

TABLE IX-1

COMPARISON OF PARAMETERS IN THE PRESENT REPORT WITH THOSE OF THE NRDC REPORT: HUMAN SALMONELLOSIS DEATHS ATTRIBUTABLE TO ANY LOW-LEVEL FARM USE OF PENICILLIN OR THE TETRACYCLINES

	<u>Present Report (Mid-Range Estimates)</u>	<u>NRDC Report (First Model)</u>
Reported cases per year in the U.S.	50,000	40,000
Resistance to penicillin and/or tetracyclines	0.15	0.20
Death rate for salmonellosis from resistant strains	0.008	0.042
Fraction associated with strains of farm origin	0.70	0.69*
Fraction caused by subtherapeutic use of penicillin and/or the tetracyclines	0.50	0.72
Product of these estimates: Deaths per year in the U.S. from salmonellae resistant to penicillin and/or the tetracyclines because of subtherapeutic use of these drugs on the farm.	30**	116

Source: Prepared by the committee using data from Table VIII-1 and from the Natural Resources Defense Council.<sup>16</sup>

\* "Traceable to animal sources."

\*\* Differs slightly from Table VIII-2, because the product of the mid-range estimates is not necessarily the median of the 243 products.

in Table VIII-1, and thereby substantially reduce composite ranges of estimates shown in Figures VIII-1 through VIII-12.

The presently available data are an incomplete "patchwork" from a variety of sources; they are not collected systematically for the nation, they are complex, they are frequently of poor quality and require extrapolation for use in risk assessment, and they are not focused on the specific points of direct interest. These characteristics of the available data are inherent in the problem of collecting data and are not the fault of any one government agency or researchers who have studied this problem over the past several years. For example, none of the sources summarized in Table VII-8 was focused on estimation of the "etiologic fraction," none presented an estimate of that fraction, each had very small samples for this use, and each was subject to substantial bias in the identification and recruitment of subjects. Similarly, there have been few opportunities for the accurate and unbiased estimation of population-wide death rates, though countless reports of salmonellae deaths and death rates have been published for use in other contexts.

We regard the model itself as neutral--this is, unbiased with respect to errors or uncertainties in the estimates it produces--though of course it reflects all the errors or uncertainties that are inherent in the parameters it uses (Table VIII-1). Although the model itself is neutral, it can perhaps be improved, especially with respect to the path implied by the column headings of Table IX-1, the number of steps (which we took to be five), and modifications to make better use of available data. We invite and urge others to prepare alternative models, and we hope that funding agencies and sponsors of research in this field will increase their support of efforts to develop improved models.

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## CONCLUSIONS

The committee has reviewed the extensive and sometimes conflicting literature pertaining to possible human health risks associated with the use of subtherapeutic concentrations of penicillin and the tetracyclines (and other antimicrobials) in animal feed. It evaluated investigations of the molecular nature of plasmids, transposons, and other bacterial antimicrobial-resistance determinants and their transfer; data on the extent of antimicrobial resistance in Salmonella species (and in other enteric pathogens) isolated from humans and farm animals; epidemiologic studies in humans and farm animals; data on reported cases of human illness and deaths due to Salmonella transmitted to humans from farm animals via meat and poultry products; information on the extent of subtherapeutic use of penicillin, the tetracyclines, and other antimicrobials in animal feed; and data from Great Britain on the effects of the restrictions placed some years ago on the use of antimicrobials in animal feeds in those countries.

The committee also reviewed the available published reports dealing with four subjects recommended for further study in the 1980 report of the National Research Council Committee to Study the Human Health Effects of Subtherapeutic Antibiotic Use in Animal Feeds: the effects of subtherapeutic and therapeutic doses of antimicrobials on the prevalence of antimicrobial-resistant enteric bacteria (including salmonellae) in farm animals; the extent of carriage of resistance-factor-containing bacteria in vegetarians and nonvegetarians (to ascertain the extent to which such carriage is associated with meat consumption); the extent of carriage of resistance-factor-containing Enterobacteriaceae in abattoir workers, their families, and neighborhood controls (to assess the association with occupational exposure to bacteria from animal sources); and the prevalence of urinary tract infections (and urinary tract infections due to resistance-plasmid-containing Enterobacteriaceae) in female workers in poultry-processing plants and a control group of women without contact with farm animals or their unprocessed meat products.

We consulted with and heard testimony from the epidemiology staff of the Centers for Disease Control, other medical epidemiologists, veterinarians, representatives of

the Animal Health Institute, microbiologists, and representatives of the pharmaceutical industry.

Using all the resources noted above, we were unable to find a substantial body of direct evidence that established the existence of a definite human health hazard in the use of subtherapeutic concentrations of penicillin and the tetracyclines in animal feeds. However, we believe that important--but as yet scant--data indicate the flow of distinct salmonella strains from farm animals, through the food processing chain, to humans in whom they cause clinical salmonellosis. In the one compelling instance of such a clear link, the multiple-antibiotic-resistant S. newport originated in farm animals exposed to chloramphenicol, a drug not approved by the Food and Drug Administration for use in feed. The committee believes that the molecular fingerprinting techniques used in this study can provide (when unique markers are present) the direct evidence needed to trace the source of antibacterial-resistant bacteria to human infection. If records of amounts of antibiotic use are maintained on farms producing food for human consumption, better evidence can be established for incriminating subtherapeutic/therapeutic doses in disease outbreaks.

