more orders of magnitude (8,10). In spite of this concentration method, the success rate in EM diagnostics using swab specimens has declined to <10%, while viral agents continue to be identified in >60% of lesions in submitted aspirates.

Because concentration methods are not always available, and in view of the sample problems identified by Marshall (3), we reviewed, in Winnipeg, whether collection of lesion fluids directly onto EM sample grids (5) improved sensitivity over aspiration into 26-gauge needles on tuberculin syringes (4). While neither method increased the number of cases identified in matched samples, the yield of virus seen in samples taken by touching the EM sample grid directly to the base of the lesion did increase, making it easier to identify viral agents in the samples (Hazelton and Louie, unpub. data). In Berlin, we also routinely find higher particle numbers on grids that have been prepared by the direct touch method. Sample preparation on EM grids is conducive to prolonged storage and transport of samples over long distances (5) and removes the risk of needle-stick accidents.

We continue to recommend examining grids touched directly to the lesion or vesicle aspirates. Where possible, infectious diseases and infection control staff contact the EM unit when a sample needs to be collected to receive instructions about methods and ensure that staff are available to conduct the examination. When the specimen needs to be transported some distance, such as between cities, smears on individually packaged glass slides or on sample grids are an alternative method for submitting vesicle aspirates. Glass slides allow the collection of samples for both polymerase chain reaction and EM examination (Charles Humphrey, personal communication). An additional advantage of smears is that interfering background proteins can be removed by drying the sample on the slide and then resuspending the viral agent. Proteins such as mucus, which interfere with staining and visualization, remain insoluble. We understand that other major viral EM diagnostic units also prefer aspirates, smears on glass slides, or lesion exudate on the final sample grid as preferred methods of submission of suspected blister material because of ease in handling and higher efficiency in examination.

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Antimicrobial Resistance

To the Editor: Davis et al. offered four reasons why local antimicrobial selection pressure in cattle may not play an important role in the dissemination of multidrug-resistant *Salmonella* from cattle to humans (1). Their conclusions differ from those of other recent studies (2-6).

The authors' first two arguments relate to the high levels of chloramphenicol resistance in the United States, despite a relative lack of

chloramphenicol use in livestock. In industrialized countries, chloramphenicol use in humans is also low because of medical and legal concerns about aplastic anemia. In Australia, the total average annual human use of chloramphenicol from 1992 to 1997 was 208 kg (6). This is lower than the annual use for most other antibiotics (e.g., sulphonamide 22,331 kg in humans and 24,869 kg in animals; tetracycline 12,677 kg in humans and 77,619 kg in animals) (6). Despite this low use in humans, chloramphenicol resistance can be common in many human pathogens, e.g., multidrug-resistant *Staphylococcus aureus* (7) and *Pneumococcus* (8). Even though tetracyclines are not used in children, children's pneumococcal isolates are often tetracycline resistant (8). With these bacteria, the use of other antibiotics (e.g., penicillins, macrolides, and cephalosporins) appears to drive chloramphenicol (and other) resistance, which is often a part of gene clusters that encode for multidrug resistance. The situation in animals for *Salmonella* is likely to be similar. In the United States, chloramphenicol resistance is higher in isolates from cattle (73% in 1995-97) than from humans (47% in 1997). Therefore, chloramphenicol resistance seen in cattle isolates is very unlikely to have come from the human use of chloramphenicol. Also, chloramphenicol-resistant isolates increased suddenly in both human and animal isolates just after 1990; resistance in cattle isolates rose from 2% to 62% (1). These points suggest that just after 1990 the same chloramphenicol-resistant strains (presumably new clones) were being shared rapidly between cattle and people. This spread is very unlikely to be from people to cattle but rather to people from cattle through food.

The third argument by Davis et al. relates to the spread of resistant strains by wildlife. Even though these strains can move easily around the world, they need to be amplified to cause a serious problem. One of the best ways to amplify resistant bacteria is to give them a selective advantage (e.g., when *Salmonella* is ingested in feed or water by animals that receive in-feed antibiotics).

The authors' fourth argument is that there is still broad dissemination of antibiotic-susceptible strains. So what? In hospitals, despite the overuse of antibiotics, we still see cross-infection with relatively sensitive strains of *S. aureus*, even when these hospitals have a high incidence of multidrug-resistant *S. aureus*. This does not mean that antibiotic use in humans is not one of the important factors in the amplification and spread of multidrug-resistant *S. aureus*.

