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Short Communication

Evaluation of a survey approach to estimating the prevalence of cattle carrying antimicrobial-resistant *Escherichia coli*

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Abstract

A random survey of farms in the Highlands and Islands of Scotland provides estimated of the prevalence of calves, finishers and cows carrying ampicillin, apramycin and/or nalidixic acid resistant *Escherichia coli*. While the survey provides information on the geographical variation in risk, the results are of limited value for interpreting causality.

Keywords: Antibiotic; Associations; Cattle, *Escherichia coli*; Prevalence

The passive surveillance of antimicrobial-resistant bacteria from livestock is a routine function of the United Kingdom's diagnostic veterinary laboratories, which examine bacterial isolates recovered from cases of clinical disease and other material submitted through the animal disease surveillance system (Teale et al., 2004; SAC, unpublished data). Although passive surveillance is of value for detecting emerging resistance, the use of submitted samples is likely to overestimate the prevalence of animals carrying antimicrobial-resistant bacteria (Gunn et al., 2003), and provides poor quality information on both the prevalence of affected farms, and associations between resistance and farm management practices. A small survey of cattle farms was therefore undertaken to evaluate the utility of a more structured approach and the results analysed to make use of improved statistical techniques.

Faeces samples from 312 calves aged less than 6 weeks, 327 stores or fattening animals nearest to finishing (finishers), and 477 cows were collected from 100 randomly selected farms throughout the Highlands and Islands of Scotland during the spring of 1998. Up to five animals from each age group were sampled from each farm. A face-to-face interview on farm management practices was completed during the visit.

Each faeces sample was tested for the presence of ampicillin, apramycin and nalidixic acid resistant *Escherichia coli*. Microflora from each sample were cultured over-night at 37 °C on four MacConkey agar number 3 (Oxoid) plates supplemented with either 16 μg/mL ampicillin, 32 μg/mL apramycin, or 15 μg/mL nalidixic acid. The fourth plate was an unsupplemented control to test for the successful culture of *E. coli*. Up to three lactose fermenting colonies were picked from each plate and *E. coli* confirmed by indole production and growth at 44 °C. Samples were characterised as containing ampicillin, apramycin and/or nalidixic acid resistant *E. coli* if one or more isolates were recovered.

The prevalence of animals carrying ampicillin, apramycin and/or nalidixic acid resistant *E. coli* was estimated and associations with farm management practices evaluated using mixed-effect models assuming a binomial error distribution and utilising the logit link function. The prevalence of carriers for each antimicrobial for each age group, including empirical 95% confidence intervals (CI), was estimated from the intercept of weighted covariance pattern models (Brown and Prescott, 1999) with animals on the same farm parameterised as random repeated measures.

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Weighting was used to take into account the differential contribution to the estimate of farms of different herd size. Additive associations between the proportion of carrier animals and farm management practices were investigated using multifactorial random effect models (Breslow and Clayton, 1993) in which farm and a farm-by-age group interaction were fitted as random effects. Prevalence estimates for farms with one or more carrier animals were corrected for false-negatives using a beta-binomial distribution, and 95% CI estimated using the non-parametric bootstrap corrected for bias and acceleration (Efron, 1987). Differences between prevalence estimates for different antimicrobial agents and animal age groups were evaluated by comparing confidence intervals.

Observed proportions and estimated prevalences for each antimicrobial and age group are presented in Table 1. The estimated prevalence of ampicillin resistant carrier animals is higher than that for apramycin and nalidixic acid for all three age groups. The estimated prevalence of ampicillin and apramycin carrier animals for finishers and cows is lower than for calves; the same trend is apparent for nalidixic acid although there is a substantial overlap in CI between age groups. The observed proportions and estimated prevalences for farms with one or more carrier animals for each antimicrobial and animal age group are also shown in Table 1. Hypothetical estimates of the expected farm prevalence assuming that the occurrence of carrier animals on farms is a random process are also presented. A comparison with estimated farm prevalences suggests that there is clustering of carrier animals on farms for at least some antimicrobial and age group combinations. This could be due to an increased risk of resistance on some farms, and/or the transmission of resistance between animals on a farm.

Statistical associations between the proportion of carrier animals and farm management practices are described for ampicillin in Table 2. Similar analyses for apramycin and nalidixic acid are not presented because of poor model fits due to the low proportion of carrier animals (Table 1). Statistically significant ($P \leqslant 0.05$) associations for the propor-

Table 1
Proportion and prevalence of carrier animals and farms with one or more carrier animals

Antimicrobial	Age group	Animal estimates			Farm estimates				
		Proportion	Prevalence (95% CI)		Proportion	Prevalence (95% CI)		Hypothetical prevalence (95% CI)	
Ampicillin (16 μg/mL)	Calves	88.8	89.6	(83.7–93.6)	97	98	(95–100)	100	(99–100)
	Finishers	48.6	44.4	(33.4–56.1)	73	74	(63–84)	100	(94–100)
	Cows	48.0	41.7	(33.3–50.7)	76	77	(69–85)	99	(97–100)
Apramycin (32 μg/mL)	Calves	16.0	26.2	(12.3–47.5)	27	29	(19–38)	79	(69–87)
	Finishers	2.8	1.8	(0.5–6.2)	6	20	(12–31)	37	(24–48)
	Cows	3.8	2.8	(1.1–6.9)	10	Invalid estimate		59	(1-69)
Nalidixic acid (15 μg/mL)	Calves	6.7	5.4	(2.8–10.0)	17	23	(15–33)	35	(26–46)
	Finishers	1.8	1.1	(0.2-6.4)	3	14	(7–24)	26	(16–37)
	Cows	1.9	1.1	(0.2–5.5)	3	9	(4–15)	34	(25–44)

