



Cross-sectional study of antimicrobial-resistant bacteria in horses. Part 1: Prevalence of antimicrobial-resistant *Escherichia coli* and methicillin-resistant *Staphylococcus aureus*

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Summary

Reasons for performing study: The increasing prevalence of antimicrobial-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and antimicrobial-resistant *Escherichia coli* represents a significant problem. However, the carriage of such bacteria by horses in the UK has not been well characterised.

Objectives: To estimate the prevalence of nasal carriage of MRSA and faecal carriage of antimicrobial-resistant *E. coli* amongst horses in the general equine community of the mainland UK.

Methods: A cross-sectional study of horses recruited by 65 randomly selected equine veterinary practices was conducted, with nasal swabs and faecal samples collected. Faecal samples were cultured for antimicrobial-resistant *E. coli*. Nasal swabs were cultured for staphylococcal species; methicillin-resistant isolates identified as *S. aureus* were characterised by SCCmec and spa gene typing. Multilevel logistic regression models were used to calculate prevalence estimates with adjustment for clustering at practice and premises levels. Spatial variation in risk of antimicrobial resistance was also examined.

Results: In total, 650 faecal samples and 678 nasal swabs were collected from 692 horses located on 525 premises. The prevalence of faecal carriage of *E. coli* with resistance to any antimicrobial was 69.5% (95% CI 65.9–73.1%) and the prevalence of extended-spectrum β -lactamase (ESBL)-producing *E. coli* was 6.3% (95% CI 4.1–9.6%). The prevalence of nasal carriage of MRSA was 0.6% (95% CI 0.2–1.5%). Spatial analysis indicated variation across the UK for risk of carriage of resistant and multidrug-resistant (resistant to more than 3 antimicrobial classes) *E. coli*.

Conclusions and potential relevance: Carriage of MRSA by horses in the community appears rare, but the prevalence of antimicrobial-resistant *E. coli* (including ESBL-producing

E. coli) is higher. A high prevalence of antimicrobial-resistant bacteria could have significant health implications for the horse population of the UK.

Introduction

Antimicrobial resistance amongst bacteria is recognised as an important and increasing problem with significant economic implications, as well as increased morbidity and mortality for both human and veterinary patients (Paladino *et al.* 2002). The situation could prove to be particularly critical for equine medicine, as the number of licensed antimicrobial drugs available is limited, leaving little scope for alternative agents to be used if bacterial resistance results in treatment failure. Resistance in bacterial isolates from horses has been reported for a number of commensal and pathogenic bacteria including *Escherichia coli*, enterococci, salmonellae and *Staphylococcus aureus* (Harihara and Barnum 1973; Devriese *et al.* 1996; Hartmann *et al.* 1997; Ward *et al.* 2005). Multidrug-resistant (MDR) bacteria (resistant to ≥ 3 antimicrobials classes), including *E. coli* and methicillin-resistant *S. aureus* (MRSA), have been responsible for disease in horses (Vo *et al.* 2007; van Duijkeren *et al.* 2010).

Escherichia coli is part of the commensal gastrointestinal flora found in most animals, including horses and man (van Duijkeren *et al.* 2000). Although considered an uncommon cause of gastrointestinal disease in horses, it is frequently exposed to antimicrobial agents used in the treatment of infections caused by other organisms, potentially allowing it to acquire resistance determinants from other bacterial species and act as a reservoir for resistance genes (Hart *et al.* 2006; Karami *et al.* 2007). Resistance to most antimicrobial agents commonly used in equine medicine has been documented in *E. coli* from horses (Anzai *et al.* 1987; Anderson *et al.* 2006; Panchaud *et al.* 2010). Extended spectrum β -lactamase (ESBL) enzymes produced by *E. coli* represent a particular concern, providing resistance to a wide range of β -lactam antimicrobials, including the third-generation cephalosporins

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(e.g. cefotaxime, ceftiofur). Recently, such bacteria have emerged as a significant problem in human healthcare (Pitout 2010) and ESBL-producing *E. coli* has been reported as a cause of nongastrointestinal infections in horses (Vo *et al.* 2007).

