



HAL
open science

Antimicrobial resistance in bacteria isolated from diseased horses in France

Clémence Bourély, Géraldine Cazeau, Nathalie Jarrige, Marisa Haenni, Emilie Gay, Agnès Leblond

► **To cite this version:**

Clémence Bourély, Géraldine Cazeau, Nathalie Jarrige, Marisa Haenni, Emilie Gay, et al.. Antimicrobial resistance in bacteria isolated from diseased horses in France. *Equine Veterinary Journal*, 2020, 52 (1), pp.112-119. 10.1111/evj.13133 . hal-02503293

HAL Id: hal-02503293

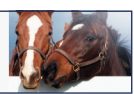
<https://hal.science/hal-02503293>

Submitted on 27 Jun 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright



Surveys and Population Studies

Antimicrobial resistance in bacteria isolated from diseased horses in France

C. BOURÉLY^{†‡§}, G. CAZEAU[‡], N. JARRIGE[‡], M. HAENNI[¶], E. GAY^{*‡}  and A. LEBLOND[§]

[†]École Nationale des Services Vétérinaires, ENSV, VetagroSup, Marcy l'Étoile, France

[‡]Laboratoire de Lyon, Unité Épidémiologie et Appui à la Surveillance, Université de Lyon, ANSES, Lyon, France

[§]Epidémiologie des maladies Animales et zoonotiques, INRA, EPIA, UMR 0346, VetAgroSup, University of Lyon, Marcy L'Étoile, France

[¶]Laboratoire de Lyon, Unité Antibiorésistance et Virulence Bactériennes, Université de Lyon, ANSES, Lyon, France.

*Correspondence email: emilie.gay@anses.fr; Received: 07.09.18; Accepted: 18.04.19

Summary

Background: Horses are one of the potential reservoirs of antimicrobial resistance (AMR) determinants that could be transferred to human subjects.

Objective: To describe the AMR patterns of major bacteria isolated from diseased horses in France.

Study design: Retrospective observational study.

Methods: Data collected between 2012 and 2016 by RESAPATH, the French national surveillance network for AMR, were analysed. Only antimicrobials relevant in veterinary and human medicine for the isolated bacteria were considered. Mono- and multidrug resistance were calculated. The resistance proportions of major equine diseases were assessed and compared. Where data permitted, resistance trends were investigated using nonlinear analysis (generalised additive models).

Results: A total of 12,695 antibiograms were analysed. The five most frequently isolated bacteria were *Streptococcus* spp., *Escherichia coli*, *Pseudomonas* spp., *Staphylococcus aureus*, *Pantoea* spp. and *Klebsiella* spp. The highest proportions of resistance to gentamicin were found for *S. aureus* (22.1%) and *Pseudomonas* spp. (26.9%). *Klebsiella* spp. and *E. coli* had the highest proportions of resistance to trimethoprim-sulfamethoxazole (15.5 and 26.2%, respectively). Proportions of resistance to tetracycline were among the highest for all the bacteria considered. Resistance to third-generation cephalosporins was below 10% for all *Enterobacteriaceae*. The highest proportions of multidrug resistance (22.5%) were found among *S. aureus* isolates, which is worrying given their zoonotic potential. From 2012 to 2016, resistance proportions decreased in *Pseudomonas* spp. isolates, but remained the same for *S. aureus*. For *Streptococcus* spp. and *E. coli*, resistance proportions to trimethoprim-sulfamethoxazole increased.

Main limitations: Since antibiograms are not systematic analyses, any selection bias could impact the results.

Conclusions: Such studies are essential to estimate the magnitude of the potential threat of AMR to public health, to design efficient control strategies and to measure their effectiveness. These findings may also guide the initial empirical treatment of horse diseases.

Keywords: horse; antimicrobial resistance; time series; multidrug resistance; RESAPATH

Introduction

Horses are one of the potential reservoirs of antimicrobial resistance (AMR) determinants that can be shared with human subjects through direct or indirect contact [1,2]. Major attention has been paid worldwide to the detection and characterisation of equine pathogens with zoonotic potential, that is *Enterobacteriaceae* producing extended-spectrum beta-lactamases (ESBL) or methicillin-resistant *Staphylococcus aureus* (MRSA) [3–8]. Conversely, other bacteria such as streptococci are poorly studied despite their importance as equine pathogens, and little is known about their resistances, especially in Europe [9–11].

While several studies focused on the genetic characterisation of specific mechanisms of resistance in horses' bacteria, studies on the global epidemiology of equine pathogenic bacteria and their associated resistance phenotypes are rare [3,12,13]. In addition, the differences between studies suggest that resistances can largely vary according to the geographical area and the clinical context considered, reflecting different practices of antimicrobial use [4,5,12].

Understanding the evolution of AMR over time is crucial to estimate the burden of resistance. In North America [4,6,9,10,12], several studies have demonstrated variation of resistance levels in pathogenic bacteria isolated from horses over several years. Unfortunately, the analysis of resistance over time remained limited due to the small sampling size or the basic methodology used. Time-series analysis methods, which were used to describe AMR in food-producing animals [14,15], could be relevantly applied to describe more precisely the evolution of resistance in equine bacteria over time. In Europe, there is clearly a gap of knowledge regarding AMR dynamics in equine bacteria, while regularly updated data on the prevalence and variations in AMR in different bacteria isolated from horses

would be necessary to estimate the potential threat to public health, design efficient control strategies and subsequently measure their effectiveness [16,17].

In western Europe, alongside Germany and the UK, France is one of the three countries with the largest equine herds in number, estimated at 1,100,000 horses [18]. Therefore, the aim of this study was to estimate the AMR pattern of the major bacteria isolated from diseased horses in France, to explore differences in AMR according to pathogenic context and to describe variations in AMR between 2012 and 2016.

Materials and methods

Data collection

This study was based on the results from bacterial culture and susceptibility testing (antibiograms) sent by French veterinary laboratories to RESAPATH, the established French national surveillance network for AMR in pathogenic bacteria from animals [14]. RESAPATH laboratories use standard bacteriological culture and methods for bacterial isolation and identification. All the laboratories perform antibiograms by the disc diffusion method, according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM).