The committee believes that there is indirect evidence implicating subtherapeutic use of antimicrobials in producing resistance in infectious bacteria that causes a potential human health hazard. The evidence is of several kinds:

- o There are extensive experimental data on the properties of R plasmids and their capacity for transfer of antimicrobial-resistance determinants, both in the test tube and in the intestinal tract, particularly in the presence of antimicrobial selective pressure.
- o There is evidence of widespread use of subtherapeutic concentrations of penicillin and the tetracyclines (and other antimicrobials) on farms and feedlots.
- o There is ample evidence of high levels of antimicrobial resistance among animal isolates of salmonellae.
- o Animal and poultry carcasses in meat-processing plants are often contaminated with Escherichia coli and other enteric pathogens. Few data are available on the frequency of antimicrobial resistance among such isolates. If the prevalence of antimicrobial resistance among reported isolates from diagnostic laboratories is a true representation of antimicrobial resistance in farm animals going to slaughter, the frequency of resistance among enteric pathogens in animal and poultry carcasses would be expected to be high. However, if the salmonella isolates reported

from diagnostic laboratories are principally from animals that are ill and have received antimicrobials, the figures would clearly overestimate the frequency of resistant isolates from meat and poultry carcasses.

- o Handling and ingestion of improperly cooked, packaged frozen or refrigerated meat and poultry contaminated with bacterial pathogens provides exposure to an infecting inoculum.

- o Experience with antimicrobial drugs in humans over the last 45 years has revealed the emergence of resistant strains associated with extensive drug use and the need to avoid unnecessary and prolonged use, particularly "prophylactic" use without clear and proven indications.

In addition, the committee has used the results provided by the risk assessment model presented to estimate quantitatively the possible risk of mortality associated with antibiotic-resistant salmonellae due to the subtherapeutic use of penicillin or the tetracyclines in animals. In the 1980 NRC report, the Committee to Study the Human Health Effects of Subtherapeutic Antibiotic Use in Animal Feeds concluded that "the postulated hazards to human health from a subtherapeutic use of antimicrobials in animal feeds were neither proven nor disproven." In other words, the risk of human health as a result of subtherapeutic use of antimicrobials in feed was not estimated.

We found the available data base on some aspects of the problem to be limited in quality and quantity; indeed, the data had not been gathered prospectively for the purpose of this type of analysis. The committee has used what it considers the best available information, indicating, where appropriate, the inherent weaknesses in the data. Admittedly, in some instances, we used only the best estimates available in the risk assessment. The assessment does indicate the presence of risk. Although it does not provide a distinct numerical "answer" to the question of the magnitude of the human health risk involved, it does provide some indication of the probable size of the risk in terms of numerical estimates or ranges. These are presented below as numbers of deaths per year attributable to the subtherapeutic use of antimicrobials (or penicillin and tetracyclines) in the listing of specific conclusions:

#### BIOLOGIC IMPACTS

- o Use of each new antimicrobial agent over the last half-century has eventually mobilized genes that encode resistance to the agent and disseminated them widely through

the world's interconnecting bacterial populations. Use of the antimicrobial agent disseminates the resistance genes in stages, each of which begins with a rare molecular event that facilitates further dissemination. Although use of antimicrobials in a patient or the patient's neighbors might have triggered overgrowth and clinical manifestation of the resistant strain, the evolution and delivery of its resistance genome was the result of prior use in many, probably distant, bacterial populations.

- o Results of surveys of isolates of salmonellae from animals and humans in the United States and restriction-  
endonuclease fragment patterns of resistance plasmids from selected isolates suggest that clones of resistant salmonellae are endemic in animals and sporadic or occasionally epidemic in humans.

- o Herds of farm animals given subtherapeutic amounts of antimicrobial agents have more antimicrobial-resistant intestinal bacteria than herds given no antimicrobials.

- o The most important determinant in the selection of antimicrobial-resistant strains in a bacterial population is exposure of that population to antimicrobials. Total duration and concentration of antimicrobial use are important in selection for resistance. Any measure that fails to reduce total use appreciably is unlikely to affect the prevalence of antimicrobial-resistant strains.

- o Resistance to antimicrobial drugs among salmonella strains can interfere with the efficacy of antimicrobial therapy of human salmonellosis. (Such resistance is usually R-plasmid-mediated, so it can involve other drugs, such as trimethoprim-sulfamethoxazole, chloramphenicol, and ampicillin.) Although such interference with the efficacy of therapy almost certainly occurs (i.e., patients are treated with an antimicrobial that is ineffective because of drug resistance), it is probably quite uncommon in nontyphoidal salmonellosis.

- o The available data are inadequate to conclude that either subtherapeutic or therapeutic concentrations of antimicrobials are more selective of drug-resistant bacteria. On theoretical grounds, it is likely that therapeutic and subtherapeutic dosages exert equal selective pressure for clonal expansion of resistance, but subtherapeutic dosages exert more pressure for conjugative transfer of drug resistance, because of the dosages and the durations of administration.

o Animal and poultry products (including veal, beef, pork, chicken, eggs, and milk) are the principal sources of human nontyphoidal salmonellosis. Also, some E. coli serotypes can also be found in the intestinal flora both of humans and of farm animals. Thus, there could be an interconnecting link between these two large pools of enteric microorganisms, facilitated by the high frequency of contamination of animal and poultry carcasses in slaughterhouses. Such a potential link would provide a means of movement of R plasmids of farm origin to the human alimentary tract. The interconnection, because of its nature, would constitute an almost exclusively one-way passage.

o The overall prevalence of resistance to any of five commonly used antimicrobials is about 4 times as great in collections of salmonella isolates from farm animal and poultry (65%) as those in collections of isolates from humans (15.5%). This difference suggests that the predominant pool of resistant salmonellae is in farm animals. Because ultimately almost all human infections with nontyphoidal salmonellae result from strains originating in farm animals, the antimicrobial resistance observed in human isolates most likely is derived from the animal pool of resistance genes, rather than from selection due to antimicrobial use in humans.