Methicillin-resistance in *S. aureus* from horses was first identified in 1996 as the cause of a series of uterine infections in a group of mares (Anzai *et al.* 1996). Since then there has been a steady rise in the number of infections reported and clusters of infection have been seen in some equine hospitals (Seguin *et al.* 1999; Weese *et al.* 2005; van Duijkeren *et al.* 2010). The majority of these have been soft tissue infections; mainly of wounds, post surgical incisions and i.v. catheter sites. Molecular typing methods such as *spa* gene typing, SCCmec typing and multilocus sequence typing (MLST) have revealed that many of the strains of MRSA carried by horses are similar and often not related to the types commonly isolated from human subjects (Weese *et al.* 2005; Cuny *et al.* 2006; Moodley *et al.* 2006).

Both MRSA and antimicrobial-resistant *E. coli* may be carried without causing disease, but the prevalence of carriage of such resistant bacteria by horses in the UK has not been well established. Nasal sampling of a limited number of horses in the community from the UK and larger numbers from Europe have identified a very low prevalence MRSA carriage from 0–1.6% (Baptiste *et al.* 2005; Busscher *et al.* 2006; Abbott *et al.* 2010a). Carriage of other staphylococci such as methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant and susceptible coagulase negative staphylococci (MR-CNS and MS-CNS, respectively) is more prevalent (Baptiste *et al.* 2005; Busscher *et al.* 2006; Burton *et al.* 2008). Large surveys of horses for faecal carriage of antimicrobial-resistant *E. coli* have not been reported, but carriage has been documented in a group of 85 nonhospitalised horses in the USA (Dunowska *et al.* 2006) and sampling of horses in the community from the UK indicated a prevalence of approximately 25% (Ahmed *et al.* 2010).

The aim of this study was to determine the prevalence of carriage of MRSA and antimicrobial-resistant *E. coli* in horses from the general equine community of the mainland UK.

Materials and methods

Study population

The study population consisted of horses from the mainland UK attended by veterinary surgeons. Veterinary practices were selected via random number generation (Excel 2007)¹ from a list of all 1261 mainland UK practices in the 2006 Royal College of Veterinary Surgeons (RCVS) Directory of Practices that indicated that they attended horses. An arbitrary target for involvement of approximately 5% of equine practices (65 veterinary practices in total) was aimed for. Preliminary work had identified an expected prevalence (P_{exp}) of antimicrobial-resistant *E. coli* of 25–50% (Ahmed *et al.* 2010) and assuming a conservative between cluster (veterinary practice) variance (V_c) of 0.01, a total (T_s) of 503 faecal samples would be required to determine the prevalence with a precision (d) of 5% and 95% confidence if 65 clusters/practices (g) were recruited:

$$T_s = \frac{1.96^2 g P_{\text{exp}} (1 - P_{\text{exp}})}{gd^2 - 1.96^2 V_c}$$

The expected prevalence of MRSA was estimated to be considerably lower at 1–2% (Baptiste *et al.* 2005; Abbott *et al.*

2010a), with a much lower between cluster variance estimated at 0.0001, meaning a total of 800 nasal swab samples would be required to estimate prevalence with a precision of 1% and 95% confidence. To allow for an overall compliance proportion of approximately 60%, each practice was asked to recruit the next 20 horses seen on visits (a total of 1300 horses). Participating practices were asked to collect one nasal swab and one faecal sample from individual horses they saw on a veterinary visit for any reason. There were no criteria for exclusion of any horses from recruitment.

A 6 page self-administered owner questionnaire was designed, edited and precoded using dedicated data capture software (TELEform System)². This questionnaire employed mostly closed questions with tick box responses, with space provided for additional written information. A 'Don't Know' response was included for all questions to enable the respondent to avoid answering incorrectly if they were uncertain. Data were collected regarding case details, reason for veterinary visit, previous veterinary history (including antimicrobial therapy prior to visit and prior hospitalisation), location and characteristics of the horse's stable yard or premises, management and surrounding land (for full details see Supporting information). The questionnaire was initially validated via completion by a convenience sample of 30 horse owners, and the full study protocol was evaluated by a small pilot study involving 10 horses and horse owners from a practice that did not participate in the main study. Ethical approval for the study was granted by the University of Liverpool's Research Ethics Sub-Committee in December 2007. Recruitment of practices commenced in May 2008 and was completed by August 2008; samples were collected until April 2009.