From the RESAPATH database, data on the bacterial genera most frequently isolated from diseased horses from 2012 to 2016 (at least 500 isolates collected over the period) were extracted, and antimicrobials of relevance in veterinary and human medicine were selected (Supplementary Item 1). The variables extracted included horse diseases, bacterial species, date of sampling, location of sampling by administrative sector and inhibition zone diameters for each antimicrobial tested.

Data analysis

For *Streptococcus* spp. isolates, analyses were performed for the *Streptococcus* spp. genus as a whole, since the data did not strictly distinguish between species. For the other genera, the main bacterial species was used if the number of isolates collected from this bacterial species was greater than 1000 (*Escherichia coli* and *Staphylococcus aureus*). For the other, less frequent, bacteria, genera were used for analysis (*Pseudomonas* spp., *Pantoea* spp., *Klebsiella* spp.).

In accordance with the approved breakpoints from the veterinary section of the CA-SFM, isolates were categorised as susceptible, intermediate or resistant, using their inhibition zone diameters. Since the event of interest is the nonsusceptibility of the isolate to a particular antimicrobial, indicating that it is no longer a wild-type strain, intermediate isolates were grouped together with resistant ones.

For each combination of bacteria and antimicrobial, the AMR indicator was defined as the ratio between the number of resistant strains and the total number of strains tested over the 2012–2016 period. Associations between disease and prevalence of resistance were tested: resistance proportions were calculated for each disease and then compared two by two using Fisher's exact test. A $P \leq 0.05$ was considered statistically significant.

Time-series data were analysed for those combinations of bacteria–antimicrobial for which we had sufficient data available (at least 25 antibiograms per time step [19] [bimonthly], all diseases combined). The ratio between the bimonthly number of resistant strains and the total number of strains submitted bimonthly was modelled using binomial regression. AMR trends for each antimicrobial [20] were modelled using generalised additive models (GAM). The analysis was carried out as described in Boireau *et al.* [14], using two splines designed to control for trend and seasonality. If the splines fitted were nonsignificant, they were removed from the model before drawing the smoothed time graphs. Version 3.4.0 of R software (21-04-2017) was used for all data processing and statistical analyses (gam4 package for GAM implementation).

Multidrug resistance (MDR) analysis was carried out according to the classical definition of MDR (isolates with acquired nonsusceptibility to at least one antimicrobial in three or more antimicrobial classes). The following proportions were estimated: those of multisusceptible isolates (susceptible to all classes of antimicrobials tested and described in Supplementary Item 1); those resistant to one or two antimicrobials from different classes; those with MDR; and those resistant to all classes of antimicrobials considered [21]. These analyses were conducted with isolates for which full records on resistance to the aforementioned antimicrobial classes (Supplementary Item 1) were available. For *Pseudomonas aeruginosa*, only two antimicrobials are commonly tested by veterinary laboratories in France (enrofloxacin and gentamicin), so the MDR analysis was limited to these two. Confidence intervals (95% CIs) for the proportions were calculated using the exact binomial method (P -values were two-sided).

Results

Between 1 January 2012 and 31 December 2016, the six most frequent bacteria isolated from diseased horses were *Streptococcus* spp., *E. coli*, *Pseudomonas* spp., *Staphylococcus aureus*, *Pantoea* spp. and *Klebsiella* spp. (Supplementary Item 2).

Resistance to antimicrobials

Over the whole period 2012–2016, resistance to penicillins was low throughout for *Streptococcus* spp. (2.2%) but higher for *E. coli* (41.1%) and *S. aureus* (42.6%) (Table 1). The proportion of resistance to third-generation cephalosporins was below 10% for all *Enterobacteriaceae*: 7.6% for *E. coli*, 5.2% for *Klebsiella* spp. and 0.7% for *Pantoea* spp. Resistance to gentamicin was very low for *Pantoea* spp. (0.1%); below 10% for *Streptococcus* spp. (1.2%), *Klebsiella* spp. (6.1%) and *E. coli* (9.0%); but higher for *S. aureus* (22.1%) and *Pseudomonas* spp. (26.9%). Proportions of resistance to tetracycline were among the highest for all bacteria considered, except *Pantoea* spp. The proportions of resistance to trimethoprim-sulfamethoxazole were very low for *Pantoea* spp. (0.3%), below 10% for

Streptococcus spp. (9.9%) and *S. aureus* (6.0%), and higher for *Klebsiella* spp. (15.5%) and *E. coli* (26.2%). Resistances to fluoroquinolones were very low for *Pantoea* spp. (0.4%) and below 5% for *E. coli* (4.5%), *S. aureus* (2.9%) and *Klebsiella* spp. (3.1%), whereas they were much higher for *Pseudomonas* spp. (40.2%) and *Streptococcus* spp. (68.4%).

Resistance by disease

The isolates originated from three major pathological contexts: reproductive diseases, respiratory diseases and diseases of the skin and mucosa (Supplementary Item 3). Proportions of resistance were significantly different for many antimicrobials, depending on the disease (Table 2), except for *Pantoea* spp. In *Streptococcus* spp. isolates, the highest proportions of resistance were identified for reproductive diseases, except for trimethoprim-sulfamethoxazole and oxacillin. For *S. aureus*, reproductive diseases likewise presented the highest proportions of resistance. For *E. coli* and *Klebsiella* spp. isolates, on the other hand, the proportions of resistance from reproductive diseases were the lowest.

Trends in resistance

The analysis of trend in the proportions of resistance over the 2012–2016 period for *Streptococcus* spp., *E. coli*, *Pseudomonas* spp. and *S. aureus* isolates did not reveal any seasonal cycle, and the seasonal component in the model was never significant (Table 1). For *S. aureus*, all resistance trends were stationary (without significant variations) over the period.

For *Streptococcus* spp., trends in resistance to erythromycin, gentamicin and enrofloxacin were stationary. Trends in resistance to oxacillin, tetracycline and trimethoprim-sulfamethoxazole, however, were neither stationary nor linear over the period (Fig 1). The proportions of resistance to tetracycline increased from January 2012 (29.5% [19.8; 39.2]) to May 2013 (65.6% [60.7; 70.5]) and then fluctuated at very high proportions, reaching 65.1% [54.0; 76.2] in December 2016. The trend in resistance to oxacillin was initially stationary from 2012 to May 2015 and then increased from 1.8% [1.2; 2.3] up to 4.7% [2.4; 6.9] in December 2016. Similarly, though it started off stable, the proportion of resistance to trimethoprim-sulfamethoxazole then sharply increased, rising from 8.1% [6.1; 10.3] in January 2016 to 53.5% [42.8; 64.2] in December 2016.

