-4515. doi:10.1128/AEM.71.8.4510-4515.2005.

30. Nemati, M., K. Hermans, U. Lipinska, O. Denis, A. Deplano, M. Struelens, L. A. Devriese, F. Pasmans, and F. Haesebrouck. 2008. "Antimicrobial Resistance of Old and Recent Staphylococcus Aureus Isolates from Poultry: First Detection of Livestock-Associated Methicillin-Resistant Strain ST398." *Antimicrobial Agents and Chemotherapy* 52 (10): 3817-3819. doi:10.1128/AAC.00613-08.

31. Ojeniyi, A. A. 1989. "Direct Transmission of Escherichia Coli from Poultry to Humans." *Epidemiology and Infection* 103 (3): 513-522.

32. Ramchandani, M., A. R. Manges, C. DebRoy, S. P. Smith, J. R. Johnson, and L. W. Riley. 2005. "Possible Animal Origin of Human-Associated, Multidrug-Resistant, Uropathogenic Escherichia Coli." *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America* 40 (2): 251-257. doi:10.1086/426819.

33. Sapkota, A.R., F.C. Curriero, K.E. Gibson, and K.J. Schwab. 2007. Antibiotic-Resistant Enterococci and Fecal Indicators in Surface Water and Groundwater Impacted by a Concentrated Swine Feeding Operation. *Environmental Health Perspectives* 115 (7): 1040 - 1045. -

34. Schraft, H., N. Kleinlein, and F. Untermann. 1992. "Contamination of Pig Hindquarters with Staphylococcus Aureus." *International Journal of Food Microbiology* 15 (1-2): 191-194.

35. Shoemaker, N. B., H. Vlamakis, K. Hayes, and A. A. Salyers. 2001. "Evidence for Extensive Resistance Gene Transfer among Bacteroides Spp. and among Bacteroides and Other Genera in the Human Colon." *Applied and Environmental Microbiology* 67 (2): 561-568. doi:10.1128/AEM.67.2.561-568.2001.

36. Smith, K. E., J. M. Besser, C. W. Hedberg, F. T. Leano, J. B. Bender, J. H. Wicklund, B. P. Johnson, K. A. Moore, and M. T. Osterholm. 1999. Quinolone-Resistant Campylobacter Jejuni Infections in Minnesota, 1992-1998. Investigation Team. *The New England Journal of Medicine* 340 (20): 1525-1532. doi:10.1056/NEJM199905203402001.

37. Smith, T. C., M. J. Male, A. L. Harper, J. S. Kroeger, G. P. Tinkler, E. D. Moritz, A. W. Capuano, L. A. Herwaldt, and D. J. Diekema. 2009. "Methicillin-Resistant Staphylococcus Aureus (MRSA) Strain ST398 is Present in Midwestern U.S. Swine and Swine Workers." *PloS One* 4 (1): e4258. doi:10.1371/journal.pone.0004258.

38. Song, W., M. Huang, W. Rumbeiha, and H. Li. 2007. "Determination of Amprolium, Carbadox, Monensin, and Tylosin in Surface Water by Liquid Chromatography/Tandem Mass Spectrometry." *Rapid Communications in Mass Spectrometry : RCM* 21 (12): 1944-1950. doi:10.1002/rcm.3042.

39. Tenover, F. C. 2006. "Mechanisms of Antimicrobial Resistance in Bacteria." *The American Journal of Medicine* 119 (6 Suppl 1): S3-10; discussion S62-70. doi:10.1016/j.amjmed.2006.03.011.

40. van Belkum, A., D. C. Melles, J. K. Peeters, W. B. van Leeuwen, E. van Duijkeren, X. W. Huijsdens, E. Spalburg, A. J. de Neeling, H. A. Verbrugh, and Dutch Working Party on Surveillance and Research of MRSA-SOM. 2008. "Methicillin-Resistant and -Susceptible Staphylococcus Aureus Sequence Type 398 in Pigs and Humans." *Emerging Infectious Diseases* 14 (3): 479-483. http://wwwnc.cdc.gov/eid/content/14/3/pdfs/v14-n3.pdf.