Sample collection and processing

From each horse, a nasal swab was collected by the visiting veterinary surgeon and a faecal sample obtained if a fresh sample was available (if unavailable the owner returned one at the earliest opportunity). The attending veterinary surgeon also completed a one page questionnaire confirming the reason for the visit and any treatment drugs the horse had recently received.

Faecal samples were inoculated onto an eosin methylene blue agar (EMBA) plate³ and MacConkey agar plate³ and 7 antimicrobial impregnated discs⁴ applied, in accordance with the direct plating method of Bartoloni *et al.* (1998, 2006). The antimicrobial discs used were: ampicillin (10 µg), co-amoxiclav (30 µg) ciprofloxacin (1 µg), gentamicin (10 µg), nalidixic acid (30 µg), tetracycline (30 µg) and trimethoprim (2.5 µg). Three further EMBA plates were inoculated; one containing cefotaxime (1 µg/ml), one containing ceftazidime (1 µg/ml) and one with no antimicrobials incorporated. After incubation, presumptive resistant bacterial growth morphologically consistent with *E. coli* was subcultured for further testing. Three bacterial colonies were randomly selected from the EMBA plate with no antimicrobial. Isolates were prepared for antimicrobial sensitivity testing in accordance with British Society for Antimicrobial Chemotherapy (BSAC) guidelines (Anon 2007), using the same 7 antimicrobial discs detailed previously. Suspected ESBL-producing isolates taken from the plates containing cefotaxime or ceftazidime were subjected to the paired disc diffusion test⁴ (M'Zali *et al.* 2000), for confirmation of ESBL production. All isolates with biochemical profiles consistent with *E. coli* were confirmed by polymerase

chain reaction (PCR) assay for the *E. coli* specific *uidA* gene (McDaniels *et al.* 1996).

Nasal swabs were enriched for 24 h at 37°C in nutrient broth containing 6% NaCl and inoculated onto Mannitol Salt agar³ containing aztreonam (6 µg/ml) and oxacillin resistance screening agar plates³. Following aerobic incubation bacterial growth morphologically consistent with staphylococci was inoculated onto Columbia Blood agar³ supplemented with 5% horse blood and 2% NaCl, with an oxacillin (1 µg) antimicrobial disc. After aerobic incubation, bacterial colonies were Gram-stained for morphology and subjected to coagulase, staphylase⁵ and catalase tests. Isolates consistent with staphylococcal species were prepared for antimicrobial sensitivity testing according to BSAC guidelines using 10 antimicrobial discs: methicillin (5 µg), gentamicin (10 µg), rifampicin (2 µg), tetracycline (10 µg), vancomycin (5 µg), teicoplanin (30 µg), ciprofloxacin (1 µg), co-trimoxazole (25 µg), fusidic acid (10 µg), mupirocin (5 µg). All unique staphylococcal isolates were subjected to previously reported PCR assays for the *mecA* gene for methicillin resistance, and the *femA* and *nuc* genes for identification of *S. aureus* (Brakstad *et al.* 1992; Vannuffel *et al.* 1995). All isolates confirmed as *S. aureus* were subjected to molecular characterisation by *spa* gene typing and SCC*mec* typing in accordance with previously published methods (Oliveira and de Lencastre 2002; Harmsen *et al.* 2003). Additionally, all *S. aureus* isolates were screened for the presence of the gene encoding the Pantone-Valentine leucocidin toxin (*pvl*) by PCR assay (Lina *et al.* 1999).