#### EPIDEMIOLOGIC FINDINGS

o Evidence is sparse that directly links the use of penicillin and tetracycline in subtherapeutic concentrations in animal feeds to human infections. Several studies have yielded reliable evidence of spread, from farm animals and poultry to humans, of E. coli strains in which antimicrobial resistance had been induced by administration of subtherapeutic concentrations of antimicrobials as feed additives. There is evidence from only one study of the direct spread of multiple-antimicrobial-resistant salmonellae from farm animals to humans via meat products. However, the antimicrobial used on the farm was chloramphenicol, a drug not approved by FDA as a feed additive in animals used for food production. It might be difficult, or impossible, to provide a total chain of evidence directly relating the majority of cases of human infection with antimicrobial-resistant salmonellae to a source on the farm or feedlot or to relate the presence of the resistance to the use of specific antimicrobials in subtherapeutic concentrations in feed. By the time a detailed investigation of an outbreak of human salmonellosis occurs, evidence of prior antimicrobial use patterns might not be available.

o It has not been possible to determine whether antimicrobial resistance of salmonellae caused by the administration of subtherapeutic concentrations of antimicrobials in animal feed increases the number of cases of human salmonellosis.

o Whether the presence of antimicrobial resistance in salmonellae increases virulence is uncertain; the available data are limited and conflicting. In special circumstances, as when R plasmids are linked with virulence genes (e.g., those for enterotoxin or hemolysin in E. coli), selection by antimicrobial agents might promote spread of virulent strains; however, such an occurrence has only rarely been reported. It is not clear whether the overall prevalence of salmonellae in food products is increased by virtue of antimicrobial resistance. However, the incidence of human salmonellosis in the United States is increasing, and the increase is unlikely to be an artifact of better reporting. As long as most strains of Salmonella are susceptible to the antimicrobials to which they are exposed, subtherapeutic administration of antimicrobials might reduce the prevalence of salmonellae in meat and poultry products that humans ingest. However, as the prevalence of resistant strains increases because of repeated and prolonged exposure to antimicrobials, subtherapeutic administration might actually favor the increase by suppressing the normal competing flora and promoting R-plasmid spread. Direct proof of this pattern in salmonellae in farm animals is lacking.

o The current frequency of R-plasmid-mediated antimicrobial resistance among isolates of E. coli and salmonellae in the intestinal contents of farm animals and poultry is high--much higher than in human isolates. It would be difficult to predict the period required, after curtailment of the use of subtherapeutic concentrations of penicillin and the tetracyclines in animal feed, for R-plasmid-mediated antimicrobial resistance to decrease in any extent in salmonellae and E. coli strains. Major decreases might occur only after the passage of years, in view of (1) the current degree of resistance, (2) the extensive environmental contamination on farms and feedlots with resistant organisms, (3) the prolonged prior subtherapeutic use of antimicrobials, which has allowed extensive permeation of resistance genes (transposons) throughout the highly colonization-adapted coliform flora of farm animals, and (4) the need to introduce competing, antimicrobial-susceptible coliform bacteria. Results of studies in confined populations of swine indicate that it could take many years for major decreases in levels of resistance to occur.

o Although the extent of antimicrobial resistance among salmonella strains isolated from humans is probably growing, it is still low enough for suitable intervention to forestall possible further increases and eventually to lower the overall extent of antimicrobial resistance.

#### ANTIBIOTIC USE PATTERNS

o The use of subtherapeutic dosages of penicillin and the tetracyclines in animal feeds is extensive in the United States. Such use is for the purpose of either growth promotion or disease prevention and often continues for a substantial portion of the growth cycle of farm animals. The specific rationale for use in a given herd at a given time is not always clear. Of over 31 million pounds of antimicrobials produced each year in the United States, about 42-48% is designated for addition to animal feeds or other unspecified (minor) uses. The best estimates (they are only estimates) indicate that penicillin and the tetracyclines account for almost 60% of the antimicrobials sold to the feed trade (and presumably ultimately used on farms and in feedlots). Of the total amount of tetracyclines produced in this country, for use in both humans and animals, approximately 70% is sold for use in livestock and poultry feeds. An estimated 88% of all antimicrobial use in livestock and poultry is in subtherapeutic concentrations. Thus, subtherapeutic use of penicillin, the tetracyclines, and other antimicrobials in animal feeds--which accounts for some 40% of antimicrobial production in the United States--constitutes a sizable segment of the total antimicrobial selective pressure (for resistant enteric microorganisms) exerted on the combined human and farm-animal intestinal bacterial populations.

o Interpretation of the results in Great Britain after banning the subtherapeutic use of penicillin and the tetracyclines in animal feed is difficult, in part because total farm use of these antimicrobials might not have decreased because use could have taken the form of therapeutic or prophylactic doses in feed for disease treatment or prevention as prescribed by a veterinarian. The appearance of new epidemic strains of antimicrobial-resistant salmonella serotypes during the period of interdiction of subtherapeutic use further confounds interpretation. It might take years for dilution of antimicrobial-resistant strains of Salmonella and E. coli in the farm animal population before any substantial changes might be observable.

