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Antimicrobial resistance in equine respiratory disease in the UK

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Abstract

Introduction: Respiratory diseases account for the highest number of clinical problems in horses compared to other body systems. While microbiological culture and sensitivity testing is essential for certain cases, knowledge of the most likely bacterial agents and their susceptibilities is necessary to inform empirical antibiotic choices. ***Methods:*** A retrospective study of microbiological and cytological results from upper and lower respiratory samples (n=615) processed in a commercial laboratory between 2002-2012 was carried out. A further study of lower respiratory samples from horses with clinical signs of lower respiratory disease from May-June 2012 was undertaken. ***Results:*** Both studies revealed *S.equi* subspecies *zooepidemicus*, *P.aeruginosa, Pasteurella* spp*, E.coli* and *B.bronchiseptica* as the most frequently isolated species. *S.equi* subspecies *zooepidemicus* and subspecies *equi* were susceptible to ceftiofur (100%) and erythromycin (99%). Resistance to penicillin (12.5% of *S.equi* subspecies *equi* from URT samples) and tetracycline (62.7%) was also detected. Gram-negative isolates showed resistance to gentamycin, trimethoprim-sulfamethoxazole and tetracycline but susceptibility to enrofloxacin (except *Pseudomonas* spp where 46.2% were resistant). MDR was detected in 1% of isolates. ***Conclusion:*** Resistance to first-choice antibiotics in common equine respiratory tract bacteria was noted and warrants continued monitoring of their susceptibility profiles. This can provide information to clinicians about the best empirical antimicrobial choices against certain pathogenic bacteria and help guide antibiotic stewardship efforts to converse their efficacy.

Introduction

Bacteria are important causes of upper and lower respiratory disease in horses, which often result in poor performance and exercise intolerance [1, 2]. Bacterial respiratory disease is mostly initiated by a viral infection which weakens the respiratory immune mechanisms [3]. A noteworthy exception to this is strangles where *S. equi subspecies equi* can cause disease of the upper respiratory tract without any predisposing factors [4]. Frequently isolated opportunistic respiratory pathogens are *S. equi* subspecies *zooepidemicus, Pasteurella spp* and *Bordetella bronchiseptica* but *Pseudomonas aeruginosa* and *Escherichia coli* are also commonly detected in respiratory tract samples [5].

The cytological examination and bacterial culture of respiratory specimens are useful tools for the diagnosis of these infections, the determination of their aetiology and the selection of adequate antibiotic treatment [1, 2]. Unfortunately culture and sensitivity of lower airway fluid samples is not always performed and treatment is often initiated on an empirical basis when a bacterial infection is suspected [6]. This is warranted as delays in the initiation of treatment can lead to poor clinical outcomes [5]. Selection of empirical therapy should be based on current knowledge of the prevalence and antibiotic susceptibility patterns of the bacteria most commonly isolated from affected horses.

The aims of this study were to provide evidence on the aetiology and antibiotic resistance of bacteria isolated from horses with respiratory disease using retrospective data from a diagnostic laboratory as well as prospective data from a sub-study and evaluation of their relevant comparisons.

Materials and methods

We conducted a retrospective review of respiratory specimens collected from horses suspected of respiratory disease and processed at NationWide Laboratories (NWL; United Kingdom) between May 2002 and May 2012. Data on bacterial culture, antibiotic susceptibility testing results, cytology reports and age and sex of the patients were obtained by record review. Samples from the same patient that had the same organisms with identical antibiotic susceptibilities and were submitted within 3 months of the original sample were excluded. The analysis focused on the aetiology of the lower and upper respiratory infections and their antibiotic susceptibilities.

In a prospective sub-study, 200 µL aliquots of 13 broncho alveolar lavage (BAL) and 1 tracheal wash (TW) samples from horses with clinical signs of lower respiratory disease received by NWL for routine

