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Antimicrobial susceptibility of *Histophilus somni* isolated from clinically affected cattle in Australia

Lauren K. Goldspink [a,b], Joanne L. Mollinger [a], Tamsin S. Barnes [b,c], Mitchell Groves [b], Timothy J. Mahony [c], Justine S. Gibson [b,*]

[a] *Biosecurity Sciences Laboratory, Biosecurity Queensland, Brisbane, Queensland 4108, Australia*
[b] *The University of Queensland, School of Veterinary Science, Gatton, Queensland 4343, Australia*
[c] *The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Centre for Animal Science, St Lucia, Queensland 4072, Australia*

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ABSTRACT

This study investigated antimicrobial resistance traits, clonal relationships and epidemiology of *Histophilus somni* isolated from clinically affected cattle in Queensland and New South Wales, Australia. Isolates ($n = 53$) were subjected to antimicrobial susceptibility testing against six antimicrobial agents (ceftiofur, enrofloxacin, florfenicol, tetracycline, tilmicosin and tulathromycin) using disc diffusion and minimum inhibitory concentration (MIC) assays. Clonal relationships were assessed using repetitive sequence PCR and descriptive epidemiological analysis was performed. The *H. somni* isolates appeared to be geographically clonal, with 27/53 (47%) isolates grouping in one cluster from one Australian state. On the basis of disc diffusion, 34/53 (64%) isolates were susceptible to all antimicrobial agents tested; there was intermediate susceptibility to tulathromycin in 12 isolates, tilmicosin in seven isolates and resistance to tilmicosin in one isolate. Using MIC, all but one isolate was susceptible to all antimicrobial agents tested; the non-susceptible isolate was resistant to tetracycline, but this MIC result could not be compared to disc diffusion, since there are no interpretative guidelines for disc diffusion for *H. somni* against tetracycline. In this study, there was little evidence of antimicrobial resistance in *H. somni* isolates from Australian cattle. Disc diffusion susceptibility testing results were comparable to MIC results for most antimicrobial agents tested; however, results for isolates with intermediate susceptibility or resistance to tilmicosin and tulathromycin on disc diffusion should be interpreted with caution in the absence of MIC results.

Introduction

Histophilus somni causes bovine respiratory disease (BRD) worldwide (Sandal and Inzana, 2010). Although it is a commensal of the nasopharynx (Corbeil, 2007), *H. somni* can be an opportunistic pathogen of cattle, predominantly causing respiratory infections, but occasionally septicaemia, myocarditis, arthritis, abortion and other systemic infections (Sandal et al., 2007).

BRD is the most economically important disease in beef cattle (Welsh et al., 2004), costing the Australian feedlot industry approximately AUD$40 million per year (Sackett et al., 2007). Antimicrobial agents including tetracycline, tilmicosin, florfenicol, tulathromycin, ceftiofur and enrofloxacin are used routinely to prevent and/or treat BRD (Welsh et al., 2004). A reliance on these drugs creates a selection pressure that may result in the emergence of drug-resistant microorganisms (Barton et al., 2003). Resistance is emerging amongst BRD pathogens, particularly to those antimicrobial agents from first generation classes (e.g. tetracycline) (Welsh et al., 2004; Portis et al., 2012). Moreover, antimicrobial resistance patterns vary according to bacterial species and geographical location (Hendriksen et al., 2008), meaning that local knowledge of susceptibilities is critical for the effective prevention and treatment of *H. somni* infections.

The aim of this study was to determine the antimicrobial susceptibilities of *H. somni* against six antimicrobial agents commonly used to control and treat bovine bacterial respiratory pathogens via both disc diffusion and minimum inhibitory concentration (MIC) testing. Although MIC is considered to be the gold-standard test method in antimicrobial susceptibility determination (Andrews, 2001), disc diffusion is commonly used in veterinary diagnostic laboratories. An additional aim of this study was to assess associations between epidemiological factors (e.g. state of origin, production type, site of isolation), clonal relationships and antimicrobial susceptibility of *H. somni* cultured from Australian cattle.