For *E. coli*, trends in resistance to gentamicin, tetracycline, enrofloxacin and ceftiofur were stationary. The trend in resistance to amoxicillin, on the other hand, was neither stationary nor linear over the period, and proportions of resistance decreased from 53.0% [43.3; 62.6] in January 2012 to 36.4% [31.8; 40.9] in May 2015 and then stabilised (Fig 2). The proportion of resistance to trimethoprim-sulfamethoxazole increased linearly from 21.6% [17.9; 25.4] in January 2012 to 30.5% [26.4; 34.5] in December 2016.

The resistance trends for *Pseudomonas* spp. also showed significant variation. The trend in resistance to gentamicin was linear and proportions of resistance dropped from 30.0% [24.0; 36.0] in January 2012 to 16.3% [11.4; 21.2] in December 2016. The trend in resistance to enrofloxacin was nonlinear, but proportions of resistance also decreased from 51.7% [45.2; 58.3] in January 2012 to 30.0% [25.0; 34.5] in December 2016 (Fig 3).

Multidrug resistance

The analysis of MDR included 9497 isolates for which complete records on resistance to the aforementioned classes of antimicrobials were available (Table 1). The MDR proportions (Table 3) were very low for *Pantoea* spp. (0.2%), below 5% for *Klebsiella* spp. (4.0%), but higher for *Streptococcus* spp. (10.1%) and *E. coli* (16.5%). *S. aureus* isolates had the highest proportions of MDR over the whole period (22.5%). The proportion of *Pseudomonas* spp. resistant to both gentamicin and enrofloxacin reached 7.7%.

Discussion

Aims and limits

The aim of our study was to estimate the AMR pattern and variations in the six bacteria most frequently isolated from diseased horses in France on the basis of 12,695 antibiograms collected by RESAPATH from 2012 to 2016. The major strength of this study was the availability of an ongoing

TABLE 1: Proportion of resistance (in % with 95% CI) of bacteria isolated from diseased horses in France over the 2012–2016 period and identification of resistance trends

Pathogen	Antibiotic	Total number of isolates	Proportion of resistance (%)	Resistance trend
<i>Streptococcus</i> spp.	Oxacillin	5222	2.0 [1.6; 2.4]	Nonlinear
	Erythromycin	5557	8.3 [7.6; 9.1]	Stationary
	Gentamicin	4810	1.2 [0.9; 1.5]	Stationary
	Enrofloxacin	5523	68.4 [67.2; 69.6]	Stationary
	Tetracycline	4810	60.1 [58.7; 61.5]	Nonlinear
	SXT	5050	9.9 [9.1; 10.8]	Nonlinear
<i>Escherichia coli</i>	Amoxicillin	3272	41.1 [39.4; 42.8]	Nonlinear
	Ceftiofur	3311	7.6 [6.7; 8.6]	Stationary
	Gentamicin	3312	9.0 [8.0; 10.0]	Stationary
	Enrofloxacin	3306	4.5 [3.8; 5.2]	Stationary
	Tetracycline	2626	23.1 [21.5; 24.7]	Stationary
	SXT	2963	26.2 [24.6; 27.8]	Linear increasing
<i>Pseudomonas</i> spp.	Gentamicin	1310	23.2 [20.9; 25.6]	Linear decreasing
	Enrofloxacin	1307	40.2 [37.6; 43.0]	Nonlinear
<i>Staphylococcus aureus</i>	Penicillin G	1113	42.6 [39.7; 45.6]	Stationary
	Cefoxitin	1020	23.0 [20.5; 25.7]	Stationary
	Erythromycin	1106	6.3 [5.0; 7.9]	Stationary
	Gentamicin	1122	22.1 [19.7; 24.6]	Stationary
	Enrofloxacin	1098	2.9 [2.0; 4.1]	Stationary
	Tetracycline	1040	33.5 [30.2; 36.9]	Stationary
<i>Pantoea</i> spp.	SXT	1016	6.0 [4.6; 7.6]	–
	Ceftiofur	713	0.7 [0.2; 1.6]	–
	Gentamicin	713	0.1 [0.0; 0.1]	–
	Enrofloxacin	713	0.1 [0.0; 0.1]	–
	Tetracycline	684	0.3 [0.0; 1.2]	–
	SXT	602	0.3 [0.0; 1.2]	–
<i>Klebsiella</i> spp.	Ceftiofur	617	5.2 [3.6; 7.2]	–
	Gentamicin	619	6.1 [4.4; 8.3]	–
	Enrofloxacin	615	3.1 [1.9; 4.8]	–
	Tetracycline	488	18.0 [14.7; 21.7]	–
	SXT	566	15.5 [12.7; 18.8]	–

SXT, Trimethoprim-sulfamethoxazole; –, no breakdown due to the low number of isolates.

nationwide surveillance system for AMR in horses among other animals, namely the RESAPATH network. However, this study had several limitations due to potential selection biases in data, since laboratories join RESAPATH on a voluntary basis and antibiograms rely on the decisions made by veterinarians during animal health care, which could lead to a lack of representativeness. Nevertheless, an assessment of the network in 2015 concluded that RESAPATH was representative [22].

For *Pseudomonas* spp. isolates, ciprofloxacin is considered to be a better marker of fluoroquinolone resistance than enrofloxacin [23], which tends to result in an overestimation of resistance proportions. Ciprofloxacin is not routinely tested by veterinary laboratories, however, because its use is restricted to human medicine. Therefore, the proportions of resistance by *Pseudomonas* spp. to fluoroquinolones might have been overestimated in our study. Nevertheless, since fluoroquinolones are considered of critical importance in human medicine, having information on resistance to this family of molecules is important, even if the information is imperfect. It is most likely that resistance to fluoroquinolones has also been overestimated in streptococcus isolates because intermediate and resistant isolates were grouped together: due to an intrinsic resistance to quinolones, some streptococci are often classified as phenotypically intermediate without carrying any selected resistance mutation. Finally, some insignificant differences in resistance proportions between horse diseases were potentially due to a lack of statistical power, related to a low number of isolates for some diseases.