RISK ANALYSIS

o The committee has been unable to find substantial direct evidence that bacterial resistance resulting from the use of subtherapeutic concentrations of penicillin or the tetracyclines in animal feed causes an excess risk to human health as a result of consumption of food products derived from the treated animals, as a result of contact with such animals, or as a result of exposure to an environment contaminated by resistant enteric bacteria from such animals. Lacking this direct evidence, the committee turned to the tools of risk assessment to develop some quantitative estimate of the probable risk to human health associated with this form of the subtherapeutic use of these antimicrobials.

o Use of penicillin and the tetracyclines in subtherapeutic concentrations in animal feed has led to increased antimicrobial resistance in foodborne commensals and pathogens. The risk analysis in this report focused only on human infection with salmonella serotypes, because available data on other species were insufficient. The committee has not assessed the potential risk to human health associated with drug resistance in other gram-negative bacillary species (Campylobacter jejuni, Yersinia enterocolitica, and enterohemorrhagic E. coli) of animal origin, because the data on human cases are too limited and because antimicrobial susceptibility data on those bacteria are not routinely obtained.

o Because the committee's risk assessments are based on estimates using sparse data, these estimates should be interpreted and used with caution. Such estimates are best seen as scientific hypotheses about the possible extent of a problem. This does not mean that they are "hypothetical" in the weak sense of being speculative. Rather, they are hypotheses that are consistent with all available information and scientific understanding, but they have not been tested by traditional scientific methods. All the estimates presented in this report should be viewed in that perspective.

o Annual numbers of deaths from salmonellosis attributable to subtherapeutic uses of any antimicrobials for prophylaxis and growth promotion have been estimated. The likeliest estimate is 70 deaths per year.

o The likeliest estimate of mortality from salmonellosis attributable to subtherapeutic uses of penicillin/ampicillin and/or tetracycline for prophylaxis and growth promotion is 40 deaths per year. Caveat--these are not necessarily "excess deaths," but rather estimates of the

yearly mortality attributable to salmonellosis of the indicated origin. The deaths might to some extent replace deaths (in the same patients or others) that occur from infections due to salmonellae susceptible to penicillin/ampicillin and tetracycline if subtherapeutic dosages of these antimicrobials had not been used in animal feed. Estimation of such "replacement" of deaths is not possible with the evidence at hand.

o The likeliest estimate of mortality from salmonellosis attributable to subtherapeutic uses of any antimicrobial for growth promotion only is 20 deaths per year. As in the preceding (and following) estimates, the caveat regarding "excess deaths" applies.

o The likeliest estimate of mortality from salmonellosis attributable to subtherapeutic uses of penicillin/ampicillin and/or tetracycline only for growth promotion is 15 deaths per year.

o The likeliest estimate of mortality from salmonellosis in the "etiologic fraction" attributable to subtherapeutic uses of any antimicrobial for prophylaxis and growth promotion is 6 deaths per year. The "etiologic fraction" is the proportion of persons exposed to an antimicrobial-resistant salmonella strain who are at increased risk of illness by virtue of recent use of antimicrobial drugs for whatever reason. Therefore, such deaths can be considered as "excess deaths"; i.e., they would not occur if the infecting salmonella strain were not antimicrobial-resistant and if its multiplication were not promoted, presumably, by suppression of growth of the competing normal antimicrobial-susceptible normal flora. In the same way, the number of foodborne pathogens (inoculum size) needed to precipitate disease might have been decreased. Whether a similar effect can be produced by prior antimicrobial use in persons infected with antimicrobial-susceptible salmonellae (due to possible differential antimicrobial susceptibility between susceptible salmonellae and normal components of the intestinal flora) is unknown, and the committee has not been able to find data bearing on this question.

o The likeliest estimate of mortality from salmonellosis in the "etiologic fraction" attributable to subtherapeutic uses of penicillin/ampicillin and/or tetracycline for prophylaxis and growth promotion is 6 deaths per year.

o The likeliest estimate of mortality from salmonellosis in the "etiologic faction" attributable to

subtherapeutic uses of any antimicrobial only for growth promotion is 2 deaths per year.

o The likeliest estimate of mortality from salmonellosis in the "etiologic fraction" attributable to subtherapeutic uses of penicillin/ampicillin and/or tetracycline only for growth promotion is 2 deaths per year.

o Infections with antimicrobial-resistant strains of Salmonella are more often fatal than infections with susceptible Salmonella. Therefore, the increased difficulty of providing effective therapy for human disease can be estimated. The increased difficulty in providing effective treatment may be due to increased virulence of antimicrobial-resistant strains, to the presence of resistance to one of the antimicrobials ordinarily used to treat such infections when they are severe or when they occur in particularly vulnerable persons, or to some other factor. The likeliest estimate of mortality from salmonellosis arising because of increased difficulty of treatment attributable to subtherapeutic uses of any antimicrobial for prophylaxis and growth promotion is 40 deaths per year.

o The likeliest estimate of mortality from salmonellosis arising because of increased difficulty of treatment attributable to subtherapeutic uses of penicillin/ampicillin and/or tetracycline for prophylaxis and growth promotion is 20 deaths per year.

o The likeliest estimate of mortality from salmonellosis arising because of increased difficulty of treatment attributable to subtherapeutic uses of any antimicrobial only for growth promotion is 8 deaths per year.

o The likeliest estimate of mortality from salmonellosis arising because of increased difficulty of treatment attributable to subtherapeutic uses of penicillin/ampicillin and/or the tetracyclines only for growth promotion is 8 deaths per year.

o Evaluation of the foregoing estimates of mortality from salmonellosis attributable to subtherapeutic uses of antimicrobials in animal feed requires consideration in a broader context. What possible benefits accrue from such subtherapeutic use of antimicrobials in food production? Would human deaths from salmonellosis be reduced by the discontinuation of subtherapeutic use of penicillin/ampicillin and/or the tetracyclines? The committee's thesis is that, although some deaths due to antimicrobial-resistant strains might be "replaced" by deaths due to susceptible strains, the total number of deaths would decrease, however,

this cannot now be proved. The committee offers no recommendations regarding policy-making because that was not part of its mandate.

## XI

### RECOMMENDATIONS FOR FUTURE RESEARCH

The committee offers no recommendations of possible solutions to risk management of the overall problem under consideration. It has directed its attention mostly to its charge to review the human health consequences and the risk associated with the use of penicillin and the tetracyclines at subtherapeutic concentrations in animal feed. Recommendation of any action would be appropriate only after regulatory agency review and weighing of both the benefits and risks of use of these antibiotics in subtherapeutic dosages.