* Corresponding author. Tel.: +61 7 54601830.
E-mail address: gibson.j@uq.edu.au (J.S. Gibson).

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Table 1
Disc diffusion distribution and susceptibility zones of 53 *Histophilus somni* isolates.

Antimicrobial agents	Number of isolates (%)			Disc diffusion zone sizes (mm)		
	Susceptible	Intermediate	Resistant	Median	Range	CLSI breakpoints
Ceftiofur	53 (100%)	0 (0%)	0 (0%)	38	26–48	R ≤ 17; S ≥ 21
Enrofloxacin	53 (100%)	0 (0%)	0 (0%)	32	24–42	R ≤ 16; S ≥ 21
Florfenicol	53 (100%)	0 (0%)	0 (0%)	40	30–50	R ≤ 14; S ≥ 19
Tilmicosin	45 (85%)	7 (13%)	1 (2%)	14	10–24	R ≤ 10; S ≥ 14
Tulathromycin	41 (77%)	12 (23%)	0 (0%)	20	16–28	R ≤ 14; S ≥ 18
Tetracycline	NA	NA	NA	28	22–36	NA

S, susceptible; R, resistant; NA, not available; CLSI, Clinical and Laboratory Standards Institute.

Materials and methods

Isolates

Fifty-three *H. somni* isolates were obtained in 2012 from bovine samples that had been submitted to the Animal Disease Surveillance Laboratory, Toowoomba, Queensland or Elizabeth Macarthur Agricultural Institute, Menangle, New South Wales, Australia. Isolates were derived from cattle with clinical signs of respiratory disease ($n = 51$), thrombotic meningoencephalitis ($n = 1$) or infertility ($n = 1$) and *H. somni* was considered to be the causal or a contributing pathogen. Isolates were recovered from lung samples (37/53, 70%), nasal swabs (6/53, 11%), brain swabs (3/53, 6%) and one each from a pleural swab, preputial swab and heart blood swab; the remaining four (8%) isolates were from unspecified sites. All isolates were confirmed as *H. somni* by clonal morphology, Gram stain and *H. somni*-specific PCR (Angen et al., 1998). The quality control strain *H. somni* ATCC 700025 was used for all testing.

A clinical history, including location, breed, sex, age, production type and if the animal was introduced onto the property or homebred, was available for all cases, together with the results of serology or molecular testing for potential contributing pathogens, including infectious bovine rhinotracheitis virus (bovine herpesvirus type 1), bovine coronavirus and bovine pestivirus (bovine viral diarrhoea virus).

Antimicrobial disc diffusion susceptibility

Disc diffusion susceptibility testing was used to determine the antimicrobial susceptibility of *H. somni* isolates against ceftiofur (30 μg), enrofloxacin (5 μg), florfenicol (30 μg), tilmicosin (15 μg) and tulathromycin (30 μg) according to Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical Laboratory Standards Institute, 2013). Since guidelines for tilmicosin were not available for *H. somni*, interpretation was based on guidelines for *Mannheimia haemolytica* (Blackall et al., 2007). Disc diffusion susceptibility testing was also performed for tetracycline (30 μg), although CLSI guidelines were not available for interpretation of these results. Tulathromycin discs were obtained from Becton Dickinson, while other antimicrobial discs were obtained from Oxoid.

Minimum inhibitory concentration susceptibility testing

The MICs of ceftiofur, enrofloxacin, florfenicol, tetracycline, and tilmicosin were determined according to CLSI guidelines for agar dilution (Clinical Laboratory Standards Institute, 2013). The MICs of tulathromycin were determined for only 43 isolates using the same guidelines, since there were delays in obtaining tulathromycin antimicrobial powder and 10 isolates could not be revived for testing. Tulathromycin was obtained from Zoetis, while other antimicrobial powders were obtained from Sigma Aldrich.