Comparison with other studies

To the authors' knowledge, this study is the first to investigate AMR in bacteria isolated from diseased horses at a national level, to compare AMR proportions between horse diseases and to apply time-series analysis

methods to AMR data. In the literature, most studies have focused on MRSA and ESBL-producing *E. coli* due to limited treatment strategies and their zoonotic potential [9]. To properly compare our results to previous ones, we should take into account methodological features (for instance, sample selection and size, the bacterial population studied or the breakpoints used), which can limit data comparison.

With respect to the common genera and species of bacteria isolated from horses, our findings are consistent with previous studies [9,12,24,25]: *Streptococcus* spp. and *E. coli* were the most common bacteria isolated over the period in France. In a study carried out in the United States using data collected between 2000 and 2010 in a pathological context, the authors reported similar proportions of resistance to beta-lactam (2.0%) and erythromycin (2.4%) in streptococci isolated from horses (2,893 isolates), but they described higher proportions of resistance to gentamicin (12.5%) and trimethoprim-sulfamethoxazole (69.1%) [10]. More recently, a study in Canada based on clinical isolates collected from horses over the 2007–2010 period mentioned that trimethoprim-sulfamethoxazole resistance was high (35%), but no isolate was resistant to beta-lactam [9]. In this same study, 57 *E. coli* isolates were collected and the authors reported higher proportions of resistance to gentamicin (25%), ceftiofur (18%) and trimethoprim-sulfamethoxazole (26.2%) than in our own findings. Like our own results, Saputra *et al.* identified *S. aureus* as the most frequent coagulase-positive staphylococci isolated from diseased horses in Australia in 2013. Resistance to tetracycline (32.1%) and amoxicillin (47.1%) was close to that reported in our study, but a lower resistance to trimethoprim-sulfamethoxazole (13.2%) was reported [26]. All the differences observed may be due to differences in study design or difference in geographical distribution of different clones, but they also suggest differences in antimicrobial use between countries.

TABLE 2: Proportion of resistance (in %) of bacteria isolated from diseased horses in France over the 2012–2016 period by disease and P-value for comparisons between proportions

Bacteria	Antimicrobial	Proportion of resistance (%) by disease			Significant difference between at least 2 proportions	P-value for each comparison		
		Reproductive	Respiratory	Skin and mucosa		Reproductive vs. respiratory	Reproductive vs. skin and mucosa	Respiratory vs. skin and mucosa
<i>Streptococcus</i> spp.	Oxacillin	1.2	1.6	1.2	No	0.7	0.9	0.5
	Erythromycin	10.7	3.6	6.2	Yes	<0.001	<0.001	0.01
	Gentamicin	1.4	0.5	0.8	Yes	0.02	0.3	0.5
	Enrofloxacin	70.3	65.7	69.7	Yes	0.005	0.8	0.08
	Tetracycline	64.6	54.8	61.4	Yes	<0.001	0.1	0.008
<i>Escherichia coli</i>	SXT	7.8	10.5	6.2	Yes	0.01	0.2	0.004
	Amoxicillin	37.5	52.0	48.7	Yes	<0.001	<0.001	0.4
	Ceftiofur	4.5	10.3	15.4	Yes	<0.001	<0.001	0.05
	Gentamicin	5.3	11.2	22.1	Yes	<0.001	<0.001	<0.001
	Enrofloxacin	1.7	5.1	14.8	Yes	<0.001	<0.001	<0.001
<i>Staphylococcus aureus</i>	Tetracycline	18.4	23.2	35.6	Yes	0.03	<0.001	<0.001
	SXT	21.0	28.6	45.0	Yes	0.002	<0.001	<0.001
	Penicillin G	57.1	42.4	36.5	Yes	0.02	<0.001	0.3
	Cefoxitin	29.6	18.1	19.3	Yes	0.04	0.001	0.9
	Erythromycin	6.0	5.4	6.1	No	1	1	1
<i>Pseudomonas</i> spp.	Gentamicin	37.6	14.1	16.4	Yes	<0.001	<0.001	0.6
	Enrofloxacin	1.5	4.5	2.7	No	0.1	0.3	0.3
	Tetracycline	45.1	15.1	18.8	Yes	<0.001	<0.001	0.5
	SXT	5.8	4.9	4.8	No	1	0.6	1
	Gentamicin	12.9	31.3	14.5	Yes	<0.001	0.05	<0.001
<i>Pantoea</i> spp.	Enrofloxacin	50.1	33.6	63.3	Yes	<0.001	0.01	<0.001
	Ceftiofur	0.0	0.4	0.0	No	1	1	1
	Gentamicin	0.0	0.0	0.0	No	1	1	1
	Enrofloxacin	0.0	0.0	0.0	No	1	1	1
	Tetracycline	0.0	0.0	0.0	No	1	1	1
<i>Klebsiella</i> spp.	SXT	0.0	0.0	0.0	No	1	1	1
	Ceftiofur	3.7	6.0	8.7	No	0.3	0.1	0.5
	Gentamicin	3.4	7.5	17.4	Yes	0.05	<0.001	0.08
	Enrofloxacin	0.8	5.3	11.1	Yes	0.004	<0.001	0.2
	Tetracycline	14.5	18.8	35.0	Yes	0.3	0.003	0.05
SXT	11.2	19.8	35.7	Yes	0.02	<0.001	0.06	

SXT, trimethoprim-sulfamethoxazole.

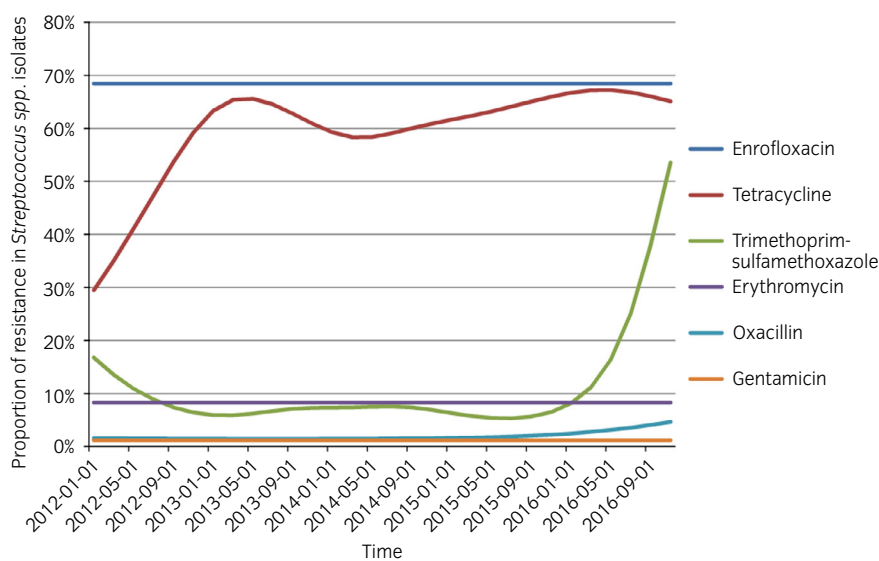


Fig 1: Trends of antimicrobial resistance (proportions in %) in *Streptococcus* spp. isolates from diseased horses during the 2012–2016 period, using a bimonthly time step.