The committee does, however, offer recommendations concerning further investigations that would be helpful in resolving the issue, which has been intensely debated for some 15-20 years. Many of the recommendations for study would remain appropriate whether current policies regarding subtherapeutic antimicrobial use remain in effect or are changed by a regulatory agency. In the former instance, the data obtained would serve to strengthen the informational underpinning of risk estimation. In the latter instance, they would make it possible to compare data on the prevalence of antimicrobial resistance among enteric pathogens and human health risks before and after institution of any change in approved antimicrobial use.

#### STUDIES TO SUPPLEMENT THE DATA BASE FOR RISK ANALYSIS

The risk assessment performed by this committee used the best available data related to the six essential elements in its risk estimates: resistance of human salmonella isolates to antimicrobials, annual reports of cases of salmonellosis, death rates associated with antimicrobial-susceptible and antimicrobial-resistant strains, fraction of human salmonellosis deaths associated with strains of farm origin, fraction of antibiotic resistance in strains of farm origin caused by subtherapeutic use of antimicrobials in animal feed, and "etiologic fraction." In compiling its risk estimates the committee was limited by the paucity of some types of data and the consequent need to extrapolate from the results of small studies to the global problem, by the fact that in some subjects reliable data were almost totally lacking and had to be substituted for with "best estimates,"

and by the nature of the available relevant data which often had been collected for other purposes. The risk assessment was confined to infections caused by Salmonella species, only a portion of the problem, because basic surveillance data on infections due to other gastrointestinal pathogens of animal origin--such as Campylobacter jejuni, Yersinia enterocolitica, and enterohemorrhagic E. coli--were not available.

The committee hopes that FDA will find the risk assessment performed by this committee to be useful in its decision-making. The committee acknowledges the qualitative and quantitative deficiencies of the primary data and the broad range of estimates used in the assessments of risk. Narrowing the range of these estimates would necessitate refining and enlarging the data base used in the risk analysis. In the committee's view, such an effort appears reasonable.

#### ANTIMICROBIAL-RESISTANT SALMONELLA STRAINS AND THEIR SOURCES

Improved surveillance of salmonella isolates in the United States is essential for better understanding and control both of bacterial resistance to antimicrobial agents and of disease due to salmonellae in humans and animals. Improved surveillance would be easy to put into place, because it would build on, and actually require only a small increment to, a large existing system.

#### The Existing System

Each year, hundreds of thousands of physicians, analyzing illnesses of millions of patients, send hundreds of thousands of stool specimens to more than 5,000 microbiology laboratories. This is a time-consuming process by which skilled technologists isolate 40,000 or more salmonella strains and conduct tests for resistance to antibacterials. Most of the salmonella isolates are then forwarded to state reference laboratories, where technologists with special training, skills, and reagents laboriously type them into more than 1,000 possible serotypes. Serotype reports are returned to the referring laboratories, where they only rarely contribute to the management of patients.

Epidemiologists in the separate states use the reports of isolated salmonella serotypes to delineate recognized outbreaks and detect others. The accumulated reports of all the state reference laboratories are collected, tabulated, and published by the Centers for Disease Control (CDC).

In a similar system, state veterinary laboratories, the National Veterinary Reference Laboratory, the Food Safety and

Inspection Service (FSIS) of the U.S. Department of Agriculture, and various university diagnostic laboratories isolate and serotype salmonellae from specimens taken from animals; however, these data are unpublished and have not been included in the data base used by this committee.

#### Additions to the System

The antimicrobial susceptibilities of each salmonella isolate need to be recorded with its serotype.

Reference laboratories need to enter their results each day into a networked computer system that analyzes all data automatically and comprehensively.

Resistant isolates classified by a computer as epidemiologically important should be forwarded to a laboratory that will catalog their plasmids.

#### Addition of Susceptibility Test Results

One good reason for adding results of susceptibility tests is to obtain accurate and complete measurements of prevalence of resistance, regional variations, trends over time, etc. All those would have been valuable to this committee, but could only be pieced together crudely from fragmentary reports of limited comparability (see Chapter V).

A second, and probably better, reason is to improve epidemiologic information, which is the only justification for the present elaborate system. Essentially, outbreaks are confirmed or recognized now by virtue of an excess of isolates of one serotype over the expected incidence. The outbreak clone boosts the serotype isolation rate above the threshold of random appearances. However, for the more commonly isolated serotypes (which account for most of the isolates), the threshold for detecting excess is high. Generally, only large outbreaks are investigated, except for outbreaks caused by rare serotypes; in fact, most outbreaks are overlooked. Coupling antibiotype to serotype permits detection of small outbreaks that are due to resistant subclones that belong to common serotypes. Two isolates of a subclone can be recognized to constitute an outbreak; without the antibiotype, dozens might be needed. Recognition of more small outbreaks or of large outbreaks sooner improves the understanding and control of salmonella disease and of the flow of resistance genes in animals and humans. Recognition of more outbreaks also provides more opportunities to trace chains of transmission.

A third reason for recording antibiotypes is that it is the key to the use of plasmid cataloging, as described below, which adds another level of subclone discrimination.

Determining the resistance of all salmonella isolates in the United States would be easy and inexpensive. Four-fifths of the human isolates exhibit no antimicrobial resistance. The ones that do nearly always have resistance at least to either tetracycline, streptomycin, sulfonamide, or ampicillin; dropping four disks on a small plate or part of a plate would screen out the susceptible (nonresistant) four-fifths, leaving 8,000 resistant isolates--an average of less than one per day per state reference laboratory. Each would need a routine disk-susceptibility test plate, which entails several minutes of work and a dollar's worth of supplies. Some 4,000-5,000 animal isolates of salmonellae would need susceptibility testing each year.