The MICs were determined as the lowest concentrations of antimicrobial agent in the plate that completely inhibited colony formation. All MICs were tested in duplicate independently on separate days. If duplicate tests were within one serial dilution of each other, they were accepted, and the MIC result was reported as the highest MIC. In all cases, duplicate MIC results were identical or within one serial dilution.

Enterobacterial repetitive intergenic consensus PCR

Clonality between the *H. somni* isolates was determined by enterobacterial repetitive intergenic consensus (ERIC) PCR (Versalovic et al., 1991). Banding patterns were analysed using GelComparII (Applied Maths) with a Dice coefficient of 0.28% and a tolerance of 2.8%. A cluster was defined as a group of isolates that shared ≥80% similarity in their ERIC-PCR patterns. Within each cluster, isolates with a similarity of >94% were considered to be a clonal group. Isolates were considered to be outliers if they were <70% similar.

Epidemiological analysis

Epidemiological analyses were performed with Epitools.[1] The effect of state (Queensland vs. New South Wales), production type (meat/feedlot vs. non-meat/feedlot) and sample site (lung vs. non-lung) for cluster 6 (the dominant cluster including 27/53 of all isolates) compared to isolates from other clusters was determined using the Fisher's exact test. Other variables were not compared, since the total number of isolates in each category were <10.

Results

Antimicrobial susceptibility testing

Using the disc diffusion method, 35/53 (66%) isolates were susceptible to all antimicrobial agents tested (Table 1). All isolates were susceptible to ceftiofur, enrofloxacin and florfenicol. Intermediate susceptibility against tulathromycin was exhibited by 12/53 (23%) isolates and against tilmicosin by 7/53 (13%) isolates; 2/53 (4%) isolates had intermediate susceptibility to both tulathromycin and tilmicosin, while 1/53 (2%) isolates exhibited resistance to tilmicosin.

MICs, percentages of resistance to each antimicrobial agent, and MIC_{50} and MIC_{90} values are shown in Table 2. One of 53 (2%) isolates was resistant to tetracycline, with an MIC of 32 μg/mL, while all other isolates were susceptible to all antimicrobial agents tested.

There was complete agreement between the results of the disc diffusion and MIC methods for ceftiofur, enrofloxacin and florfenicol; all isolates were identified as susceptible with both methods. The isolate which exhibited tetracycline resistance in the MIC (32 μg/mL) had a corresponding disc diffusion of 22 mm (Fig. 1).

Using CLSI breakpoints for *M. haemolytica*, all *H. somni* isolates were susceptible to tilmicosin on MIC (Fig. 1). Seven isolates had intermediate susceptibility to tilmicosin by disc diffusion, with zone diameters of 12–13 mm (intermediate breakpoints 11–13 mm); these isolates had MIC values of 2–8 μg/mL (susceptible breakpoint ≤8 μg/mL). The one resistant isolate had a zone diameter of 10 mm (resistant breakpoint ≤10 mm) and a corresponding MIC of 8 μg/mL.

All 43 isolates tested were susceptible to tulathromycin on MIC testing (Fig. 1); 11/43 (26%) isolates had intermediate susceptibility to tulathromycin by disc diffusion, all with a zone diameter of 16 mm (intermediate breakpoints 15–17 mm). These isolates had MIC values of 4–16 μg/mL (susceptible breakpoint ≤16 μg/mL).

Clonal relationships

Using ERIC-PCR, 10 clusters were identified amongst the 53 *H. somni* isolates (Fig. 2). If four outlying clusters (clusters 1, 2, 9 and 10) were removed, the remaining isolates had a similarity level of >72% (Fig. 2). Twenty-seven of 52 (51%) isolates aligned with cluster

[1] See: http://epitools.ausvet.com.au (accessed 1 December 2014).