Data analysis

All questionnaire-derived information and microbiological data were entered into a spreadsheet program (Excel 2007)¹. ArcMap (ArcGIS system 9.1)⁶ geographical information system (GIS) was used to link postcode data to geographical loci and produce maps of summarised data showing geographical variations in the locations of horses and premises with samples positive for the outcomes being considered. Formal spatial analysis of the presence of antimicrobial resistance in a sample was conducted using the R statistical software program⁷, implementing the 'spatialkernel' package. A spatially varying risk surface $p(x)$ was estimated using kernel smoothing, with an optimal band-width chosen to maximise a cross-validated log-likelihood criterion, as described by Diggle *et al.* (2005); $p(x)$ denotes the probability that a horse at location x will have antimicrobial resistance. Monte Carlo sampling (using 999 simulations) was used to test the null hypothesis of a spatially constant risk for the outcomes being considered.

Due to the nature of sampling, data were clustered within horse premises and veterinary practices and so the simple calculated sample prevalence for the outcomes considered may not accurately approximate the true prevalence for horses in the UK. Therefore, the prevalence of antimicrobial-resistant *E. coli* or staphylococci was estimated using separate multilevel models with a binomial distribution and logit link function. Samples were considered the level one unit of interest; the binary outcome for each faecal sample was the presence or absence of an *E. coli* isolate with resistance to one of 7 antimicrobials. Resistance to each of the 7 antimicrobials was considered as a separate outcome. Additionally, the presence in a sample of an *E. coli* with multidrug resistance (to 3 or more antimicrobial classes) or with an ESBL-producing phenotype were considered as 2 further outcomes. For nasal swabs, the 4 separate

binary outcomes considered were the presence or absence of MRSA, MSSA, MR-CNS and MS-CNS in sample.

Data were analysed using the MLwiN statistical software package (MLwiN Version 2.1)⁸. Three-level multilevel models were constructed for each outcome, with calculations performed using penalised quasi-likelihood estimates (second order PQL). Within-premise and within-practice clustering were accounted for by incorporation of second- and third-level random intercept terms in all 3-level models. The true prevalence (P_T) was estimated using the formula below, by incorporating the constant parameter estimate (β_0) derived from the random intercept-only 3-level models constructed for each of the outcomes considered:

$$P_T = \frac{e^{\beta_0}}{1 + e^{\beta_0}}$$

95% confidence intervals for all adjusted prevalence estimates were constructed by examination of the standard errors of β_0 of the intercept-only model parameters. All remaining statistical analyses were performed in the SPSS software package (SPSS 16.0 for Windows)⁹.

Results

Description of study population

A total of 121 veterinary practices were contacted in order for 65 to agree to participate in the study and 64 of these practices managed to recruit at least one horse. The median number of horses enrolled per practice was 11 (interquartile range 6.0–15.8). In total, 1328 samples were collected from the 692 horses enrolled in the study, which were located on 525 premises. Of these, 650 were faecal samples and 678 were nasal swabs, with 645 usable questionnaires returned: an overall response proportion of 49.6% of the 1300 sampling packs sent to the 65 veterinary practices. The location of the recruiting veterinary practices and premises where one or more horses were sampled is shown in Figure 1.

Results of survey

The mean \pm s.d. age of the horses in the population sampled was 11.4 \pm 6.6 years. Mares and geldings comprised approximately equal proportions of the horses, with a small number of stallions. Thoroughbred or Thoroughbred-cross animals were the most prevalent breed at 22.6% of the recruited population, along with Welsh or Welsh-cross breeds. Most horses were kept either on a private yard (45.9%) or part-livery yard (25.0%) and the most common size for a yard or premises was 2–5 horses (39.2%). Further details of the reported breeds, primary use of the horses and premises types and size are detailed in Table 1.

Veterinary treatment: Most of the horses (76.9%) were being attended by a veterinary surgeon for routine reasons (defined as vaccination, dental treatment or examination/sedation for a nonveterinary procedure) or lameness examination. Only 4.9% of horses were on antimicrobial therapy at the time of sampling, with 9.5% of horses having received antimicrobial therapy in the 10 days prior to sampling. Of the horses, 8.7% had been hospitalised within the 6 months prior to sampling. Further details of antimicrobial drug treatments administered and other veterinary treatment are detailed in Table 2.



Fig 1: Geographical distribution of recruiting veterinary practices (large grey circles) and locations with at least one sampled horse (smaller black circles) in a cross-sectional survey of horses in the mainland UK.