101 microbiological analysis from May to June 2012 were frozen at -20 C for processing at UCL Centre for Clinical
102 Microbiology (CCM).
103
104 Samples were processed by NWL and CCM using standard protocols for aerobic and anaerobic semi-
105 quantitative bacterial culture using the calibrated loop method. The level of bacterial growth was reported
106 as follows: no growth; scanty (colonies limited to the initial sector); moderate (colonies on sectors 1-3); or
107 profuse (colonies on all 4 sectors).
108
109 Microorganism identification was done by NWL using Gram-staining and standard biochemical procedures
110 while CCM used Matrix-assisted laser desorption/ionisation (MALDI-TOF) and proceeded to standard
111 biochemical procedures for the isolates where MALDI-TOF was unsuccessful. The Biotyper 3 Wizard program
112 was employed to analyse the mass spectrum profiles of each isolate and parallel them against the Bruker
113 taxonomy library to identify each organism by pattern matching. Isolates with a match log score of over 2
114 were considered to have a valid genus and species identification while a score between 1.7 and 2 was
115 marked as a valid identification of the genus only and the species were written in brackets.
116
117 The identity of only one isolate of each bacterial species was confirmed and testing for susceptibility was
118 only carried out when the organism was isolated in moderate or profuse growth. Antimicrobial susceptibility
119 testing was performed by the Kirby-Bauer method in accordance with the guidelines from the Clinical and
120 Laboratory Standards Institute [7, 8, 9]. Herein, multiple drug resistance (MDR) was defined as resistance to
121 three or more antimicrobial drug classes as proposed by Magiorakos *et al* [10].
122
123 Cytology slides were prepared by cytocentrifugation, stained with Wright-Giemsa and examined by clinical
124 pathologists at NWL. The cytology reports reviewed by the researchers included overall cellularity, cell types
125 and appearances and interpretation of findings.
126
127 **Results**
128 Bacterial culture results were available for 615 samples: 120 TW, 2 BAL, 473 nasal swabs (NS), 19
129 nasopharyngeal swabs (NPS) and 1 guttural pouch wash (GPW). Bacteria were isolated from 91 (75.8%) of
130 the TW samples, both of the BAL samples, 450 (95.1%) of the NS samples, 17 (89.5%) of the NPS samples and
131 the GPW sample. The mean age of horses with culture-positive lower respiratory samples (TW and BAL) was
132 8.2±6.7 years in females and 10.0±9.0 years in males. For those with culture-positive upper respiratory
133 samples (NS, NPS and GPW), the mean age at diagnosis was 10.6±6.8 years in females and 9.4±7.3 years in
134 males.

In samples from the upper respiratory tract, *Streptococcus equi* (37%) (subspecies *zooepidemicus* 22.9%, subspecies *equi* 14.1%), *E. coli* (17.5%), coagulase-negative staphylococci (17.3%), and *S. equi* subspecies *equi* (14.1%) were the bacterial species most frequently isolated. In lower respiratory samples, there was a predominance of *Streptococcus equi* (29.7%) (subspecies *zooepidemicus* 25.3%, subspecies *equi* 4.4%,), *Pasteurella* spp (28.6%), *Pseudomonas* spp (20.9%) and *Escherichia coli* (13.2%), (Table 1). In the prospective study (May – July 2012) *Streptococcus equi* was demonstrated to be the most common isolate, 8 of 14 processed samples (57%).

Polymicrobial growth was observed in 53 (58.2%) TW, 311 (69.1%) NS and 13 (76.5%) NPS samples. In TW samples, the most common combinations involved *S. equi* subspecies *zooepidemicus* (present in 11 samples), *Pasteurella* spp (12) or both (8). In NS and NPS samples with mixed growth, *S. equi* subspecies *zooepidemicus* (74) and *E. coli* (67) were the bacterial species most frequently isolated. These were often in combination with staphylococci, particularly *Staphylococcus aureus* or coagulase-negative staphylococci. When only one organism was present, *Pasteurella* spp (20.0%) and *Pseudomonas* spp (15.0%) were the most frequently isolated in lower respiratory samples and *S. equi* subspecies *zooepidemicus* (23.1%) and *S. equi* subspecies *equi* (18.9%) in upper respiratory samples. Anaerobes were only present in TW samples (5.5%), mainly in combination with aerobic bacteria (60%) (Table 1). Polymicrobial growth was noted in 13 out of the 14 TW and BAL prospective samples with the most common combinations including *Streptococcus* and *Staphylococcus spp*.

Cytology reports were available for 78 samples from which 26 had evidence of bacterial infection (i.e. increased numbers of degenerate neutrophils and presence of intracellular bacteria). Twenty-two of these samples (18 from TW samples and 4 from NS samples) were culture-positive. *Pasteurella* spp (22.2%) and *S. equi* subspecies *zooepidemicus* (14.8%) were the most prevalent bacterial species in culture- and cytology-positive TW samples and *S. equi* subspecies *zooepidemicus* (25%), coagulase-negative staphylococci (12.5%) and *E. coli* (12.5%) in NS samples.