#### An Integrated Computer System

Salmonella outbreaks often appear as a small number of isolates scattered across several states. Accordingly, continuing analysis of all U.S. data in an integrated system is needed to detect these outbreaks early or at all. Such a system would become more important as antibiotyping and plasmid cataloging began to discriminate more salmonella subclones. It would also be helpful in developing or adapting shared software, such as automatic notification whenever any clone or subclone in any state or combination of states exceeded its outbreak threshold. Integration should be considered soon, before individual reference laboratories acquire various incompatible systems.

#### Plasmid Cataloging

Plasmid cataloging would be a useful diagnostic tool and should begin with a survey of the restriction endonuclease digestion patterns of resistance plasmids from animal and human salmonella isolates representing prevalent antibiotypes for a number of serotypes (see Table V-2). Experience has shown each set of plasmids from isolates of one antibiotype-serotype combination would be expected to have one or more restriction patterns. Wholly different restriction patterns in any set would discriminate subclones presumed to be unrelated. Patterns with small differences undoubtedly represent evolutionary variants of one clone and may be distinguished with microepidemiologic studies. For example, plasmids from six human isolates from different parts of one state over a two month period had identical restriction patterns, whereas those from humans or animals in other states all differed slightly from those six and from one another.

As the catalog of U.S. salmonella plasmids might build, in parallel with a computerized isolate data base, the epidemiology of salmonellae in animals and humans could begin to emerge at a new level of detail and quantitation. The distribution of specific clones and subclones among animal populations could be delineated, and rates of appearance in humans could be established. That would provide better understanding of the epidemiology of salmonellae in animal and human outbreaks, give a basis for each of them at the outset to known plasmid families with known prior distributions, and hence provide early clues to possible chains of spread.

One laboratory could catalog the salmonella plasmids and explore technology for improved cataloging of plasmids; restriction endonuclease profiling, although workable now, is likely to be supplemented (if not replaced) by newer methods that would be faster and provide more critical molecular detail. In particular, as more is learned about the stages of spread of resistance through bacterial populations and the molecular changes that accompany those stages, it will be of great value to find correlates in the data on salmonellae.

#### Implications of Change in Use of Animal Feed Additives

It can be questioned whether a different program of salmonella surveillance would be needed if use of animal feed additives changed. The program recommended above would probably be a sensitive monitor of such change, as well as providing improved observation of the present situation.

#### HUMAN MORBIDITY AND MORTALITY DUE TO ENTERIC PATHOGENS OF FARM ANIMAL ORIGIN

"Salmonella deaths" might be construed as deaths due primarily to salmonellosis (with bacteremia and shock, endarteritis, metastatic abscesses, and severe gastroenteritis with dehydration, usually in infants or elderly); those in patients with underlying diseases in whom salmonella gastroenteritis contributed to the death, but was not the primary cause of it; and those in patients from whom salmonellae were isolated, but in whose deaths had other causes. In cases with other causes, hospitalization might have been initiated by salmonella infection that had subsided and their deaths have had unrelated causes. Similarly, salmonella gastroenteritis and asymptomatic carriage can occur as almost incidental matters in patients with other major medical problems to which they succumb.

Often, the above distinctions have not been made in published series of cases and reports of outbreaks, and

information on death certificates is not of sufficient quality to allow such distinctions to be drawn. Such data can probably be developed only with expanded studies of selected counties of the type performed by CDC.<sup>7</sup> However, it will likely require more detailed analysis of hospital records and information (based on chart-directed recall) from attending physicians to categorize "salmonella deaths" more definitively. In addition, definitive classification of those deaths as to contribution of salmonella infection (primary cause, contributing cause, of unknown relevance, or unrelated) should be stratified according to antimicrobial susceptibility pattern of the strain involved (susceptible, resistant to penicillin or tetracycline, resistant to multiple antimicrobials, etc.). In the characterization of strains, primary attention should be on resistances to antimicrobials known to be R-plasmid-mediated.

The selected-counties study of the Salmonella Surveillance System is the only current system of surveillance of infection with Salmonella of which the committee is aware. Additional useful information could be obtained through the system if the following modifications were introduced:

- o Expansion of the number of communities to yield a larger data base.
- o Continuation of the selected-counties study in the form of annual surveillance, rather than monitoring every 4-5 years.
- o Determination of morbidity (days of diarrhea, hospitalization rates, etc.) associated with infection caused by antimicrobial-susceptible and antimicrobial-resistant strains.
- o Inclusion of infections with Campylobacter jejuni based on the same kinds of epidemiologic questionnaires (given the increasingly evident impact of campylobacter infection).

#### QUANTITIES OF ANTIMICROBIAL DRUGS USED IN SUBTHERAPEUTIC CONCENTRATION IN ANIMAL FEEDS

Amounts of antimicrobials in the aggregate (and of individual antibiotics, such as penicillin and the tetracyclines) used in animal feed are not known. The best available information comes from the Animal Health Institute and from industrial sources as estimates that are admittedly rough. Valuable data could be provided by monitoring and surveillance to determine actual subtherapeutic use of

antimicrobials in animal and poultry feeds, perhaps through sampling of farms and feedlots. Sampling should reflect characteristics of a cross section of users of antimicrobial feed additives.

o Characteristics of facilities to be monitored.

Sampling should include large and small farms, appropriate geographic areas, the major animal sources of meat products (beef cattle, veal cattle, pigs, and poultry), and (in the case of cattle) widely different methods of animal rearing (confined, high-density herds, and open-range grazing). It should include the spectrum from small local facilities to huge industrial operations and existing data bases, such as APHIS-USDA.

o Rationales for subtherapeutic use of antimicrobials given by user at each administration.