Prevalence of carriage of antimicrobial-resistant *E. coli* and MRSA

The prevalence of *E. coli* and staphylococcal carriage was high: at least one isolate of *E. coli* was recovered from 646 (99.4%) of the 650 faecal samples and staphylococcal species were isolated from 622 (91.7%) of the 678 nasal swabs. *E. coli* with resistance to at least one antimicrobial were isolated from 450 (69.2%) faecal samples and at least one methicillin-resistant staphylococcal isolate recovered from 206 (30.4%) nasal swabs. After correction for clustering, the sample prevalence of resistance to each antimicrobial, multidrug resistance and presence of an ESBL-producing *E. coli* are detailed in Table 3. Table 4 details the adjusted and simple sample prevalence of nasal carriage of MRSA, MR-CNS, MSSA and MS-CNS.

The location of all of the premises with one or more horses with faecal carriage of a multidrug-resistant or ESBL-producing *E. coli* is shown in Figure 2. The prevalence of MRSA in nasal samples and ESBL-producing *E. coli* in faecal samples was too low to justify formal spatial analysis; however, analysis was performed for the outcomes of resistance to any antimicrobial and multidrug resistance. A small number of locations in Northern Scotland were

TABLE 1: Summary of horse characteristics and details of their stabling premises provided by respondents in a cross-sectional survey of 645 horses in the mainland UK

		Percent	
Breed	Thoroughbred	14.5	
	Thoroughbred-cross	8.1	
	Welsh and Welsh-cross	15.6	
	Warmblood and Warmblood-cross	12.7	
	Irish Draught and Irish Draught-cross	10.6	
	Native	10.2	
	Cob and cob-cross	8.1	
	Unknown breed	7.5	
	Arab and Arab-cross	5.4	
	Other breed	4.9	
Sex	Draught and draught-cross	2.2	
	Female entire	46.9	
	Male entire	4.4	
	Male neutered	48.7	
Use	Hacking	37.7	
	Hacking and amateur competition	25.7	
	Retired	16.2	
	Breeding	8.7	
	Professional competition	5.9	
	Racing	2.4	
	Riding school	2.1	
	Other use	1.4	
	Premises	Private yard	45.9
		'Do-it-yourself' livery	25.0
Field only		9.0	
Full livery		5.6	
Stud		5.6	
Riding school		5.0	
Farm		2.0	
Racing yard		1.9	
Size of yard		1 horse	1.9
		2–5 horses	39.2
	6–10 horses	20.2	
	11–20 horses	16.8	
	21–50 horses	14.1	
	51–100 horses	7.2	
	>100 horses	0.6	

excluded because they were isolated from the majority of sampled premises and coastally located, rendering them unreliable for inclusion for spatial analysis. Analysis was therefore restricted to a polygonal region approximating to England, Wales and southern Scotland, which was re-scaled for convenience. The P value for the null hypothesis of constant risk was 0.002 for any resistance and 0.001 for multidrug resistance, so spatial variation in risk is highly significant for both of these outcomes, with both showing wide spatial variation in their estimated type-specific probabilities (see Fig 3). The risk of resistance was high in areas approximately corresponding to East Anglia, Humberside, North Yorkshire and Cumbria, and lower throughout most of Wales and Scotland.

Although the prevalence of ESBL-producing *E. coli* was too low for formal analysis, visually there appears to be some variation with horses with ESBL-producing *E. coli* almost exclusively located in the southern part of the UK, with only one such horse from Scotland. This does not appear to merely reflect the underlying sampling distribution as several intensively sampled regions in northern England and Scotland yielded no samples positive for ESBL-producing *E. coli*.