The antibiotic resistance profiles of the respiratory isolates are presented on Tables 2 and 3. Two *S. equi* subspecies *zooepidemicus* and four *S. equi* subspecies *equi* isolates from NS samples were resistant to penicillin. All isolates of these β-haemolytic Group C streptococci were susceptible to ceftiofur and (with the exception of one isolate from each species) to erythromycin. In contrast, resistance to tetracycline was common, particularly in isolates from lower respiratory samples (more than 90% of *S. equi* subspecies *zooepidemicus* and 66.7% of *S. equi* subspecies *equi* were resistant).

Enrofloxacin showed good *in vitro* activity against Gram-negative isolates except those belonging to *Pseudomonas* spp (46.2% resistant). Also in Gram-negative isolates, resistance to gentamycin, trimethoprim-sulfamethoxazole and tetracycline was prevalent. From the 1,342 isolates included in our study, only 1% were MDR. Multiple drug resistance was observed in *E. coli* (7 isolates), *Acinetobacter* spp (4) and *Pseudomonas* spp (3).

Six *Streptococcus equi* (subspecies not characterised during this study) were identified and isolated from the in-house lab work (CCM) carried out on the BAL and TW samples. All of these were found to be sensitive to the β-lactams tested (penicillin and ampicillin), trimethoprim/sulfamethoxazole, erythromycin and cefoxitin, and resistant to tetracycline and streptomycin. These findings were in agreement and reinforced the findings of the retrospective work carried out. There were variable susceptibilities to rifampicin with 4 of the isolates being intermediately resistant, 1 being sensitive and another resistant. One of the *Streptococcus equi* was found to be MDR, with resistance to tetracycline, streptomycin, rifampicin, and kanamycin.

Discussion

Our findings are largely in agreement with previous reports on the detection of bacteria in the respiratory tracts of horses with respiratory disease, albeit with minor differences in terms of the prevalence of each bacterial species [1, 5, 11, 12, 13, 14, 15]. Most isolates belonged to environmental or commensal species capable of opportunistic infection when the host's defence mechanisms are compromised, which complicates the interpretation of culture results. Moderate to heavy bacterial growth, especially if in pure culture, is generally considered to be more likely to represent true infection [1]. However, a considerable proportion of cytology-positive samples (41.2%) in our study only yielded the growth of small numbers of bacteria. *Pseudomonas aeruginosa* and *E. coli* are often detected in upper respiratory tract samples from horses but are not necessarily the reason for clinical disease. Alternatively, *Pastereulla* spp are often cultured with *S. equi* subspecies *zooepidemicus* and are more likely to be associated with inflammation of the lower respiratory tract [16]. Furthermore, although the lower airways in a healthy horse are considered sterile, the passage of an endoscope during BAL sampling can introduce oropharyngeal contamination or nasopharyngeal bacteria during TW sampling. This indicates that antibiotics susceptibility tests should be analysed with caution depending on the organism(s) isolated and highlights the importance of cytology in the evaluation of these patients.

The use of antibiotics for the treatment of strangles remains controversial and studies to indicate the use and appropriate timing of antibiotics are lacking. Prospective studies observing horses on antibiotic