Was it for growth promotion, disease prevention, or some other purpose? Did the farmer know, in fact, whether feeds contained an antimicrobial additive and, if so, what it was? For what fraction of the total growth cycle is the regular use of penicillin and tetracyclines (and other antimicrobials) in subtherapeutic concentrations in feed a regular practice?

o Concentrations of penicillin and tetracyclines (and other antimicrobials) achieved in feed on farms and in feed lots.

Are the prescribed subtherapeutic concentrations being achieved after mixing on farm and feedlot? Are they being exceeded unwittingly?

o Would routine record-keeping of antimicrobial use on farms and feedlots be feasible?

Such records would be of value, after antimicrobial-resistant strains involved in outbreaks of human salmonellosis were traced to the farm source (by plasmid fingerprinting techniques), in determining relationship to prior use of subtherapeutic concentrations of antimicrobials in feed given to the farm animals involved.

ROLE OF PRIOR EXPOSURE TO ANTIMICROBIALS IN INFECTION BY ANTIMICROBIAL-RESISTANT STRAINS OF SALMONELLAE ("ETIOLOGIC FRACTION")

Studies of the role of the "etiologic fraction" in humans should be expanded to provide a larger statistical basis for the estimates of risk to human health. Further data might be gleaned both from outbreaks, as has already been done, and from the Salmonella Surveillance System for selected counties. It would also be of interest to develop data on the morbidity and mortality rate in patients who constitute the etiologic fraction. Do these patients exhibit

more or less severe illness than other patients overall? The selection of matched controls must be carried out with great care, because the patients in the etiologic fraction are likely to differ from the general population at risk of salmonellosis by virtue of their recent intake of antimicrobial drugs, their greater age, their underlying illnesses, and by their receipt of a smaller inoculum.

As far as we are aware, the term "etiologic fraction" has been applied only to infections in humans, but there might also be an etiologic fraction in animals--i.e., decreased colonization and infection of animals by susceptible strains, an effect that might be termed a "negative etiologic fraction." Indeed, evidence supports the latter hypothesis.<sup>3</sup> A negative etiologic fraction for infections caused by susceptible strains will increase the proportion of infections caused by resistant strains.

A more crucial question concerns the net effect of the subtherapeutic administration of antibiotics on the overall prevalence of carriage of salmonellae (resistant or susceptible) in animals. Studies to evaluate that question could be carried out by passive observation of naturally occurring infection during the administration of antibiotics or by deliberate feeding of salmonella strains under controlled conditions. The results would probably be highly influenced by the choice of antibiotics for administration, as well as by the dosage and frequency of administration, the degree of resistance (or susceptibility) of the infecting strains, and the degree of resistance of the normal flora (possibly including both aerobes and anaerobes). Small differences in any those variables would influence the subtle interaction between the suppressive effect of a drug on a bacterial pathogen and on the normal bacterial flora and the interaction between these bacteria.

A negative etiologic fraction might also be hypothesized in humans. Indeed, some have suggested that the inappropriate use of antibiotics for such illnesses as viral pharyngitis, although attended by potential adverse effects, might have a beneficial effect in preventing secondary infection or complications of infection caused by susceptible pathogens. This committee's charge does not deal with the administration of antibiotics to humans, so that subject has not been pursued in this report.

#### MORBIDITY AND MORTALITY ASSOCIATED WITH UNREPORTED CASES OF SALMONELLOSIS

There is reliable evidence that only about 1-10% of cases of salmonellosis are identified in outbreaks; a comparably small fraction is thought to be reported in sporadic cases. Perhaps more important, it is often commonly

assumed that the unrecognized or unreported cases are generally less severe than the recognized or reported ones. The committee, in estimating risk, had no data on salmonellosis morbidity and of course could use data on salmonellosis deaths only if such deaths were reported.

It might be possible for CDC to conduct telephone surveys during outbreaks of salmonellosis to detect not only the unreported proportion of cases, but also to obtain data on morbidity in those cases; the results for both mortality and morbidity could be compared with data on reported cases. If morbidity were similar in the reported and unreported cases--i.e., chance, rather than severity of illness, would be determining whether an infection is reported--this would suggest that the impact of salmonellosis in our risk assessment, which is based on 40,000-65,000 cases per year, should be scaled sharply upward to take account of unreported cases.

STUDIES TO DETERMINE EXISTENCE OF DIRECT  
EVIDENCE OF HUMAN HEALTH HAZARD ASSOCIATED  
WITH SUBTHERAPEUTIC USE OF PENICILLIN AND  
TETRACYCLINES IN ANIMAL FEEDS

Only CDC is in a position to perform such studies. The use of molecular techniques to characterize salmonella isolates clonally and to identify antimicrobial-resistant strains through the food chain from farm animal to human consumer has proved successful. Do the one or two examples reported constitute clear evidence of a common phenomenon, or are they exceptions? The weak point in the argument is the lack of a direct demonstration of the role of subtherapeutic use of approved antibiotics, such as penicillin or tetracyclines, as the farm origin of the infecting salmonella clone. More salmonella outbreaks should be studied with molecular fingerprinting techniques and with conventional epidemiologic methods, including documentation of the qualitative and quantitative aspects of antimicrobial use on the implicated farms or feedlots.

OTHER STUDIES

ANTIMICROBIAL RESISTANCE AMONG, AND FREQUENCY OF DISEASE  
DUE TO, OTHER FOODBORNE ANIMAL PATHOGENS THAT AFFECT HUMANS

Recognition over the last decade that other bacterial enteric pathogens, such as Campylobacter jejuni and E. coli 0157:H7, can spread from animals to humans and that Campylobacter might infect humans more often than any other intestinal pathogen, raises the question of whether

monitoring the frequency with which they cause human and animal infections might illuminate the issues faced by this committee.