Molecular characterisation of MRSA

Methicillin-resistant *S. aureus* was isolated from the nasal swabs of 4 horses, with all 4 positive for *mecA*, *femA* and *nuc*

TABLE 2: Summary of veterinary treatment details of horses provided by respondents in a cross-sectional survey of 645 horses in the mainland UK

	Percent	
Reason for veterinary visit	Routine	62.1
	Lame/musculoskeletal	14.7
	Medical	3.9
	Reproductive	3.6
	Soft-tissue surgery	3.5
	Wound	2.9
	Abscess	2.2
	Respiratory disease	2.2
	Eye/skin	1.7
	Gastrointestinal disease	1.7
	Other	1.4
On antimicrobial treatment	No current treatment	95.0
	Currently on antimicrobial treatment	5.0
Recent antimicrobials	No recent treatment	90.5
	Antimicrobial treatment last 10 days	9.5
Antibiotic type last 10 days	None	90.2
	Cotrimoxazole	5.3
	Penicillin	1.9
	Other	1.2
	Cephalosporins	0.7
	Enrofloxacin	0.7
Hospitalised recently	Within last 10 days	3.7
	Within last one month	0.8
	Within last 3 months	2.1
	Within last 6 months	2.1
	Over 6 months ago	91.3

TABLE 3: Number and sample prevalence (adjusted for clustering within practice and premises) for *E. coli* with antimicrobial resistance isolated from faecal samples obtained in a cross-sectional survey of 650 horses in the mainland UK

Outcome	Number of positive samples	Prevalence adjusted for clustering (95% CI)
Any resistance	452	72.2% (66.8–77.0%)
Multidrug resistance	233	37.6% (33.8–41.5%)
ESBL-mediated resistance	42	6.3% (4.1–9.6%)
Trimethoprim resistance	363	55.9% (50.6–61.1%)
Tetracycline resistance	335	50.7% (44.9–56.6%)
Ampicillin resistances	298	45.6% (40.4–51.0%)
Gentamicin resistance	73	9.3% (6.6–13%)
Co-amoxiclav resistance	59	8.8% (6.7–11.4%)
Nalidixic acid resistance	73	8.5% (5.8–12.4%)
Ciprofloxacin resistance	42	5.4% (3.5–8.2%)

ESBL = extended spectrum β -lactamase.**TABLE 4: Number and sample prevalence (adjusted for clustering within practice and premises and simple) for staphylococci isolated from nasal swab samples obtained in a cross-sectional survey of 678 horses in the mainland UK**

Outcome	Number of positive samples	Prevalence adjusted for clustering (95% CI)
MRSA	4	0.6% (0.2–1.5%)
MR-CNS	202	29.0% (24.3–34.1%)
MSSA	25	3.7% (2.5–5.4%)
MS-CNS	570	84.8% (80.9–88%)

MRSA = methicillin-resistant *S. aureus*; MR-CNS = methicillin-resistant coagulase negative staphylococci; MSSA = methicillin-susceptible *S. aureus*; MS-CNS = methicillin-susceptible coagulase negative staphylococci.

genes, but negative for the *pvl* gene. All isolates carried SCCmec type IV. The *spa* gene typing analysis identified 3 *spa* types; 2 were *spa* type t064, one was type t451 and one was type t032.

Discussion

The main aim of this study was to estimate the prevalence of carriage of antimicrobial-resistant bacteria by horses in the mainland UK. The prevalence identified for nasal carriage of MRSA by horses was very low at 0.6%, which is comparable with that reported by other studies that have sampled horses in the community, including those in the UK, mainland Europe and North America (Busscher *et al.* 2006; Anderson and Weese 2007; Burton *et al.* 2008; Tokatelloff *et al.* 2009; Abbott *et al.* 2010a). Carriage of methicillin-resistant coagulase-negative staphylococci was more frequent, as has been previously demonstrated by others (Busscher *et al.* 2006; Bagcigil *et al.* 2007). The significance of this is difficult to assess. Coagulase-negative staphylococci may be capable of causing disease under some circumstances, such as in immunocompromised animals or those otherwise at risk of opportunistic infections, but appear much less frequently associated with clinical disease than *S. aureus* (Kloos and Bannerman 1994; Corrente *et al.* 2009). Two of the *spa* types of the MRSA isolates identified in this study reflect the types commonly recovered from horses, with t451 and t064 both having been reported on multiple occasions from horses in the UK, Europe and North America (Baptiste *et al.* 2005; Tokatelloff *et al.* 2009; van Duijkeren *et al.* 2010; Abbott *et al.* 2010b). Type t032 *spa* has only been reported from horses once previously (Abbott *et al.* 2010b), but is more frequently identified in small animals (Moodley *et al.* 2006; Strommenger *et al.* 2006) and is associated with one of the predominant hospital-acquired MRSA strains of human patients in the UK (Khandavilli *et al.* 2009).