As Davis et al. point out, antibiotic-resistant bacteria spread worldwide in many ways, including by wild animals and human travel. We need to prevent this spread; however, the central issue is antibiotic use in animals and how it amplifies resistant bacteria (e.g., *Salmonella enterica* serovar Typhimurium DT104). For every antibiotic Davis et al. tested, the level of resistance was higher in *Salmonella* isolates from cattle than from humans (1). The figures supplied by the authors clearly show that antibiotic resistance in cattle and human isolates is related and that resistance in *Salmonella* is and has been more of a problem in cattle than in humans, presumably as a result of widespread use of antibiotics in cattle.

Antibiotic resistance over the medium- to long-term is an inevitable consequence of antibiotic use. Ciprofloxacin and similar fluoroquinolones are the most effective drugs for treating many serious infections in humans, including some *Salmonella* infections (such as bacteremia or osteomyelitis). The prevalence of resistance to fluoroquinolones in human infections acquired from animals through the food chain is increasing (2,4). We should therefore avoid entirely the use of "last-line" human antibiotics such as fluoroquinolones (i.e., antibiotics for which there may be no alternatives if resistance develops) in livestock. All other antibiotics should be used only when there is no other way to prevent or treat infections.

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Changes in Antimicrobial Resistance in *Salmonella enterica* Serovar Typhimurium

To the Editor: The conclusion by Davis and colleagues (1) that use of antimicrobial agents in agriculture is unlikely to have contributed to the emergence of multidrug-resistant *Salmonella* serotype Typhimurium DT104 (MR-DT104) is contrary to available evidence. Use of antimicrobial agents in aquaculture in Asia may have contributed to the emergence of DT104. The resistant determinants of MR-DT104 reside on the chromosome, apparently within a transferrable element (2-4). Chloramphenicol resistance in MR-DT104 is due to *floR*, a florfenicol resistance gene (5); florfenicol is a veterinary antimicrobial agent that, although not approved in the United States until 1996, has been used in aquaculture in Asia since the early 1980s. *FloR* was first identified in *Photobacterium damsela,* a bacterium found in fish (5). Furthermore, tetracycline resistance in MR-DT104 is due to a class G resistance gene first identified in *Vibrio anguillarum*, a pathogen of fish (4,6). The molecular sequence where the class G and *floR* determinants reside on the DT104 chromosome is closely related (94% identity) to a plasmid in *Pasteurella piscicida*, another pathogen of fish (7). These data suggest that the resistance determinants of MR-DT104 may have emerged among bacteria in aquaculture and been horizontally transferred to *S.* Typhimurium DT104.

Spread of MR-DT104 between regions during international travel, as Davis and colleagues suggest, is unlikely because in industrialized countries *Salmonella* is seldom transmitted from person to person (8). Once MR-DT104 emerged, it spread rapidly to many regions through unknown means. The rapid emergence of MR-DT104 suggests a means of spread more efficient than person-to-person transmission. Possibilities include movement of infected breeding or "multiplier" stock or shipment of contaminated feed ingredients; such movements may not be as limited as Davis et al. suggest. For example, the international spread of *Salmonella* serotype Agona was traced to the global distribution of contaminated fish meal from Peru (9).

Once MR-DT104 is introduced into food animals in a region, use of antimicrobial agents in animals would contribute to further dissemination of MR-DT104 (8). If MR-DT104 is present on a farm, the use on the farm of any antimicrobial agent to which MR-DT104 is resistant would contribute to its persistence. An example of such use in cattle in the United States is the tetracycline-containing milk "replacement" commonly fed to dairy calves. This product could kill susceptible gastrointestinal flora while allowing tetracycline-resistant flora such as MR-DT104 to survive and proliferate. Once MR-DT104 proliferates on a farm, dissemination to other farms in the region is facilitated, particularly if the other farms are using an antimicrobial agent to which MR-DT104 is resistant.

Increasing antimicrobial resistance in *Salmonella* contributes to its spread and threatens the use of clinically important antimicrobial agents. To slow the emergence and dissemination of resistant *Salmonella,* measures should be implemented to ensure that antimicrobial agents are used prudently in food-producing animals (10).