41. van Duijkeren, E., D. J. Houwers, A. Schoormans, M. J. Broekhuizen-Stins, R. Ikawaty, A. C. Fluit, and J. A. Wagenaar. 2008a. "Transmission of Methicillin-Resistant Staphylococcus Intermedius between Humans and Animals." *Veterinary Microbiology* 128 (1-2): 213-215. doi:10.1016/j.vetmic.2007.11.016.

42. van Loo, I. H., B. M. Diederen, P. H. Savelkoul, J. H. Woudenberg, R. Roosendaal, A. van Belkum, N. Lemmens-den Toom, C. Verhulst, P. H. van Keulen, and J. A. Kluytmans. 2007. "Methicillin-Resistant Staphylococcus Aureus in

Meat Products, the Netherlands." *Emerging Infectious Diseases* 13 (11): 1753-1755. http://wwwnc.cdc.gov/eid/article/13/11/pdfs/07-0358.pdf.

43. van Rijen, M. M., P. H. Van Keulen, and J. A. Kluytmans. 2008. "Increase in a Dutch Hospital of Methicillin-Resistant Staphylococcus Aureus Related to Animal Farming." *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America* 46 (2): 261-263. doi:10.1086/524672.
44. Voss, A., F. Loeffen, J. Bakker, C. Klaassen, and M. Wulf. 2005. "Methicillin-Resistant Staphylococcus Aureus in Pig Farming." *Emerging Infectious Diseases* 11 (12): 1965-1966. http://wwwnc.cdc.gov/eid/content/11/12/pdfs/v11-n12.pdf.
45. White, D. G. et al. 2001. "The Isolation of Antibiotic-Resistant Salmonella from Retail Ground Meats." *New England Journal of Medicine* 345 (- 0028-4793 (Print); - 0028-4793 (Linking)): 1147.
46. White, P. L., A. L. Naugle, C. R. Jackson, P. J. Fedorka-Cray, B. E. Rose, K. M. Pritchard, P. Levine, et al. 2007. "Salmonella Enteritidis in Meat, Poultry, and Pasteurized Egg Products Regulated by the U.S. Food Safety and Inspection Service, 1998 through 2003." *Journal of Food Protection* 70 (3): 582-591.
47. Witte, W., B. Strommenger, C. Stanek, and C. Cuny. 2007. "Methicillin-Resistant Staphylococcus Aureus ST398 in Humans and Animals, Central Europe." *Emerging Infectious Diseases* 13 (2): 255-258.
48. Wray, C. and J. C. Gnanou. 2000. "Antibiotic Resistance Monitoring in Bacteria of Animal Origin: Analysis of National Monitoring Programmes." *International Journal of Antimicrobial Agents* 14 (4): 291-294.