The prevalence of faecal carriage of antimicrobial-resistant *E. coli* was considerably higher, and there are few other reports to compare this with. Two studies examining antimicrobial resistance in commensal *E. coli* have included groups of horses from the community, the first from the USA (Dunowska *et al.* 2006) and the second from northwest England (Ahmed *et al.* 2010). Neither of these studies reported the prevalence of resistant *E. coli* at the horse level and in both cases a variable number of samples were collected per horse. However, 17.7% of recovered *E. coli* isolates were found to have some form of resistance by Dunowska *et al.* (2006) and 25% of faecal samples were found to contain *E. coli* with resistance to at least one antimicrobial by Ahmed *et al.* (2010). A *post mortem* study has identified a much higher prevalence of gastrointestinal carriage of resistant *E. coli* of 75% of horses on sampling at slaughter (Bucknell *et al.* 1997).

The results of this study demonstrate that when considering antimicrobial-resistance in bacteria from horses, awareness should not just be restricted to MRSA, as extensive resistance was identified in *E. coli*. The *E. coli* recovered in this study were not causing disease and probably represent commensal strains. However, under certain circumstances such strains may be able to cause disease, either through the acquisition of virulence determinants or via inoculation of an extra-intestinal site (such as a post surgical wound) (Meng *et al.* 1998; Vo *et al.* 2007). The multidrug-resistant *E. coli* isolates identified in this study would prove refractory to treatment by most of the antimicrobial drugs



Fig 2: Geographical distribution of locations with at least one sampled horse (grey circles) in a cross-sectional survey of horses in the mainland UK: a) locations with at least one horse with resistance to any antimicrobial (black circles); b) locations with at least one multidrug-resistant *E. coli* positive horse (black circles); c) locations with at least one extended-spectrum β -lactamase producing *E. coli* positive horse (black circles).

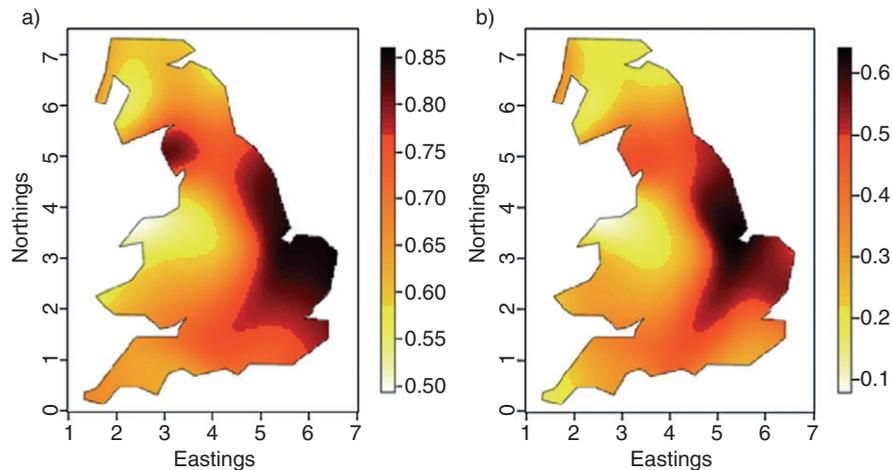


Fig 3: Estimated type-specific probabilities across the mainland UK (excluding northern Scotland) for the presence of antimicrobial resistance in a horse's faecal sample: a) resistance to any antimicrobial; b) multidrug resistance.

currently available for use in equine medicine if involved in infection. In particular, the prevalence of carriage of ESBL-producing *E. coli* identified in this study was higher than expected, although there are no reports on the prevalence of ESBL-producing bacteria in horses for comparison. However, ESBL-producing *E. coli* have been identified in uterine, ocular and soft-tissue infections in horses (Vo *et al.* 2007; Ewers *et al.* 2010) and a prevalence of carriage of 19% has been identified in hospitalised horses (Dolejska *et al.* 2008). Routine testing for ESBL production is not thought to be normal practice for many veterinary diagnostic laboratories, so it is possible that infections with ESBL-producing *E. coli* are underdiagnosed and under-reported (Hunter *et al.* 2010). There remains a possibility of

bacterial contamination of samples during collection by the owner or veterinary surgeon, although gloves were provided to limit this.