203 treatment and without treatment are warranted [17]. Nevertheless most strangles cases recover
204 uneventfully without antibiotics but are indicated in certain cases such as marked lymphadenopathy and
205 dyspnoea. Their use is also advocated in acutely infected horses with high fever and lethargy in order to
206 prevent abscess formation as well as cases of 'bastard strangles' and guttural pouch infections to eliminate
207 the carrier state [2, 18].
208
209 Penicillin is currently regarded as the drug of choice for the treatment of infections by non-pneumococcal
210 streptococci in horses and benzylpenicillin administration topically and systemically has appeared to improve
211 treatment success rates for strangles carriers [2, 18]. The emergence of penicillin-resistant strains of *S. equi*
212 subspecies *equi* should therefore be closely monitored and contrary to similar studies on isolates of equine
213 origin conducted in the UK and elsewhere [15, 19, 20, 21] we detected penicillin resistance in *S. equi*
214 subspecies *equi* .
215
216 Tetracyclines are sometimes recommended as alternative agents for the treatment of upper respiratory
217 infections in horses [22] but our results suggest that a significant proportion of *S. equi* subspecies *equi* and
218 subspecies *zooepidemicus* responsible for these infections might be resistant.
219
220 Ceftiofur has also been proposed for off-license use in *Streptococcus equi* infections. Studies have shown
221 both *S. equi* subspecies *zooepidemicus* and subspecies *equi* to be susceptible in vitro to ceftiofur while
222 another has indicated that sustained release ceftiofur suspension was effective against lower respiratory
223 tract infections associated with *S. equi* subspecies *zooepidemicus* [23, 24, 25]. From our findings, all β-
224 haemolytic Group C streptococci tested were susceptible to ceftiofur. In order to preserve their efficacy and
225 ensure appropriate antibiotic cascade use, cephalosporins should be reserved for cases indicated by culture
226 and where clinical signs and disease progression necessitate treatment [18].
227
228 Given the multiplicity of agents that can cause lower respiratory tract infections in horses and the possibility
229 of mixed aerobic/anaerobic infections, a broad-spectrum antibiotic regimen is usually recommended for the
230 empirical treatment of more severe cases [1]. A combination of gentamicin for Gram-negative coverage and
231 penicillin for Gram-positive and anaerobic coverage (with or without metronidazole) is often used.
232 Gentamicin showed good to moderate *in vitro* activity against the Gram-negative isolates from lower
233 respiratory samples included in our study (resistance ranging from 10.9-26.3%, depending on the bacterial
234 species). The emergence of gentamicin resistance in *E. coli* of equine origin was documented in a recent
235 study [20] and should be further monitored. Gentamicin is sometimes substituted by enrofloxacin in adult
236 horses [1]. Whilst enrofloxacin resistance remained low amongst isolates from most Gram-negative species,

approximately half of the *Pseudomonas* spp were refractory. Information on susceptibility patterns of Gram-negative isolates in equine respiratory tracts are useful in observing trends but care should be taken in their interpretation as they may not be clinically significant to the specific case. There is a drive to reduce enrofloxacin and third and fourth generation cephalosporins in equine medicine and since the introduction of antibiotic cascade guidelines by the British Equine Veterinary Association [PROTECT ME toolkit, 26] a 90-95% decrease in prescribing enrofloxacin has been shown in one clinical setting without impacting clinical results and a 30% decrease in national sales of third and fourth generation cephalosporins have been achieved [27].

Our findings provide evidence on the aetiology and antibiotic resistance of bacteria isolated from horses with respiratory disease in the UK and highlights the importance of cytology in the interpretation and analysis of these samples. The emergence and spread of antibiotic resistance in the bacterial agents most commonly implicated in infectious respiratory disease in horses can have serious impacts on animal welfare (higher morbidity and mortality associated with treatment failure) and increase the costs of treatment. It has become an important issue affecting public health and antibiotic use by veterinarians has become a concern in recent years leading to the introduction of antimicrobial protocols [28]. Research indicates that factors affecting veterinary prescribing behaviours and the judicious use of antimicrobials include costs of culture and sensitivity, lack of rapid and cost-effective diagnostic tests and client pressure [29, 30]. Further research into factors influencing these behaviours as well as continued monitoring of the susceptibility profiles of these infections is not only necessary to inform clinicians about the best empirical antibiotic choices but also to help guide antibiotic stewardship efforts to converse antibiotic efficacy.

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References

1. Reuss SM, Giguère S. Update on Bacterial Pneumonia and Pleuropneumonia in the Adult Horse. *Veterinary Clinics of North America: Equine Practice* 2015;**31**(1):105–120.

2. Sweeney CR, Timoney JF, Newton JR, et al. *Streptococcus equi* Infections in Horses: Guidelines for Treatment, Control, and Prevention of Strangles. *J Vet Intern Med* 2005;**19**(1):123–134.

3. Dixon PM, Railton DI, McGorum BC. Equine pulmonary disease: a case control study of 300 referred cases. Part 3: Ancillary diagnostic findings. *Equine Veterinary Journal* 1995;**27**(6):428-435.