Information on those pathogens seems sparse, compared with information on salmonellae. Serotyping does not yet subdivide them as elaborately as it does salmonellae and is in any case not yet routinely practiced by a national network of laboratories. Resistance does not seem to impede therapy often or to be as varied as in salmonellae. Further work on these pathogens needs to be supported, but surveillance data on them are not likely to influence antimicrobial use greatly in the near future.

#### SELECTIVE EFFECTS OF THERAPEUTIC AND SUBTHERAPEUTIC DOSAGES OF ANTIMICROBIAL AGENT

Given the paucity of in vitro data of the kinds required to predict the effects of different dosages of drugs on selection of antibiotic-resistant strains, much more work is recommended. In particular, in a mixed bacterial population, what are the effects of different dosages of antimicrobials on the growth characteristics of resistant and susceptible bacteria, and on the genetic spread of R plasmids, particularly between species? Such studies require careful determination of growth rates and conjugative transfer rates under well-controlled conditions. The resulting information should make it possible to develop appropriate computer models to allow prediction of dosage effects on the emergence of drug-resistant organisms.

#### EFFECT OF ANTIBIOTIC RESISTANCE ON VIRULENCE OF FOODBORNE PATHOGENS OR SEVERITY OF DISEASE PRODUCED

Recently, some plasmids in salmonellae have been identified as being involved in the expression of virulence.<sup>1,2,8</sup> Plasmids in S. typhimurium (90 kilobases), in S. dublin (75 kb), and in S. enteritidis (54 kb) are necessary for these three serotypes to express virulence in mice. Plasmid-free "cured" strains lose virulence in mice, and reintroduction of the plasmids into the "cured" strain, or into a naturally occurring plasmid-free isolate, restores virulence.

Studies defining the prevalence of virulence plasmids in the common Salmonella serotypes (antimicrobial-susceptible strains) isolated from farm animals (and humans) not exposed to antibiotics will be helpful in providing baseline information concerning possible effects of subtherapeutic administration of antimicrobials in feed. Virulence plasmids might not be present in most strains of antimicrobial-

susceptible salmonellae in farm animals; in that case the possible effect of antimicrobial selection or persistence and expansion of the population of virulence plasmids in farm animals receiving subtherapeutic concentrations of antibiotics warrants investigation. Similarly, direct comparison of human (and animal) isolates of antibiotic-susceptible and -resistant salmonella isolates of specific serotypes in mouse-virulence assays bears directly on the question of whether antibiotic resistance in such a foodborne pathogen can affect its virulence.

#### BACTERIOLOGIC CONTAMINATION OF ANIMAL FOODSTUFFS WITH ENTERIC PATHOGENS

FSIS has the responsibility for ensuring the safety of meat and poultry products. FSIS has an overall goal of ensuring that meat, poultry, and their products are wholesome, unadulterated, and properly labeled and do not constitute a health hazard to the consumer. In 1983, FSIS asked NRC to evaluate the scientific basis of the current system for inspecting meat, poultry, and meat and poultry products. The 1985 report<sup>4</sup> that resulted from that request offered numerous recommendations, not all of which are directly relevant to the present study. However, one of the major conclusions of the report states that Salmonella and Campylobacter species are major causes of diseases transmissible to humans through the consumption of meat and poultry products and that current postmortem inspection methods are not adequate to detect these organisms. The report recommended that efforts to control and eliminate contamination with microorganisms include evaluation of rapid diagnostic procedures for detecting Salmonella and Campylobacter especially. Postmortem inspection methods have been relatively effective for the detection of unwholesome meats; before these methods are abandoned, FSIS should determine the effectiveness of the methods that would replace them.

At the request of FSIS, another study was done<sup>5</sup> to evaluate the current FSIS poultry inspection programs in the framework of a risk-assessment model incorporating statistical procedures. The report of that study drew several conclusions and offered recommendations that are relevant to the current study. "There is conclusive evidence that microorganisms pathogenic to humans (such as Salmonella and Campylobacter) are present on poultry at the time of slaughter and at retail." The report stated that "the critical control points at which known pathogenic microorganisms such as Salmonella and Campylobacter may be introduced into the poultry system should be identified and monitored, preferably as a part of an HACCP (Hazard Analysis

Critical Control Point) program" and that FSIS begin to lay the groundwork for a more comprehensive program with statistically based sampling that modifies the traditional bird-by-bird inspection.

In 1969, the NRC Committee on Salmonella<sup>6</sup> recognized that there was no way to be absolutely certain that a particular lot of nonsterile food is free of salmonellae. It recommended the development of a sampling plan to provide adequate assurance that the number of salmonellae present, if any, is below a statistically defined limit that reflects minimal hazard to the consumer. A subcommittee of that committee supported the HACCP concept as an effective and rational approach to the assurance of safety.

FSIS is now evaluating the effectiveness of the HACCP procedure for analyzing the slaughter of poultry and the procedures for handling finished products. FSIS plans to determine where the critical control points are and what procedures would be best for controlling bacterial contamination from the time when the animal reaches the processing plant to the time when the finished product goes to market. Today there is no routine microbiologic sampling of meat or poultry. To help in evaluating the critical control points in processing of poultry, an agency pilot plant in Puerto Rico is gathering baseline data at each critical control point in processing. Investigators will look at microbiologic contamination before animals reach the processing plant and during processing.

The present committee recommends that, with respect to microbiologic sampling of meats, consideration be given not only to the identification of pathogenic organisms, but also to their testing for antimicrobial susceptibilities. The resulting microbiologic data should be available to researchers for use in studying the relationship of antibiotic use to drug resistance in pathogens isolated from foodborne outbreaks of human disease.

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