The spatial variation in risk seen for faecal carriage of *E. coli* with resistance to any antimicrobial and multidrug resistance warrants further investigation. Visual assessment of the location of ESBL-producing *E. coli* suggests that there might be a similar (or perhaps more extreme) spatial variation, although it was not possible to verify this formally. The degree of variation identified by this study could reflect similarity of risk factors in geographical regions (surrounding land use, size or type of premises, characteristics of owners), underlying factors such as density of human or horse populations or perhaps localised clusters of 'infection' with specific

types or strains of antimicrobial-resistant *E. coli*. The degree of clustering of outcomes within premises and practices, and some of the risk factors that may relate to the spatial variation identified, are further considered in Maddox *et al.* (2011).

Faecal samples were collected in numbers in excess of those suggested by the sample size calculation, but 122 fewer nasal samples than the intended number were obtained. The smaller sample size reflects the lower than anticipated overall response proportion of 49.6%. This mainly appears to stem from a small number of practices only recruiting a low number of participants; once recruited, completed questionnaires were returned for 645 (92.3%) of the 692 enrolled horses. Information is not available on whether this is because owners refused to participate or practices failed to approach sufficient owners. All participating practices were contacted every 4 weeks to encourage enrolment and practices that were slow in recruitment were contacted on a fortnightly basis, but this appeared to do little to increase the response of the practices concerned. The smaller sample size means that less confidence can be placed in the estimates for the prevalence of nasal carriage of MRSA. However, regardless of this, it still seems likely that the prevalence of nasal carriage of MRSA of horses in the UK is very low.

The recruitment of participants by veterinary surgeons may have introduced some selection bias to this study. Firstly, by definition, all horses participating in this study will have been receiving some form of veterinary attention. Secondly, although participating vets were asked to enrol the next 20 cases they visited, it is possible that participating horses and owners were not selected in a sufficiently random manner to be truly representative of a practice's client list. Additionally, not all horses and owners will be registered with a veterinary practice and hence available to be recruited. However, the difficulties associated with sampling large numbers of horses, especially when microbiological samples are required, means that there are few other feasible methods of participant recruitment available. Nevertheless, the sample population enrolled appears to be broadly comparable with that recruited by other large-scale surveys of horse population in the UK, with similar mean ages and sex/breed proportions (Mellor *et al.* 1999; Hotchkiss *et al.* 2007). Additionally, the large number of animals seen for routine reasons tends to indicate that there was not a high degree of active case selection by the recruiting veterinary surgeons.

The findings of this survey indicate that the prevalence of MRSA carriage is low for horses in the community, but that carriage of antimicrobial-resistant *E. coli* is considerably higher. For both of these, it should be noted that only carriage of such organisms has been assessed, the situation is likely to be different when infection and clinical disease are present. The information obtained from this survey and the results of faecal sampling permits the evaluation of risk factors related to faecal carriage of antimicrobial-resistant *E. coli*, as detailed in Maddox *et al.* (2011). However, the very low prevalence of MRSA identified unfortunately precludes a similar analysis for identifying risk factors associated with MRSA carriage.

Authors' declaration of interests

No conflicts of interest have been declared.

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- ¹Microsoft, Redmond, Washington, USA.
²Cardiff Software Inc., Vista, California, USA.
³Lab M, Bury, Lancashire, UK.
⁴MAST Ltd, Bootle, Merseyside, UK.
⁵PRO-LAB Diagnostics, Neston, Cheshire, UK.
⁶ESRI, Redlands, California, USA.
⁷<http://cran.r-project.org>
⁸Centre for Multilevel Modelling, University of Bristol, UK.
⁹SPSS Inc, Chicago, Illinois, USA.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig S1: Example of the 6 page questionnaire completed by the horse owner.

Fig S2: Example of the one page questionnaire completed by the attending veterinary surgeon.

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