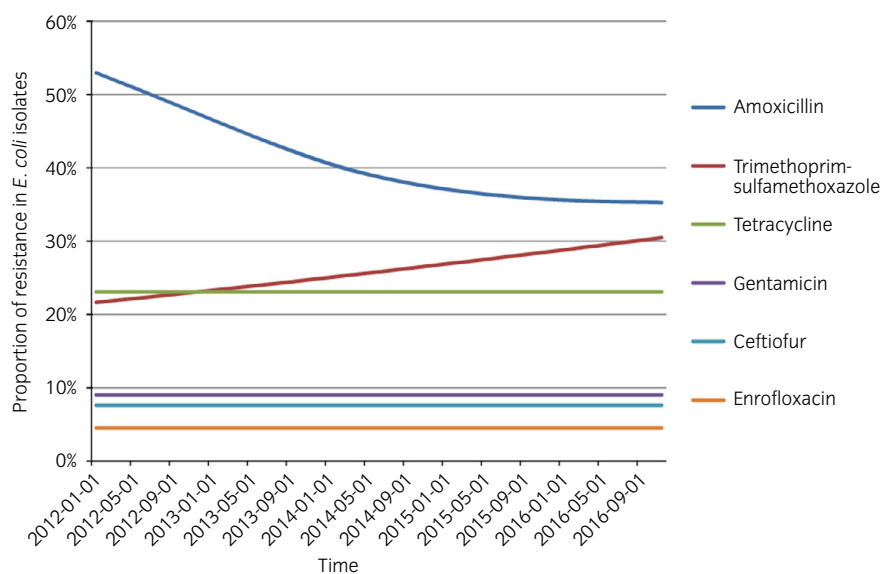


Fig 2: Trends of antimicrobial resistance (proportions in %) in *Escherichia coli* isolates from diseased horses during the 2012–2016 period, using a bimonthly time step.

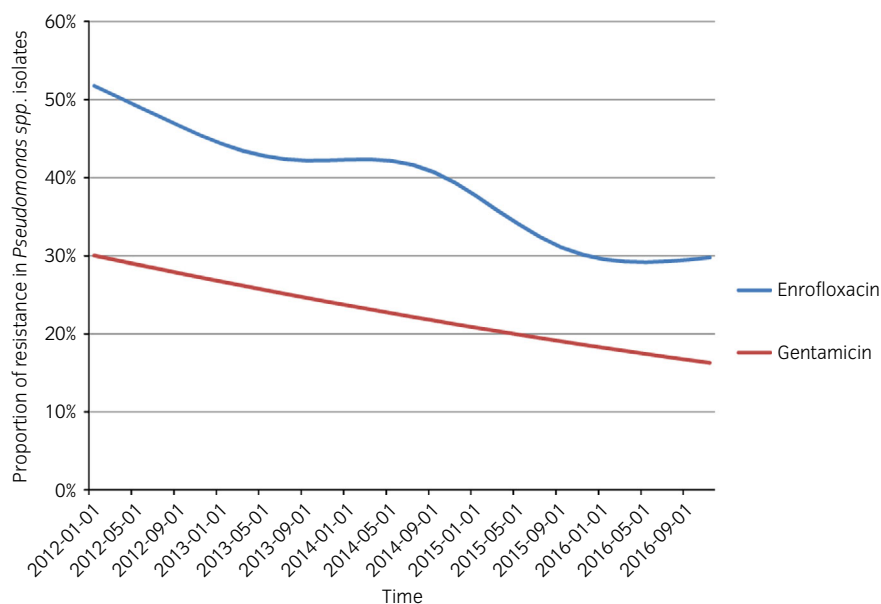


Fig 3: Trends of antimicrobial resistance (proportions in %) in *Pseudomonas* spp. isolates from diseased horses during the 2012–2016 period, using a bimonthly time step.

Few studies have so far investigated resistance trends in bacterial isolates from horses: there has been one in France [17], one in the UK [27], two in Canada [9,12], three in the United States [4,6,10] and one in South Africa [13]. Except for Davis *et al.*, these authors analysed annual proportions or proportions grouped together over several years in order to detect linear trends, and thus, they were not able to identify nonlinear variations in trends over time. Davis *et al.* focused on uterine samples from mares undergoing a routine breeding examination or examined because of a reproductive disorder. They used the generalised linear model to investigate AMR trends between 2003 and 2008, but their sample sizes per time step were very low (less than 10 isolates, whereas at least 25 are recommended) [19]. Given that in our study, we applied GAM to larger samples, and we were able to more accurately describe the trends and

changes; econometric methods such as time-series analysis offer a valuable approach for understanding the epidemiology of AMR.

Finally, this is the first study to explore resistance in *Pantoea* spp. isolates from horses. *Pantoea* spp. was the third most frequently isolated bacteria from reproductive diseases in France, and its resistance over the period was very low, unlike that of other *Enterobacteriaceae* studied.

Public health concern

AMR pathogens in domestic animals such as dogs, cats and horses can be cross-transmitted to human subjects, and close contact can foster the dissemination of AMR determinants [28]. The study of resistance in pathogenic strains carried by these animals is therefore of major interest.

