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Short communication

Evidence of antimicrobial and disinfectant resistance in a remote, isolated wild pig population

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ABSTRACT

The spread of antimicrobial resistant *E. coli* within the environment is a global concern. Wildlife such as feral pigs have been identified as a possible reservoir of antimicrobial resistant bacteria. A cross-sectional survey of free-ranging, feral pigs within the Kimberley region of northwestern Australia was conducted to estimate the prevalence of antimicrobial and disinfectant resistant *E. coli* in this population. Of the 493 faecal samples collected, 115 *E. coli* isolates were randomly selected and their identity confirmed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS). Isolates were screened for susceptibility to 14 veterinary antimicrobials (including antimicrobials not permitted for use in Australia) using MIC broth microdilution using Sensititre™ (BOPO6F) and breakpoints according to CLSI and EUCAST guidelines. Isolates also underwent disinfectant susceptibility testing to six disinfectants at their recommended concentration for use as well as at a twofold dilution, based on methods adapted from the CLSI agar plate dilution method. A moderate prevalence of resistance was observed to sulfadimethoxine (50.4%; 58/115) and florfenicol (27.0%; 31/115). A low prevalence of resistance was estimated to chlortetracycline (5.2%; 6/115) and multi-drug resistance was only identified in 1.7% (2/115) of *E.coli* isolates tested. Isolates were susceptible to five of the six disinfectants screened. Feral pigs could potentially act as a reservoir of antimicrobial resistance in the environment with possible implications for domestic livestock. The role that feral pigs might play in transmission of antimicrobial resistance requires further investigation, and the occurrence of resistance in such isolated populations needs to be considered when attempting to infer source attribution of antimicrobial resistance in livestock and human populations.

1. Introduction

Pigs were first introduced into Australia by early European settlers and by the 1880s were established in many parts of the continent (NSW Government, 2015). Due to their robust nature, their large home range and ability to cover large distances, pigs became well adapted to Australia's harsh climate. They are now found across about 38% of the continent (Hone, 1990), and the population is estimated at approximately 4–24 million (Cutler and Holyoake, 2007). Feral pigs are a pest species and have negative impacts on ecosystems and native flora and fauna, including predation, habitat degradation, competition and disease transmission, and have been estimated to cost the Australian agriculture sector more than $100 million per year (Choquenot et al., 1996), and their control is extremely difficult.

The Kimberley region of northwestern Australia is a vast natural landscape, sparsely populated and with a diverse range of flora and fauna (Pepper and Keogh, 2014). Feral pigs are found across approximately 26,000 km^2 of the Kimberley region (Cowled et al., 2009; Woolnough et al., 2004). They are isolated from other feral pig populations to the south and east (West, 2008). Feral pigs are often found as solitary boars, or in groups including adult females and juveniles, with an approximate density of three to eight pigs per km^2 (Twigg et al., 2005). Due to the arid environment and low human population density, agriculture is extensive. Beef cattle (Brahman and Braham cross) are reared in this region but compete with the feral pigs for habitat and food resources (Twigg et al., 2005). The extensive nature of cattle grazing with little human contact means that few treatments are administered to cattle, with likely no antimicrobial use.

Wildlife can be a source of infection for domestic livestock and human populations, and infections are likely to persist in such wildlife populations (Kramer-Schadt et al., 2009). Australian feral pigs are known to carry many endemic diseases that could threaten livestock and human health such as brucellosis and leptospirosis (Ridoutt et al., 2014). In Australia research on feral pigs has focused on zoonotic diseases such as methicillin-resistant *Staphylococcus aureus* (Groves et al., 2014) and *Salmonella* (Ward et al., 2013). However, limited data exists

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about *E. coli* isolated from feral pigs in Australia.

Recently there have been growing concerns about antimicrobial resistance within the environment. Resistance has been found in *Enterococci* spp. isolated from wild boar in urban environments in Barcelona (Navarro-Gonzalez et al., 2013a), presumably indicating spread of resistance from human and domestic animal populations to wildlife. Identifying a source and understanding antimicrobial resistance movement throughout the environment is paramount to keeping resistance low in wildlife populations. Antimicrobial resistance in *E. coli* isolated from feral animals could be used to anticipate any potential threat to the Australian domestic pig industry, wildlife, domestic animals, livestock and public health. There is little available information on feral pig carriage or transmission of antimicrobial resistance genes (Greig et al., 2015).

Comparing feral pigs, which have had limited to no exposure to antimicrobials, to domestic pigs can provide further insight into the mechanisms and spread of antimicrobial resistance occurring in the environment. The aim of this study was to determine susceptibility of *E. coli* isolated from an isolated, remote population of feral pigs in northwestern Australia to veterinary antimicrobials and to determine if disinfectants used for cleaning in domestic pig farms are bactericidal to these *E.coli* isolated from feral pigs.