APPENDIX 5: REVIEW STUDIES

1. Aarestrup, F.M. and A.M. Seyfarth. 2000. "Effect of Intervention on the Occurrence of Antimicrobial Resistance.", Acta Vet Scand Suppl. 2000;93:99. http://www.ncbi.nlm.nih.gov/pubmed/10822862.
2. Anderson, A. D., J. M. Nelson, S. Rossiter, and F. J. Angulo. 2003. "Public Health Consequences of use of Antimicrobial Agents in Food Animals in the United States." *Microbial Drug Resistance (Larchmont, N.Y.)* 9 (4): 373-379. doi:10.1089/107662903322762815.
3. Bailar, J. C.,3rd and K. Travers. 2002. "Review of Assessments of the Human Health Risk Associated with the use of Antimicrobial Agents in Agriculture." *Clinical Infectious Diseases* 34 Suppl 3: S135-43. doi:10.1086/340252.
4. Barker, K. F. 1999. "Antibiotic Resistance: A Current Perspective." *British Journal of Clinical Pharmacology* 48 (2): 109-124.
5. Barza, M. 2002. "Potential Mechanisms of Increased Disease in Humans from Antimicrobial Resistance in Food Animals." *Clinical Infectious Diseases* 34 Suppl 3: S123-5. doi:10.1086/340249.
6. Burkholder, J., B. Libra, P. Weyer, S. Heathcote, D. Kolpin, P. S. Thorne, and M. Wichman. 2007. "Impacts of Waste from Concentrated Animal Feeding Operations on Water Quality." *Environmental Health Perspectives* 115 (2): 308-312. doi:10.1289/ehp.8839.
7. Catry, B., H. Laevens, L. A. Devriese, G. Opsomer, and A. De Kruif. 2003. "Antimicrobial Resistance in Livestock." *Journal of Veterinary Pharmacology and Therapeutics* 26 (2): 81-93.
8. CDC/FDA/NIH Interagency Task Force on Antimicrobial Drug Resistance. 2001. *A Public Health Action Plan to Combat Antimicrobial Resistance.*
9. Centner, T. J. 2006. "Governmental Oversight of Discharges from Concentrated Animal Feeding Operations." *Environmental Management* 37 (6): 745-752. doi:10.1007/s00267-005-0130-5.
10. Cole, D., L. Todd, and S. Wing. 2000. "Concentrated Swine Feeding Operations and Public Health: A Review of Occupational and Community Health Effects." *Environmental Health Perspectives* 108 (8): 685-699.
11. Collignon, P. *The use of Antibiotics in Food Production Animals - does this Cause Problems in Human Health?*.
12. Committee on Human Health Risk Assessment of Using Subtherapeutic Antibiotics in Animal Feeds. 1988. *Institute of Medicine, Report of a Study: Human Health Risks with the Subtherapeutic use of Penicillin Or Tetracyclines in Animal Feed*. Washington, D.C.: National Academy Press.
13. Cosgrove, S. E. 2006. "The Relationship between Antimicrobial Resistance and Patient Outcomes: Mortality, Length of Hospital Stay, and Health Care Costs." *Clinical Infectious Diseases* 42 Suppl 2: S82-9. doi:10.1086/499406.
14. Council,Committee on Drug Use in Food Animals, Panel on Animal Health, Food Safety, and Public Health, Board on Agriculture,National Research. 1999. *The use of Drugs in Food Animals: Benefits and Risks*. Washington, D.C.: National Academy Press.
15. Courvalin, P. 2005. "Antimicrobial Drug Resistance: "Prediction is very Difficult, especially about the Future"." *Emerging Infectious Diseases* 11 (10): 1503-1506.
16. Donham, K. J., S. Wing, D. Osterberg, J. L. Flora, C. Hodne, K. M. Thu, and P. S. Thorne. 2007. "Community Health and Socioeconomic Issues Surrounding Concentrated Animal Feeding Operations." *Environmental Health Perspectives* 115 (2): 317-320. doi:10.1289/ehp.8836.
17. doPico GA. 1986. "Workgroup on Diseases, Report on Diseases." *American Journal of Industrial Medicine* 10 (3): 261-265.
18. Engberg, J., F. M. Aarestrup, D. E. Taylor, P. Gerner-Smidt, and I. Nachamkin. 2001. "Quinolone and Macrolide Resistance in Campylobacter Jejuni and C. Coli: Resistance Mechanisms and Trends in Human Isolates." *Emerging Infectious Diseases* 7 (1): 24-34. http://wwwnc.cdc.gov/eid/article/7/1/70-0024_article.htm.

19. European Commission. 1999. *Opinion of the Scientific Steering Committee on Antimicrobrial Resistance*.

20. FAO/OIE/WHO. a. *Joint FAO/OIE/WHO Expert Workshop on Non-Human Antimicrobial Usage and Antimicrobial Resistance: Scientific Assessment* http://Www.Who.Int/Foodsafety/Publications/Micro/En/Amr.Pdf

21. FAO/OIE/WHO . b. *Second Joint FAO/ OIE/WHO Expert Workshop on Non-Human Antimicrobial Usage and Antimicrobial Resistance: Management Options* http://Web.Oie.Int/Downld/WHO-CDS-CPE-ZFK-2004.8.Pdf

22. Food and Drug Administration. "The Judicious use of Medically Important Antimicrobrial Drugs in Food-Producing Animals.", accessed 6/6/2011, 2011, http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM216936.pdf.