TABLE 3: Proportions (in % with 95% CI) of isolates that were multisusceptible, resistant to one or two classes of antimicrobials, multidrug-resistant or resistant to all classes of antimicrobials selected for analysis over the 2012–2016 period

Bacteria (total number of isolates)	Susceptibility patterns of isolates	Number of isolates	Proportion (% [CI])
<i>Streptococcus</i> spp. (3788)	Multisusceptible	594	15.7 [14.5; 16.9]
	Resistant to one or two classes	2812	74.2 [72.8; 75.6]
	Multidrug-resistant	382	10.1 [9.1; 11.1]
	Resistant to all classes of antimicrobials selected	2	0.1 [0.0; 0.2]
<i>Escherichia coli</i> (2230)	Multisusceptible	1048	47.0 [44.9; 49.1]
	Resistant to one or two classes	815	36.5 [34.5; 38.6]
	Multidrug-resistant	367	16.5 [14.9; 18.1]
	Resistant to all classes of antimicrobials selected	42	1.9 [1.4; 2.5]
<i>Pseudomonas</i> spp. (1304)	Multisusceptible	577	44.2 [41.5; 47.0]
	Resistant to enrofloxacin and gentamicin	101	7.7 [6.4; 9.3]
<i>Staphylococcus aureus</i> (859)	Multisusceptible	452	52.6 [49.2; 56.0]
	Resistant to one or two classes	214	24.9 [22.1; 27.9]
	Multidrug-resistant	193	22.5 [19.7; 25.4]
	Resistant to all classes of antimicrobials selected	2	0.2 [0.0; 0.8]
<i>Pantoea</i> spp. (577)	Multisusceptible	570	98.8 [97.5; 99.5]
	Resistant to one or two classes	6	1.0 [0.4; 2.2]
	Multidrug-resistant	1	0.2 [0.0; 1.0]
	Resistant to all classes of antimicrobials selected	0	0.0 [0.0; 0.6]
<i>Klebsiella</i> spp. (429)	Multisusceptible	325	75.8 [71.4; 79.7]
	Resistant to one or two classes	87	20.3 [16.6; 24.4]
	Multidrug-resistant	17	4.0 [2.3; 6.3]
	Resistant to all classes of antimicrobials selected	3	0.7 [0.1; 2.0]

In Gram-negative bacteria, resistance to fluoroquinolones and last-generation cephalosporins is the most challenging issue. *Pseudomonas* infections are of particular concern because *Pseudomonas aeruginosa* is intrinsically resistant to many antimicrobials including beta-lactams, tetracycline, chloramphenicol, trimethoprim, kanamycin and quinolones [29], and it is also known for its capacity to rapidly acquire additional resistance determinants [30]. Data on the epidemiology of resistance in *Pseudomonas* spp. isolates have been limited in veterinary medicine, however, and have been mostly documented in dogs and cats [31,32]. Our study has shown that *Pseudomonas* spp. has frequently been isolated from horses, thereby stressing its importance as an equine pathogen. *Pseudomonas* resistance has previously been investigated in France [33] and in Brazil [11] but using a limited number of isolates, or mixing isolates from different species [33]. Our study therefore constitutes a major contribution to knowledge about *Pseudomonas* resistance in horses.

Currently, among Gram-positive bacteria, MRSA is of particular concern due to the limited treatment options (resistance to beta-lactamases), its

zoonotic potential [34] and the importance of this pathogen in nosocomial infections in both human and veterinary medicine [35,36]. MRSA prevalence in horses reportedly ranges between 0 and 7% [7,37], and hospitalised horses are more at risk. In our study, despite a lack of further genetic investigations, the proportion of phenotypically methicillin-resistant *S. aureus* was high (23.0%) and stable over the period studied. Even if this approximation is clearly an overestimation of the real proportion of MRSA, our results indicated that it is not as rare to isolate MRSA from infected horses in France as previously thought, a fact that is of particular concern. These findings are in accordance with a previous French study estimating the proportion of MRSA isolates at 6.1% (confirmed by genetic investigation) in horse infections from 2007 to 2013 [5].

Streptococcus spp. bacteria are commensal, opportunistic pathogens for human subjects and animals, and constitute the clinical bacteria most frequently isolated from horses. Even if streptococcus resistance is not a major public health concern (zoonotic pathogens being rare), our findings pointed out that streptococcus resistance can sharply increase over a short period. The current trend for resistance to trimethoprim-sulfamethoxazole can probably be explained by its wide use to treat horses in France. Indeed, this is preferentially prescribed in the case of prolonged therapy since it can be administered orally by owners. Considering the clear increase in trimethoprim-sulfamethoxazole resistance both for streptococci and for *E. coli* isolates, bacterial identification and susceptibility testing should be recommended as soon as clinical deterioration occurs.

Conclusion

This study provides new knowledge about the AMR pattern of the major causative agents of horse diseases isolated in France. Such knowledge of AMR prevalence and trends enables the judicious and prudent use of antimicrobials in animals: our findings provide veterinarians with comprehensive data to guide their initial empirical therapy. Our results emphasise the necessity to conduct further monitoring studies to estimate the proportion of AMR in horses and provide important recommendations regarding the use of some frequently used antimicrobials to treat common diseases. In the future, it will be necessary to collect accurate data on antimicrobial use in the equine sector to enable comparison with AMR trends. Ultimately, these results contribute to risk assessment and constitute objective evidence on which to evaluate the efficacy of control measures implemented to limit AMR.

Authors' declaration of interests

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Ethical animal research

Research ethics committee oversight not currently required by this journal: procedures were performed as part of clinical investigations.

Owner informed consent

Explicit owner informed consent for participation in this study was not stated.

Source of funding

This work was partially funded by the French Ministry of Agriculture (<http://agriculture.gouv.fr>).

Acknowledgements

The authors would like to thank all the laboratories that voluntarily collected and transmitted antibiogram results over several years. The

authors are particularly grateful to Christelle Philippon (RESAPATH's secretary) for her meticulous follow-up of laboratories and careful data collection and Jean-Luc Vinard (RESAPATH's data architect) for his careful management of the database.

Author contributions

C. Bourély, G. Cazeau, E. Gay and A. Leblond have designed the study. C. Bourély, G. Cazeau and A. Leblond have executed the study. All the authors have contributed to the data analysis and interpretation. All the authors have contributed to the preparation of the manuscript. All the authors have read and approved the final manuscript.

