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Zoonotic fecal pathogens and antimicrobial resistance in county fair animals

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ABSTRACT

Livestock fairs present a unique opportunity for the public to experience close contact with animals, but may also expose people to zoonotic pathogens through contact with animal feces. The goal of this study was to screen cattle, sheep, goat, chicken, rabbit and horse feces from a livestock fair in California for the potentially zoonotic pathogens *Escherichia coli* O157:H7, *Salmonella*, *Campylobacter*, *Vibrio*, *Cryptosporidium* and *Giardia* spp., as well as determining the level of antimicrobial resistance in *E. coli* and *Salmonella*. Notably, *E. coli* O157:H7 was reported for the first time in a pig at a county fair in California. *Campylobacter jejuni* as well as *Salmonella enterica* serovars Derby and Thompson were also isolated from pigs, cattle, sheep, goats or chickens, whereas horses and rabbits were negative for all target pathogens. The prevalence of antimicrobial resistance as well as multi-drug resistance patterns were highest for *E. coli* and *Salmonella* spp. cultured from pigs and chickens, were generally widespread but at lower levels for other animal groups, and included resistance to ampicillin and streptomycin, two antimicrobial drugs of importance for human medicine. This study provides data that highlight the importance of practicing good hygiene in livestock fair settings to avoid transmission of zoonotic microbes, particularly pathogens with antimicrobial resistance, to fair visitors and among animal populations.

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1. Introduction

The importance of considering complex connections among humans, animals and their environments when preventing zoonotic disease infections is increasingly being recognized. Such a "One Health approach" to disease prevention has relevance in any setting where disease transmission between animals and people is possible. Agricultural fairs and petting zoos provide unique educational opportunities for both young and old from diverse backgrounds to experience contact with farm animals. However, fairs also pose risks for exposure to zoonotic fecal pathogens if sufficient hygiene is not practiced.

Several outbreaks of diarrheal disease due to *Escherichia coli* O157:H7, *Salmonella* and *Cryptosporidium* spp. have been reported in visitors to petting zoos, farms, and fairs [1,2]. An additional concern for both human and animal health is the transmission of antimicrobial resistant bacteria [3], especially for drugs that are classified as 'critically important' (e.g. streptomycin, gentamicin, ampicillin) or 'highly important' (e.g. sulfamethoxazole, sulfisoxazole, trimethoprim, chloramphenicol, spectinomycin, tetracyclines) to human medicine [4]. The major factor associated with the emergence of antimicrobial resistance in livestock is thought to be the use of antimicrobials in animal production and medical settings [4,5]. Transmission of resistant bacteria from animals to humans can occur through direct contact with animal feces carrying resistant strains, as well as indirectly through contaminated food products or water [5].

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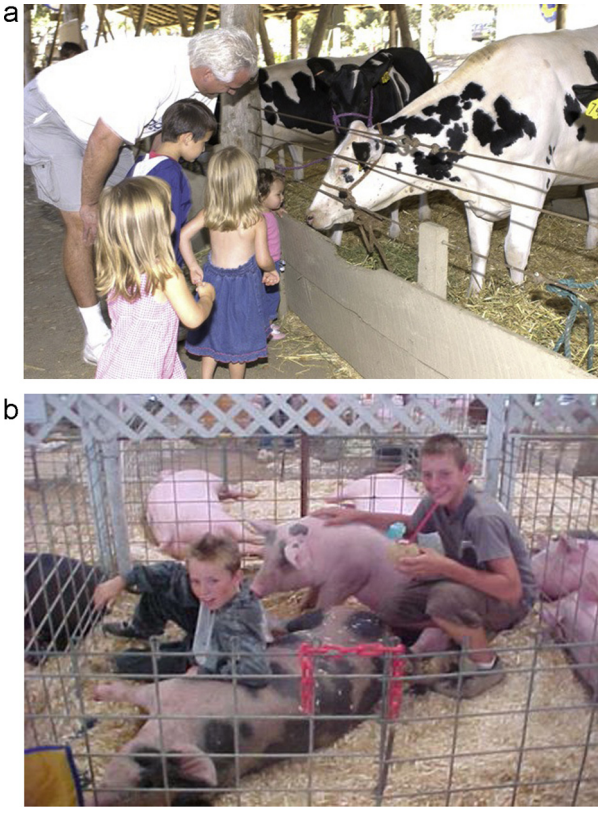

Fig. 1. Human–animal interactions come in many forms at livestock fairs including daily visitors with limited direct animal contact but potentially high risk behaviors or indirect contact (a) and animal caretakers with extensive contact (b).