23. Gilchrist, M. J., C. Greko, D. B. Wallinga, G. W. Beran, D. G. Riley, and P. S. Thorne. 2007. "The Potential Role of Concentrated Animal Feeding Operations in Infectious Disease Epidemics and Antibiotic Resistance." *Environmental Health Perspectives* 115 (2): 313-316. doi:10.1289/ehp.8837.

24. Goforth, Robyn L. and Carol R. Goforth. 2000. "Appropriate Regulation of Antibiotics in Livestock Feed." *Boston College Environmental Affairs Law Review* 28 (1): 39.

25. Gorbach, Sherwood L. 2001. "Antimicrobial use in Animal Feed — Time to Stop." *New England Journal of Medicine* 345 (16): 1202-1203. http://dx.doi.org.ezp2.lib.umn.edu/10.1056/NEJM200110183451610.

26. Gray, G. C., D. W. Trampel, and J. A. Roth. 2007. "Pandemic Influenza Planning: Shouldn'T Swine and Poultry Workers be Included?" *Vaccine* 25 (22): 4376-4381. doi:10.1016/j.vaccine.2007.03.036.

27. Harrison, P. F., J. Lederberg. 1998. Institute of Medicine . Forum on Emerging Infections. 1998. *Antimicrobial Resistance: Issues and Options : Workshop Report*. Washington, D.C.: National Academy Press.

28. Horrigan, L., R. S. Lawrence, and P. Walker. 2002. "How Sustainable Agriculture can Address the Environmental and Human Health Harms of Industrial Agriculture." *Environmental Health Perspectives* 110 (5): 445-456.

29. Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine. 1969. *Report of the Joint Committee on the use of Antibiotics in Animal Husbandry and Veterinary Medicine*. London: Her Majesty's Stationery Office.

30. Khachatourians, G. G. 1998. "Agricultural use of Antibiotics and the Evolution and Transfer of Antibiotic-Resistant Bacteria." *CMAJ : Canadian Medical Association Journal = Journal De L'Association Medicale Canadienne* 159 (9): 1129-1136. http://www.cmaj.ca/content/159/9/1129.full.pdf+html.

31. Knobler, Stacey and Institute of Medicine . Forum on Emerging Infections. 2003. *The Resistance Phenomenon in Microbes and Infectious Disease Vectors: Implications for Human Health and Strategies for Containment : Workshop Summary*. Washington, D.C.: National Academies Press. http://www.nap.edu/catalog/10651.html.

32. Levy, S. B. 1987. Antibiotic use for Growth Promotion in Animals: Ecologic and Public Health Consequences. *Journal of Food Protection (USA)* 50 (7): 616-620.

33. Levy, S. B. 1998. The Challenge of Antibiotic Resistance. *Scientific American* 278: 46-53..

34. Martel, J. L., F. Tardy, A. Brisabois, R. Lailler, M. Coudert, and E. Chaslus-Dancla. 2000. "The French Antibiotic Resistance Monitoring Programs." *International Journal of Antimicrobial Agents* 14 (4): 275-283.

35. McEwen, S. A. and P. J. Fedorka-Cray. 2002. Antimicrobial use and Resistance in Animals. *Clinical Infectious Diseases* 34 Suppl 3: S93-S106. doi:10.1086/340246.

36. O'Brien, T. F. 2002. Emergence, Spread, and Environmental Effect of Antimicrobial Resistance: How use of an Antimicrobial Anywhere can Increase Resistance to any Antimicrobial Anywhere Else. *Clinical Infectious Diseases* 34 Suppl 3: S78-84. doi:10.1086/340244.

37. Salyers, A. A. "How are Human and Animal Ecosystems Interconnected?", accessed 6/9/2011, 2011, http://www.keepantibioticsworking.com/new/Library/UploadedFiles/How_Are_Human_and_Animal_Ecosystems_Interconne.htm.