2. Material and methods

2.1. Study and ecological environment

The sampling of feral pigs has been described previously (Ward et al., 2013). In brief, a cross-sectional survey was conducted between August and October 2010 of a population of feral pigs located within the Kimberley region of northwestern Australia. The study site is a remote, sparsely human-populated region (latitude 18.3644°S, longitude 125.6194°E, elevation 114 m). The sampling area was focused on the 5 grazing properties surrounding the town of Fitzroy Crossing (2011 residential population: 1013 (Australian Bureau of Statistics, 2012)). The landscape is open savannah woodland, characterised by clay soils and the Fitzroy and Margaret Rivers and their tributaries. This region experiences a tropical monsoonal climate, and when sampling took place the study site was experiencing the end of the 'dry' season. The habitat suitable for feral pigs was estimated during an aerial survey of the study site to cover an area of 6818 km^2.

2.2. Sampling feral pigs

Feral pigs (n = 493) were sampled using helicopter harvesting. An observer and a Robinson R44 helicopter were used to search permanent water sources, as feral pigs were known to congregate in these areas (Cowled et al., 2009). Helicopter culling is permitted in Western Australia, and the feral pigs sampled in this study were culled as part of a Department of Agriculture and Food Western Australia program (Sharp, 2012). Following the cull of 10–50 pigs during a flight, a sampling team was then flown to the site(s) where measurements, hand-held GPS location and samples were collected, usually within 30 min of culling. Demographic data were recorded for each animal and diagnostic samples were collected. Faecal samples (approximately 30 g) were collected from each pig from the rectum or start of the descending colon within 30 min of death and were immediately placed on ice until refrigeration at 4 °C, usually within one hour of sampling. Diagnostic samples were transported from the study site to the laboratory at 4 °C, within 24–72 h, and stored at −80 °C for long term storage.

2.3. Rejuvenating samples

A total of 493 faecal samples previously collected in 2010 and stored at −80 °C were brought to room temperature. A sterile 10 µL loop was then used to homogenise the sample which was transferred into a 96 well plate in 100 µL of Super optimal broth (SOC) with catabolite repression (Sigma-Aldrich) and mixed well before being enriched overnight at 37 °C. A sterile 10 µL loop was used to culture on CHROMagar Orientation (CHROMagar™) and one *E. coli* isolate per sample were confirmed by MALDI-TOF MS (Microflex LT MALDI BioTyper; Bruker Biosciences, Preston, VIC, Australia).

Table 1
Susceptibility to veterinary antimicrobials of *E. coli* (n = 115) isolated from feral pigs in northwestern Australia.

Veterinary Antibiotic	Number of resistant isolates	% resistant isolates
Gentamicin	0	nil
Neomycin	0	nil
Oxytetracycline	0	nil
Chlortetracycline	6	5.2
Tulathromycin[a]	0	nil
Ampicillin	0	nil
Ceftiofur[b]	0	nil
Danofloxacin[c]	0	nil
Enrofloxacin	0	nil
Florfenicol	31	27.0
Sulfadimethoxine	58	50.4
Trimethoprim-sulfamethoxazole	0	nil
Spectinomycin[d]	0	nil
Tylosin	0	nil

[a] ≥64 µg/mL.
[b] ≥8 µg/mL.
[c] ≥0.25 µg/mL.
[d] ≥128 µg/mL.

2.4. Antimicrobial and disinfectant susceptibility testing

Of the 493 feral pig faecal samples screened, *E. coli* isolates (n = 115) were screened against 14 veterinary antimicrobials (Table 1) (including antimicrobials not permitted for use in Australia) using Sensititre™ (BOPO6F) according to veterinary CLSI (CLSI, 2012) and EUCAST (EUCAST, 2016) guidelines.

The same *E. coli* isolates were also tested against six disinfectants (Table 2), five available for use in the Australian pig industry and one awaiting registration. The six disinfectants were tested at their recommended concentration for use as well as at a twofold dilution, based on methods adapted from the CLSI agar plate dilution method (CLSI, 2012).

3. Results

3.1. Antimicrobial susceptibility

The recovery rate of *E. coli* was low with only 115 of 493 samples

Table 2
Susceptibility to disinfectants of *E. coli* (n = 115) isolated from feral pigs in northwestern Australia.

Disinfectant	Manufacture	Concentration	*E. coli* resistance (%)
Virkon	Du Pont Ltd	1:100	0.0
		1:200	0.0
Farm Fluid S	Antec International Ltd	1:100	0.0
		1:200	0.0
Nu-quat	Bunzl Distribution	1:50	0.0
	Midcentral Inc.	1:100	0.0
Microtech 7000	Artech Technologies Pty	1:500	0.0
	Ltd	1:1000	0.0
F10	Health and Hygiene Pty	1:100	0.0
	Ltd	1:200	0.0
Iodophore	Not currently commercial	1:85	100.0
	available	1:170	100.0