Due to the many opportunities for contact between animals and humans, livestock fairs and petting zoos are settings where transfers of zoonotic pathogens or resistant bacteria are possible (Fig. 1). Behavioral studies of fair visitors in Tennessee and Canada showed that a majority of visitors came into direct contact with animals or contaminated surfaces, and that some visitors conducted risk behaviors for transmission such as eating and drinking near the animals, carrying baby items that could come into contact with infant mouths into animal areas, or practicing insufficient hand hygiene [6,7]. These behavioral studies highlight the importance of more comprehensively considering the animal, human and environmental linkages for successful disease prevention strategies to be implemented. In order to better understand the risk stemming from fecal shedding of zoonotic pathogens and bacteria with antimicrobial resistance in fair animals, this study assessed the fecal prevalence of *E. coli* O157:H7, *Salmonella*, *Campylobacter*, *Vibrio*, *Cryptosporidium* and *Giardia* spp. at a California county fair, and characterized the antimicrobial resistance patterns in *E. coli* and *Salmonella* spp. strains. All of the target pathogens are of potential zoonotic importance and have caused human disease in the past [2,8,9]. Information on the prevalence of fecal shedding and occurrence of antimicrobial resistant bacteria can serve as indicators for the risk for human exposure and contribute to the planning of effective prevention measures.

2. Materials and methods

Fecal samples were collected using convenience sampling from individual dairy cattle, sheep, goats, pigs, poultry, rabbits, and horses at a California county fair in August 2005. The maximum target of 50 fecal samples per animal host group allowed for detection of up to 19% prevalence with 95% confidence and 9% precision when assuming 100% sensitivity and specificity of diagnostic tests. The sampled animals were housed in species-specific open-air barns with individual barred pens for large animals and individual cages for small animals. Direct visitor to animal contact was possible in most animal areas. A 10–50 g fresh fecal sample was collected from the ground with sterile tongue depressors, stored at 4 °C, and transported overnight to the laboratory. Samples were screened for the enteric bacteria *E. coli* O157:H7, *Salmonella*, *Campylobacter*, and *Vibrio* spp. (*Vibrio cholerae*, *Vibrio parahaemolyticus*) using culture and biochemical identification methods as described previously [10]. *Salmonella* isolates were serotyped while *Campylobacter* species-specific PCR assays were used to confirm the identity of *Campylobacter jejuni* and *Campylobacter coli* isolates [11]. Confirmation of *E. coli* O157:H7 was based on PCR assays specific to the O157 and H7 genes [12,13]. Screening for *Cryptosporidium* oocysts and *Giardia* cysts in fecal samples was done by sieving 5 g fecal samples, centrifuging the suspenion at 1000 × *g* for 10 min and examining a 10 μl slide smear using a direct fluorescent antibody (DFA) test [14].

Additionally, non-O157:H7 *E. coli* were isolated on MacConkey agar and identified based on biochemical characteristics including colony color, spot oxidase and indole tests, and reactions in triple sugar iron agar as well as Christensen's urea agar. Antimicrobial resistance testing of 10 *E. coli* isolates per host species for dairy cattle, sheep, goats, pigs, and chickens was performed at the UC Davis Veterinary Medical Teaching Hospital. The antimicrobial susceptibility panel included amikacin, amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim-sulfamethoxazole (TMS) (http://trekds.com/products/sensititre/files/CMV1AGNF.pdf; Trek Diagnostic Systems, Cleveland, OH). The isolates were classified as resistant using established standards [15], except for streptomycin where organisms with MIC ≥64 μg/ml were classified as resistant. Similarly, *Salmonella* isolates were screened for antimicrobial resistance using an antimicrobial susceptibility panel including ampicillin, ceftiofur, chlortetracycline, clindamycin, danofloxacin, enrofloxacin, florfenicol, gentamicin, oxytetracycline, spectinomycin, sulphachloropyridazine, suphathiazole, tiamulin, tilmicosin, TMS and tylosin (http://trekds.com/products/sensititre/files/BOPO6F_CUST.pdf) and classified as resistant using established standards [15].