38. Sapkota, A. R., L. Y. Lefferts, S. McKenzie, and P. Walker. 2007. "What do we Feed to Food-Production Animals? A Review of Animal Feed Ingredients and their Potential Impacts on Human Health." *Environmental Health Perspectives* 115 (5): 663-670. doi:10.1289/ehp.9760.

39. Shea, K. M., American Academy of Pediatrics Committee on Environmental Health, and American Academy of Pediatrics Committee on Infectious Diseases. 2004. "Nontherapeutic use of Antimicrobial Agents in Animal Agriculture: Implications for Pediatrics." *Pediatrics* 114 (3): 862-868. doi:10.1542/peds.2004-1233. http://pediatrics.aappublications.org/content/114/3/862.full.

40. Silbergeld, E. K., J. Graham, and L. B. Price. 2008. "Industrial Food Animal Production, Antimicrobial Resistance, and Human Health." *Annual Review of Public Health* 29: 151-169. doi:10.1146/annurev.publhealth.29.020907.090904.

41. Smith, KE, JB Bender, and MT Osterholm. 2000. "Antimicrobial Resistance in Animals and Relevance to Human Infections." Chap. 25, In *Campylobacter*, edited by I. Nachamkin and MJ Blaser. 2nd ed. Washington, DC: ASM Press.

42. Smith, D. L., J. Dushoff, and J. G. Morris. 2005. "Agricultural Antibiotics and Human Health: Does Antibiotic use in Agriculture have a Greater Impact than Hospital use?" *PLoS Medicine* 2 (8): e232. doi:10.1371/journal.pmed.0020232.

43. Smolinski, Mark S., Margaret A. Hamburg, and Joshua Lederberg. 2003. *Microbial Threats to Health: Emergence, Detection, and Response*. Washington, D.C.: National Academies Press. Access electronic version http://books.nap.edu/books/030908864X/html/.

44. Spellberg, B., R. Guidos, D. Gilbert, J. Bradley, H. W. Boucher, W. M. Scheld, J. G. Bartlett, J. Edwards Jr, and Infectious Diseases Society of America. 2008. "The Epidemic of Antibiotic-Resistant Infections: A Call to Action for the Medical Community from the Infectious Diseases Society of America." *Clinical Infectious Diseases* 46 (2): 155-164. doi:10.1086/524891.

45. STOKSTAD, E. L. 1954. "Antibiotics in Animal Nutrition." *Physiological Reviews* 34 (1): 25-51.

46. Summers, A. O. 2002. "Generally Overlooked Fundamentals of Bacterial Genetics and Ecology." *Clinical Infectious Diseases* 34 Suppl 3: S85-92. doi:10.1086/340245.

47. Suzuki, S. 1994. "Pathogenicity of Salmonella Enteritidis in Poultry." *International Journal of Food Microbiology* 21 (1-2): 89-105.

48. Swartz, M. N. 2002. "Human Diseases Caused by Foodborne Pathogens of Animal Origin." *Clinical Infectious Diseases* 34 Suppl 3: S111-22. doi:10.1086/340248.

49. Talbot, G. H., J. Bradley, J. E. Edwards Jr, D. Gilbert, M. Scheld, J. G. Bartlett, and Antimicrobial Availability Task Force of the Infectious Diseases Society of America. 2006. Bad Bugs Need Drugs: An Update on the Development Pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. *Clinical Infectious Diseases* 42 (5): 657-668. doi:10.1086/499819.

50. Teuber, M. 2001. Veterinary use and Antibiotic Resistance. *Current Opinion in Microbiology* 4 (5): 493-499. http://www.sciencedirect.com/science/article/pii/S1369527400002411.

51. Thu, K. M. 2002. "Public Health Concerns for Neighbors of Large-Scale Swine Production Operations." *Journal of Agricultural Safety and Health* 8 (2): 175-184.

52. Travers, K. and M. Barza. 2002. "Morbidity of Infections Caused by Antimicrobial-Resistant Bacteria." *Clinical Infectious Diseases* 34 Suppl 3: S131-4. doi:10.1086/340251.