4. Chapman RS, Green C, Main JPM, et al. Retrospective study of the relationships between age, inflammation and the isolation of bacteria from the lower respiratory tract of thoroughbred horses. *Veterinary Record* 2000;**146**(4):91-95.

5. Racklyeft DJ, Love DN. Bacterial infection of the lower respiratory tract in 34 horses. *Aust Vet J* 2000;**78**(8):549–559.

6. Christley RM, Rose RJ, Hodgson DR, et al. Attitudes of Australian veterinarians about the cause and treatment of lower-respiratory-tract disease in racehorses. *Preventive Veterinary Medicine* 2000;**46**(3):149-59.

7. NCCLS (1991). Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. M31-A. Wayne, PA, USA.

8. NCCLS (2004). Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Informational Supplement. M31-S1. Wayne, PA, USA.

9. CLSI (2008). Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. M31-A3. Wayne, PA, USA.

10. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012;**18**(3):268-281.

11. Boguta L, Gradzki Z, Borges E, et al. Bacterial flora in foals with upper respiratory tract infections in Poland. *J Vet Med B* 2002;**49**(6):294–297.

12. Sweeney CR, Holcombe SJ, Barningham SC, et al. Aerobic and anaerobic bacterial isolates from horses

306 with pneumonia or pleuropneumonia and antimicrobial susceptibility patterns of the aerobes. *Journal of the*
307 *American Veterinary Medical Association* 1991;**198**(5):839–842.
308 13. Whitwell KE, Greet TRC. Collection and evaluation of tracheobronchial washes in the horse. *Equine*
309 *Veterinary Journal* 1984;**16**(6):499–508.
310 14. Wood JLN, Newton JR, Chanter N, et al. Association between Respiratory Disease and Bacterial and Viral
311 Infections in British Racehorses. *J Clin Microbiol* 2005;**43**(1):120–126.
312 15. Erol E, Locke SJ, Donahoe JK, et al. Beta-hemolytic *Streptococcus* spp. from horses. *J Vet Diagn Invest*
313 2012;**24**(1):142–147.
314 16. Wood JL, Burrell MH, Roberts CA, et al. Streptococci and *Pasteurella* spp. associated with disease of the
315 equine lower respiratory tract. *Equine Vet J* 1993;**25**(4):314-8.
316 17. Ramey D. Does early antibiotic use in horses with “strangles” cause metastatic *Streptococcus equi*
317 bacterial infections? *Equine Veterinary Education* 2010;**19**(1):14–15.
318 18. Boyle AG, Timoney JF, Newton JR, et al. Streptococcus equi Infections in Horses: Guidelines for
319 Treatment, Control, and Prevention of Strangles-Revised Consensus Statement. *J Vet Intern Med*
320 2018;**32**(2):633-647.
321 19. Clark C, Greenwood S, Boison JO, et al. Bacterial isolates from equine infections in western Canada
322 (1998-2003). *The Canadian Veterinary Journal = La Revue Veterinaire Canadienne* 2008;**49**(2):153–160.
323 20. Johns IC, Adams E-L. Trends in antimicrobial resistance in equine bacterial isolates: 1999-2012.
324 *Veterinary Record* 2015;**176**(13):334–334.
325 21. Kirinus JK, Pötter L, Gressler LT, et al. Perfil fenotípico e susceptibilidade antimicrobiana de Streptococcus
326 equi isolados de equinos da região Sul do Brasil. *Pesquisa Veterinária Brasileira* 2011;**31**(3):231–238.
327 22. British Equine Veterinary. Protect Me - Practice policy. 2016.
328 https://www.beva.org.uk/Portals/0/Documents/Resources/1beva-antimicrobial-policy-template-
329 distributed.pdf (accessed 9 January 2019)
330 23. Bade D, Portis E, Keane C, et al. In vitro susceptibility of ceftiofur against *Streptococcus*
331 *equi*subsp *zooepidemicus* and subsp *equi* isolated from horses with lower respiratory disease in Europe since
332 2002. *Veterinary therapeutics: research in applied veterinary medicine* 2009;**10**(4):E1-E10.
333 24. Bade D, Sibert G, Hallberg J, et al. Ceftiofur susceptibility of *Streptococcus equi* subsp *zooepidemicus*
334 isolated from horses in North America between 1989 and 2008. *Veterinary therapeutics: research in applied*
335 *veterinary medicine* 2009;**10**(4):E1-E7.
336 25. McClure S, Sibert G, Hallberg J, et al. Efficacy of a 2-dose regimen of a sustained release ceftiofur
337 suspension in horses with *Streptococcus equi* subsp. *zooepidemicus* bronchopneumonia. *J Vet Pharmacol*
338 *Ther* 2011;**34**(5):442–447.