References

- Schmiedel, J., Falgenhauer, L., Domann, E., Bauerfeind, R., Prenger-Berninghoff, E., Imirzalioglu, C. and Chakraborty, T. (2014) Multiresistant extended-spectrum β -lactamase-producing Enterobacteriaceae from humans, companion animals and horses in central Hesse, Germany. *BMC Microbiol.* **14**, 187.
- EMA (2015) *Reflection Paper on the Risk of Antimicrobial Resistance Transfer from Companion Animals*, European Medicine Agency, London.
- Maddox, T.W., Clegg, P.D., Williams, N.J. and Pinchbeck, G.L. (2015) Antimicrobial resistance in bacteria from horses: epidemiology of antimicrobial resistance. *Equine Vet. J.* **47**, 756-765.
- Davis, H.A., Stanton, M.B., Thungrat, K. and Boothe, D.M. (2013) Uterine bacterial isolates from mares and their resistance to antimicrobials: 8,296 cases (2003-2008). *J. Am. Vet. Med. Ass.* **242**, 977-983.
- Guérin, F., Fines-Guyon, M., Meignen, P., Delente, G., Fondrinier, C., Bourdon, N., Cattoir, V. and Léon, A. (2017) Nationwide molecular epidemiology of methicillin-resistant *Staphylococcus aureus* responsible for horse infections in France. *BMC Microbiol.* **17**, 104. Available at: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5415774/> (Accessed 2 August 2017).
- Theelen, M.J.P., Wilson, W.D., Edman, J.M., Magdesian, K.G. and Kass, P.H. (2014) Temporal trends in in vitro antimicrobial susceptibility patterns of bacteria isolated from foals with sepsis: 1979-2010. *Equine Vet. J.* **46**, 161-168.
- Maddox, T.W., Pinchbeck, G.L., Clegg, P.D., Wedley, A.L., Dawson, S. and Williams, N.J. (2012) Cross-sectional study of antimicrobial-resistant bacteria in horses. Part 2: risk factors for faecal carriage of antimicrobial-resistant *Escherichia coli* in horses. *Equine Vet. J.* **44**, 297-303.
- Weese, J.S., Archambault, M., Dick, H., Hearn, P., Kreiswirth, B.N., Said-Salim, B., McGeer, A., Likhoshvay, Y., Prescott, J.F. and Low, D.E. (2005) Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel, 2000-2002. *Emerg. Infect. Dis.* **11**, 430-435.
- Malo, A., Cluzel, C., Labrecque, O., Beauchamp, G., Lavoie, J.-P. and Leclere, M. (2016) Evolution of in vitro antimicrobial resistance in an equine hospital over 3 decades. *Can. Vet. J.* **57**, 747-751.
- Erol, E., Locke, S.J., Donahoe, J.K., Mackin, M.A. and Carter, C.N. (2012) Beta-hemolytic *Streptococcus* spp. from horses: a retrospective study (2000-2010). *J. Vet. Diagn. Invest.* **24**, 142-147.
- Leigue, L., Montiani-Ferreira, F. and Moore, B.A. (2016) Antimicrobial susceptibility and minimal inhibitory concentration of *Pseudomonas aeruginosa* isolated from septic ocular surface disease in different animal species. *Open Vet. J.* **6**, 215-222.
- Clark, C., Greenwood, S., Boison, J.O., Chirino-Trejo, M. and Dowling, P.M. (2008) Bacterial isolates from equine infections in western Canada (1998-2003). *Can. Vet. J.* **49**, 153-160.
- Chipangura, J.K., Chetty, T., Kgoete, M. and Naidoo, V. (2017) Prevalence of antimicrobial resistance from bacterial culture and susceptibility records from horse samples in South Africa. *Prev. Vet. Med.* **148**, 37-43.
- Boireau, C., Morignat, É., Cazeau, G., Jarrige, N., Jouy, É., Haenni, M., Madec, J.-Y., Leblond, A. and Gay, É. (2018) Antimicrobial resistance trends in *Escherichia coli* isolated from diseased food-producing animals in France: a 14-year period time-series study. *Zoonoses Public Health* **65**, 86-94.
- Boireau, C., Cazeau, G., Jarrige, N., Calavas, D., Madec, J.-Y., Leblond, A., Haenni, M. and Gay, É. (2018) Antimicrobial resistance in bacteria isolated from mastitis in dairy cattle in France, 2006-2016. *J. Dairy Sci.* **101**, 9451-9462.
- Bowen, M. (2013) Antimicrobial stewardship: time for change. *Equine Vet. J.* **45**, 127-129.
- Robinson, T.P., Bu, D.P., Carrique-Mas, J., Fèvre, E.M., Gilbert, M., Grace, D., Hay, S.I., Jiwakanon, J., Kakkar, M., Kariuki, S., Laxminarayan, R., Lubroth, J., Magnusson, U., Thi Ngoc, P., Van Boeckel, T.P. and Woolhouse, M.E.J. (2017) Antibiotic resistance: mitigation opportunities in livestock sector development. *Animal* **11**, 1-3.
- IFCE. (2017). Filière équine française : chiffres clés 2017.
- Barlow, J. (2011) Mastitis therapy and antimicrobial susceptibility: a multispecies review with a focus on antibiotic treatment of mastitis in dairy cattle. *J. Mammary Gland Biol. Neoplasia* **16**, 383-407.
- Wood, S.N. (2008) Fast stable direct fitting and smoothness selection for generalized additive models. *J. R. Stat. Soc. Series B Stat. Methodol.* **70**, 495-518.
- Magioragos, A.-P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D.L., Rice, L.B., Stelling, J., Struelens, M.J., Vatopoulos, A., Weber, J.T. and Monnet, D.L. (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.* **18**, 268-281.
- Boireau, C., Jarrige, N., Cazeau, G., Jouy, É., Haenni, M., Philippon, C., Calavas, D., Madec, J.-Y., Leblond, A. and Gay, É. (2018) Représentativité et couverture du réseau d'épidémiologie de surveillance de l'antibiorésistance des bactéries pathogènes animales. *Bull. Epidemiol. Santé Anim. Aliment.* **80**, 10-14.
- Wildermuth, B.E., Griffin, C.E., Rosenkrantz, W.S. and Boord, M.J. (2007) Susceptibility of *Pseudomonas* isolates from the ears and skin of dogs to enrofloxacin, marbofloxacin, and ciprofloxacin. *J. Am. Anim. Hosp. Ass.* **43**, 337-341.
- Davies, S.C., Fowler, T., Watson, J., Livermore, D.M. and Walker, D. (2013) Annual Report of the Chief Medical Officer: infection and the rise of antimicrobial resistance. *Lancet* **381**, 1606-1609.
- Frontoso, R., De Carlo, E., Pasolini, M.P., van der Meulen, K., Pagnini, U., Iovane, G. and De Martino, L. (2008) Retrospective study of bacterial isolates and their antimicrobial susceptibilities in equine uteri during fertility problems. *Res. Vet. Sci.* **84**, 1-6.
- Saputra, S., Jordan, D., Worthing, K.A., Norris, J.M., Wong, H.S., Abraham, R., Trott, D.J. and Abraham, S. (2017) Antimicrobial resistance in coagulase-positive staphylococci isolated from companion animals in Australia: a one year study. *PLoS One* **12**, e0176379.
- Johns, I.C. and Adams, E.-L. (2015) Trends in antimicrobial resistance in equine bacterial isolates: 1999-2012. *Vet. Rec.* **176**, 334-334.
- Pomba, C., Rantala, M., Greko, C., Baptiste, K.E., Catry, B., van Duijkeren, E., Mateus, A., Moreno, M.A., Pyörälä, S., Ruzauskas, M., Sanders, P., Teale, C., Threlfall, E.J., Kunsagi, Z., Torren-Edo, J., Jukes, H. and Törneke, K. (2017) Public health risk of antimicrobial resistance transfer from companion animals. *J. Antimicrob. Chemother.* **72**, 957-968.
- Li, X.-Z., Plésiat, P. and Nikaido, H. (2015) The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. *Clin. Microbiol. Rev.* **28**, 337-418.
- Breidenstein, E.B.M., de la Fuente-Núñez, C. and Hancock, R.E.W. (2011) *Pseudomonas aeruginosa*: all roads lead to resistance. *Trends Microbiol.* **19**, 419-426.
- Hariharan, H., Coles, M., Poole, D., Lund, L. and Page, R. (2006) Update on antimicrobial susceptibilities of bacterial isolates from canine and feline otitis externa. *Can. Vet. J.* **47**, 253-255.
- Mekić, S., Matanović, K. and Šeol, B. (2011) Antimicrobial susceptibility of *Pseudomonas aeruginosa* isolates from dogs with otitis externa. *Vet. Rec.* **169**, 125.
- Haenni, M., Hocquet, D., Ponsin, C., Cholley, P., Guyeux, C., Madec, J.-Y. and Bertrand, X. (2015) Population structure and antimicrobial susceptibility of *Pseudomonas aeruginosa* from animal infections in France. *BMC Vet. Res.* **11**, 9.
- Islam, M.Z., Espinosa-Gongora, C., Damborg, P., Sieber, R.N., Munk, R., Husted, L., Moodley, A., Skov, R., Larsen, J. and Guardabassi, L. (2017) Horses in Denmark are a reservoir of diverse clones of methicillin-