Animal proportions are the observed percentage ratio of the number of animals carrying resistant *E. coli* to the number of animals tested. Animal prevalences are the percentage probability that a randomly sampled animal from the population will carry resistant *E. coli* taking into account unequal farm sizes. Farm proportions are the observed percentage ratio of farms with P 1 animals carrying resistant *E. coli* to the number of farms sampled. Farm prevalences are the percentage probability that a randomly sampled farm from the population will include P 1 animals carrying resistant *E. coli* and incorporate a correction for false negative farms caused by the small number of individuals sampled. The algorithm did not converge to provide a farm prevalence estimate for apramycin. The hypothetical prevalence estimates the percentage probability that a randomly sampled farm will include P 1 animals carrying resistant *E. coli* assuming that there is no clustering of carrier animals on farms.

Table 2
Statistical associations between the proportion of animals containing ampicillin resistant *E. coli* and farm management practices

Explanatory variable	Overall	Class	Odds ratio
Age group	$F_{2,165} = 33.71$, $P < 0.001$	Calf	12.22 (6.58–22.71)
		Cow	1.07 (0.62–1.84)
		Finishing	Reference
Farm location	$F_{3,83} = 6.16$, $P < 0.001$	Caithness and Sutherland	4.91 (2.29-10.51)
		Orkney	1.27 (0.64–2.50)
		Ross-shire	1.05 (0.37–3.00)
		Moray	Reference
Use of ampicillin-related compounds[a]	$F_{1,187} = 1.09$, $P = 0.298$		
Animals housed[b]	$F_{1,181} = 2.00$, $P = 0.159$		
Animals bought in[b]	$F_{1,173} = 0.87$, $P = 0.351$		

[a] Use of ampicillin-related compounds on the farm (not necessarily on individuals sampled) within the previous 12 months for prevention or treatment purposes regarded as an indicator variable for increased use of ampicillin-related compounds resulting in increased antimicrobial resistance.

[b] Animals housed at time of sampling and any of the age group (not necessarily individuals sampled) bought in regarded as an indicator variable for transmission of antimicrobial resistance between animals.

Table 3
Proportion of calves carrying resistant *E. coli* using different sampling strategies

Investigation	Type of farm	Number and percentage of carrier animals (carriers/total (percent positive))					
		Ampicillin		Apramcyin		Nalidixic acid	
Gunn et al., 2003	Diarrhoeic	53/56	(95%)	12/55	(22%)	6/56	(11%)
	Non-diarrhoeic	28/40	(70%)	1/42	(2%)	0/42	(0%)
Current study	Random	277/312	(89%)	50/312	(16%)	21/312	(7%)
Percentage of range between diarrhoeic and non-diarrhoeic farms (%)		76		70		64	

The study area for Gunn et al. (2003) is a subset of the area of the current study. The original data from Gunn et al. (2003) have been checked to ensure they are comparable given a small difference in laboratory testing. The percentage of range is the ratio of the difference between random and non-diarrhoeic farms multiplied by 100 to the difference between diarrhoeic and non-diarrhoeic farms; this gives an indication of whether the results from the current study are closer to the diarrhoeic or non-diarrhoeic farms in the previous study.

tion of animals carrying ampicillin resistant *E. coli* with age group and farm location were observed. The association with age group is consistent with the decrease in the prevalence of ampicillin resistant carrier animals with age, and has been observed in other studies (Larsen and Larsen, 1975; Hoyle et al., 2004). Although the association with farm location suggests some geographical variation in risk, this is not in itself an adequate biological explanation. Indicator variables were therefore included as proxies to test for possible relationships between the proportion of carrier animals with antimicrobial use, and/or the transmission of resistance between animals (Table 2). Associations for none of these variables approached statistical significance (*P* P 0.10).

This study provides an opportunity to evaluate whether samples submitted through the UK disease surveillance centres result in biased prevalence estimates. A previous survey during 1996 compared the proportion of calves carrying antimicrobial-resistant *E. coli* from case farms experiencing an outbreak of calf diarrhoea with control farms from a part of the region used by the current study (Gunn et al., 2003). The results (Table 3) suggest that the proportions of calves carrying antimicrobial-resistant *E. coli* in the current study are consistently lower than those from the 'diarrhoeic' case farms in the previous study. Although the hypothesis remains to be experimentally tested, this result is consistent with the idea that current surveillance estimates are higher than the true prevalence in the calf population.

The advantages and limitations of structured surveys for investigating antimicrobial resistance are illustrated by this study. Structured surveys are potentially able to provide unbiased estimates of both individual and farm prevalence for targeted populations, although this analysis assumes a laboratory test sensitivity and specificity of 100%. They also provide information on the geographical variation in risk, but are limited as a tool for understanding causality. Despite their advantages, structured surveys are expensive to execute, and an important issue to be resolved is the balance of resources required to provide targeted, unbiased estimates of prevalence, while retaining the current system for monitoring emerging resistance.

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