26. BEVA. Protect me toolkit. 2018. https://www.beva.org.uk/Resources-For-Vets-Practices/Medicines-Guidance/Protect-me (accessed 9 December 2019).
27. Bowen M, Marr CM, Clegg PD. Letter to the Editor: Equine Veterinary Journal's antimicrobial stewardship policy. *Equine Veterinary Journal* 2016;**48**(4):532-533.
28. Hardefeldt LY, Gilkerson JR, Bilman-Jacobe H, et al. Antimicrobial labelling in Australia: a threat to antimicrobial stewardship?. *Aust Vet J* 2018;**96**(5):151-154.
29. Norris JM, Zhuo A, Govendir M, et al. Factors influencing the behaviour and perceptions of Austalian veterinarians towards antibiotic use and antimicrobial resistance. *PLoS One* 2019;**14**(10):e0223534.
30. Raidal SL. Antimicrobial stewardship in equine practice. *Aust Vet J* 2019;**97**(7):238-242.

Bacterial species	Lower respiratory samples				Upper respiratory samples			
	Samples with moderate/profuse growth (n=43)		Total number of samples (n=91)		Samples with moderate/profuse growth (n=359)		Total number of samples (n=468)	
	N	%	n	%	N	%	n	%
***Acinetobacter* spp.**	4	9.3	5	5.5	38	10.6	57	12.2
α-haemolytic streptococci	2	4.7	9	9.9	31	8.6	47	10.0
Anaerobe	2	4.7	5	5.5	0	0	0	0
β-haemolytic strep	0	0	5	5.5	0	0	2	0.4
***Bordetella* spp.**	3	7.0	6	6.6	4	1.1	6	1.3
***Enterobacter* spp.**	7	16.3	9	9.9	21	5.8	29	6.2
E. coli	4	9.3	12	13.2	60	16.7	82	17.5
***Pasteurella* spp.**	15	34.9	26	28.6	35	9.7	44	9.4
***Pseudomonas* spp.**	7	16.3	19	20.9	32	8.9	58	12.4
S. aureus	1	2.3	4	4.4	54	15.0	76	16.2
Coagulase negative staphylococci	1	2.3	2	2.2	55	15.3	81	17.3
Coagulase positive staphylococci	1	2.3	2	2.2	39	10.9	63	13.5
S. pseudintermedius	0	0	1	1.1	7	1.9	58	12.4
S. dysgalactiae* subsp. *equisimilis	1	2.3	2	2.2	11	3.1	12	2.6
S. equi* subsp. *equi	1	2.3	4	4.4	55	15.3	66	14.1
S. equi* subsp. *zooepidemicus	15	34.9	23	25.3	80	22.3	107	22.9

Table 1 – Bacterial species most commonly isolated from respiratory samples from horses.
N – number of isolates, % - number of isolates of each bacterial species divided by the number of samples.
Only one isolate of each bacterial species was identified and testing for susceptibility was only carried out when the organism was isolated in moderate or profuse growth.

		% Resistant Isolates											
Bacterial species	**Growth level**	**P**	**AMP**	**SXT**	**CEF**	**TE**	**ENR**	**MAR**	**CN**	**S**	**C**	**E**	**RD**
E. coli	Moderate/Profuse	N/A	25	0	0	0	0	0	25	76.6	0	N/A	N/A
	Total	N/A	9.1	28.6	0	42.9	0	0	14.3	77.8	0	N/A	N/A
***Pasteurella* spp.**	Moderate/Profuse	N/A	0	16.7	0	0	0	0	23	57	0	N/A	N/A
	Total	N/A	9.1	14.3	0	0	5	0	26.3	57.9	0	N/A	N/A
***Pseudomonas* spp.**	Moderate/Profuse	N/A	84.6	80	80	50	50	5.7	12.5	75.8	65	N/A	N/A
	Total	N/A	80.9	58.3	69.2	30.8	46.2	2.6	10.9	76.2	67.6	N/A	N/A
Coagulase-negative staphylococci	Moderate/Profuse	76.6	N/A	0	0	34.7	0	0	0	0	0	0	0
	Total	75	N/A	0	0	33.3	0	0	0	0	0	0	0
S. equi* subsp. *equi	Moderate/Profuse	0	N/A	50	0	100	N/A	N/A	100	100	0	0	0
	Total	0	N/A	25	0	66.7	N/A	N/A	100	100	0	0	0
S. equi* subsp. *zooepidemicus	Moderate/Profuse	0	N/A	30	0	90.9	N/A	N/A	100	100	0	0	40
	Total	0	N/A	21.4	0	92.9	N/A	N/A	100	100	0	0	30
Total	Moderate/Profuse	29	35.2	34.3	26.2	29.9	11.5	1.6	13.9	53.1	9.5	14.9	47.4
	Total	30.5	34.3	32.5	24.1	28.8	12	3.1	16.8	56.7	13	18.2	37.3