- resistant and -susceptible *Staphylococcus aureus*. *Front. Microbiol.* **8**, 543.
35. Leonard, F.C. and Markey, B.K. (2008) Methicillin-resistant *Staphylococcus aureus* in animals: a review. *Vet. J.* **175**, 27-36.
 36. Wieler, L.H., Ewers, C., Guenther, S., Walther, B. and Lübke-Becker, A. (2011) Methicillin-resistant staphylococci (MRS) and extended-spectrum beta-lactamases (ESBL)-producing Enterobacteriaceae in companion animals: nosocomial infections as one reason for the rising prevalence of these potential zoonotic pathogens in clinical samples. *Int. J. Med. Microbiol.* **301**, 635-641.
 37. Tirosh-Levy, S., Steinman, A., Carmeli, Y., Klement, E. and Navon-Venezia, S. (2015) Prevalence and risk factors for colonization with methicillin resistant *Staphylococcus aureus* and other Staphylococci species in hospitalized and farm horses in Israel. *Prev. Vet. Med.* **122**, 135-144.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Supplementary Item 1: Selected antimicrobials, the corresponding antimicrobial classes and the potency of antimicrobial disc used according to the CA-SFM.

Supplementary Item 2: Number of isolates collected from horses between 2012 and 2016 per genera and bacterial species identified.

Supplementary Item 3: Number of isolates from horses collected by RESAPATH during the 2012–2016 period by pathology.

Robinson's Current Therapy in Equine Medicine

7th Edition

Editors: Kim A. Sprayberry and N. Edward Robinson

Publisher: Elsevier, September 2014 • Hardback, 1104 pages

Stay up-to-date on the latest advances and current issues in equine medicine!

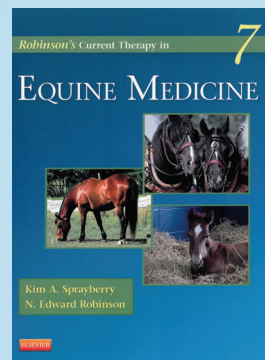
With coverage of current issues and emerging trends, this all-new reference helps you recognise, diagnose and treat equine disorders and conditions. The Current Therapy format focuses on treatment protocols and diagnostic updates in areas that have seen significant advances in the last 5 years.

Features of this new edition:

- 212 concise, NEW chapters include both a succinct guide to diagnosis of disorders and a detailed discussion of therapy.
- Cutting edge topics include new sections on trauma, pain management, and the sport horse; and chapters on hoof lameness in foals, upper airway diseases, and colic diagnosis that describe use of new imaging, endoscopy, and other diagnostic procedures.
- A body systems organisation makes it easy to find solutions for specific conditions and disorders.
- Updated Table of Common Drugs and Approximate Dosages in the appendix provides a handy quick reference - and adds all-new coverage of drug dosages for donkeys and mules.
- Nearly 200 expert contributors represent private equine practices, referral hospitals and academia, providing perspectives from around the world.

Kim A. Sprayberry, DVM, DACVIM, Hagyard Equine Medical Institute, Lexington, Kentucky, USA

N. Edward Robinson, BVetMed, PhD, MRCVS Docteur Honoris Causa (Liege), Matilda R. Wilson Professor, Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, Michigan, USA



BEVA Member: £92.34
Non Member: £102.60

BEVA Bookshop
www.beva.org.uk • 01638 723555 • bookshop@evj.co.uk