53. United States Government Accountability Office. Antibiotic Resistance: Federal Agencies Need to Better Focus Efforts to Address Risk to Humans from Antibiotic use in Animals. http://www.gao.gov/new.items/d04490.pdf.

54. United States Government Accountability Office. Food Safety: The Agricultural use of Antibiotics and its Implications for Human Health. http://www.gao.gov/archive/1999/rc99074.pdf.

55. USDA. MRSA Factsheet., last modified December 2007 http://www.aphis.usda.gov/animal_health/emergingissues/downloads/mrsa_122007.pdf.

56. van den Bogaard, A. E. and E. E. Stobberingh. 2000. "Epidemiology of Resistance to Antibiotics. Links between Animals and Humans." *International Journal of Antimicrobial Agents* 14 (4): 327-335.

57. Vidaver, A. K. 2002. Uses of Antimicrobials in Plant Agriculture. *Clinical Infectious Diseases* 34 Suppl 3: S107-10. doi:10.1086/340247.

58. Walker, P., P. Rhubart-Berg, S. McKenzie, K. Kelling, and R. S. Lawrence. 2005. Public Health Implications of Meat Production and Consumption. *Public Health Nutrition* 8 (4): 348-356.

59. Wegener, H. C., F. M. Aarestrup, P. Gerner-Smidt, and F. Bager. 1999. "Transfer of Antibiotic Resistant Bacteria from Animals to Man." *Acta Veterinaria Scandinavica.Supplementum* 92: 51-57.

60. WHO. 2011. Impacts of Antimicrobial Growth Promoter Termination in Denmark. http://www.who.int/gfn/en/Expertsreportgrowthpromoterdenmark.pdf.

61. WHO. 1997. The Medical Impact of Antimicrobial use in Food Animals. Report of a WHO Meeting. Berlin, Germany, 13-17 October 1997.", http://whqlibdoc.who.int/hq/1997/WHO_EMC_ZOO_97.4.pdf.

62. WHO. 2000. *World Health Organization Report on Infectious Diseases 2000: Overcoming Antimicrobial Resistance* *http://Www.Who.Int/Infectious-Disease-Report/2000/*

63. WHO. 2003. Impacts of Antimicrobial Growth Promoter Termination in Denmark. Foulum, Denmark, 6-9 November 2002; http://www.who.int/gfn/en/Expertsreportgrowthpromoterdenmark.pdf

64. Wierup, M. 1998. "Preventive Methods Replaced Antibiotic Growth Promoters: Ten Years Experience from Sweden." *APUA Newsletter* 16 (2): 1-4.

65. Wierup, M. 2000. "The Control of Microbial Diseases in Animals: Alternatives to the use of Antibiotics." *International Journal of Antimicrobial Agents* 14 (4): 315-319.

66. Wierup, M. 2001. The Swedish Experience of the 1986 Year Ban of Antimicrobial Growth Promoters, with Special Reference to Animal Health, Disease Prevention, Productivity, and Usage of Antimicrobials. *Microbial Drug Resistance (Larchmont, N.Y.)* 7 (2): 183-190. doi:10.1089/10766290152045066.

67. Woolhouse, M. E. and S. Gowtage-Sequeria. 2005. "Host Range and Emerging and Reemerging Pathogens." *Emerging Infectious Diseases* 11 (12): 1842-1847. http://wwwnc.cdc.gov/eid/content/11/12/pdfs/v11-n12.pdf.

68. Woolhouse, M. E., L. H. Taylor, and D. T. Haydon. 2001. "Population Biology of Multihost Pathogens." *Science (New York, N.Y.)* 292 (5519): 1109-1112.

69. Zetola, N., J. S. Francis, E. L. Nuermberger, and W. R. Bishai. 2005. "Community-Acquired Meticillin-Resistant Staphylococcus Aureus: An Emerging Threat." *The Lancet Infectious Diseases* 5 (5): 275-286. doi:10.1016/S1473-3099(05)70112-2.