Table 2 – Antibiotic resistance patterns of bacteria isolated from lower respiratory samples.
P – penicillin, AMP – ampicillin, SXT – trimethoprim-sulfamethoxazole, CEF – ceftiofur, TE – tetracycline, ENR – enrofloxacin, MAR – marbofloxacin, CN – gentamicin, S – streptomycin, C – chloramphenicol, E – erythromycin, RD – rifampicin, N/A – not available. Results are shown as percentage of resistant isolates per total number of isolates tested.
Moderate growth= colonies on sectors 1-3, profuse growth= colonies on all 4 sectors.

		% Resistant Isolates											
Bacterial species	**Growth level**	**P**	**AMP**	**SXT**	**CEF**	**TE**	**ENR**	**MAR**	**CN**	**S**	**C**	**E**	**RD**
E. coli	Moderate/Profuse	N/A	50	30.5	2.1	33.3	0	0	5.1	59.3	5.9	N/A	N/A
	Total	N/A	42	26.2	2.9	30	0	0	6.2	53.7	5.3	N/A	N/A
***Pasteurella* spp.**	Moderate/Profuse	N/A	8.6	12.1	5.6	4.3	17.4	0	5.7	40	0	N/A	N/A
	Total	N/A	9.5	12.5	8.3	4	16	0	7.1	42.9	0	N/A	N/A
***Pseudomonas* spp.**	Moderate/Profuse	N/A	87.5	62.9	68.2	49.3	37.5	5.3	9.4	40.6	56.2	N/A	N/A
	Total	N/A	86	63.2	64.6	42.9	25	2.9	5.4	36.8	58.6	N/A	N/A
Coagulase-negative staphylococci	Moderate/Profuse	21.8	N/A	3.7	6.7	5.6	13.5	2.9	1.9	13.7	0	9.4	1.9
	Total	19.8	N/A	6.2	4.4	7.7	9.3	4.1	1.3	10.5	0	12.8	2.5
S. equi* subsp. *equi	Moderate/Profuse	12.5	N/A	16.4	0	36.8	N/A	N/A	89.5	91.7	0	0	9.1
	Total	6.1	N/A	13.8	0	33.3	N/A	N/A	91.1	92.7	0	1.5	7.7
S. equi* subsp. *zooepidemicus	Moderate/Profuse	0	N/A	10.3	0	79.5	N/A	N/A	80	92.1	2.6	0	6.5
	Total	1.9	N/A	12.4	0	74	N/A	N/A	86	92.6	2	1	5.9
Total	Moderate/Profuse	23.9	36.6	18.6	7.1	25	12.4	2	16.8	49.9	6.1	18.2	30.2
	Total	24.3	32.2	17.7	8.6	20.7	12	1.8	15.6	45.7	8.1	19.1	30.6

Table 3 – Antibiotic resistance patterns of bacteria isolated from upper respiratory samples.
P – penicillin, AMP – ampicillin, SXT – trimethoprim-sulfamethoxazole, CEF – ceftiofur, TE – tetracycline, ENR – enrofloxacin, MAR – marbofloxacin, CN – gentamicin, S – streptomycin, C – chloramphenicol, E – erythromycin, RD – rifampicin, N/A – not available. Results are shown as percentage of resistant isolates per total number of isolates tested.
Moderate growth= colonies on sectors 1-3, profuse growth= colonies on all 4 sectors.