Veterinary Anaesthesia and Analgesia, 2007, **34,** 190–199 doi:10.1111/j.1467-2995.2006.00320.x

RESEARCH PAPER

An investigation of the bacterial contamination of small animal breathing systems during routine use

Ludovic Pelligand* CertVA, Doc Vet, MRCVs, **Richard Hammond†** BSc, PhD, DVA, Diplomate ECVA, BvetMed, MRCVS & **Andrew Rycroft‡** BSc, PhD, C Biol, FI Biol

*Anaesthesia Service, The Royal Veterinary College, Hertfordshire, UK

†The School of Veterinary Medicine and Science, The University of Nottingham, Sutton Bonington Campus, Sutton Bonington, Leicestershire, UK

‡Department of Pathology & Infectious Diseases, Royal Veterinary College, Hertfordshire, UK

Correspondence: Ludovic Pelligand, Anaesthesia Service, Queen Mother Hospital, The Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield AL9 7TA, Hertfordshire, UK. E-mail: lpelligand@rvc.ac.uk

Abstract

Objective To investigate the need for sterilization of anaesthetic breathing systems to prevent cross-infection between animals due to the re-use of anaesthetic circuit tubing.

Study design Prospective microbiological study.

Methods Bacteriology samples were taken from 37 sterile breathing systems, each used for 1 day, at two sampling sites (one proximal and one distal to the animal). The number of patient connections, cumulative anaesthesia time, culture results, number of colony-forming units and the number of different species were recorded. Secondly, four sterile breathing systems were used for 2 months under routine conditions and sampled every 2 weeks and the same parameters recorded. Finally, the inner surfaces of four sterile breathing systems were inoculated with a known load of canine oropharyngeal bacteria. Bacteriology samples were taken at 1 minute, 1 hour and 1 day post-deposition. The number of colonies identified was compared with the initial load.

Results Only a very small number of micro-organisms were isolated and these were generally organisms of low pathogenic potential. The proximal site was found to be significantly more colonized than the distal site ($p < 0.001$). Neither longer daily connection time ($p = 0.54$), nor a higher number of connections ($p = 0.81$) increased the incidence of proximal site colonization. Over the 2-month study period, the bacterial population did not increase. There was no correlation between cultures isolated from successive samples taken from the same tubing. There was rapid loss of viability of the micro-organisms deliberately inoculated onto the tubing surface: the number of colonies isolated from the breathing system after 1 minute was significantly lower than in the inoculum ($p = 0.042$).

Conclusions and clinical relevance Sterile anaesthesia breathing systems were colonized by environmental micro-organisms of low pathogenicity. Although long-term survival of recognized pathogens in a dry environment is still possible, the use of a bacterial filter or a sterilized breathing system for routine veterinary anaesthesia cannot be supported by current evidence.

Keywords anaesthesia, bacterial contamination, breathing system, environmental colonization, nosocomial, sterilization.

Introduction

Pneumonia is the second most common type of nosocomial infection in human hospitals. Death can

occur as a result of nosocomial pneumonia in up to 50% of patients (Johnson 2002). In the literature, it is recognized that respiratory equipment used in intensive care units (ICU), especially ventilators, can harbour pathogenic micro-organisms and may act as fomites in nosocomial pneumonia transmission (Irwin et al. 1980; Hovig 1981). In a study on ventilated patients in an ICU, 95% of the ventilator breathing systems became contaminated after 24 hours (Malecka-Griggs et al. 1989). The colonization of ICU ventilator tubing appeared to be rapid, occurring in 33% of the ventilators after only 2 hours (Craven et al. 1982).

The question of patient cross-contamination via anaesthetic apparatus is not new and has been reviewed (Hogarth 1996). Anaesthetic breathing systems deliver anaesthetic agents and dry carrier gases to the patient and take expired gases away from the patient. They are usually made of corrugated semi-disposable plastic tubing, which makes them prone to the retention of micro-organisms (Murphy et al. 1991). If pathogenic bacteria can survive in anaesthesia breathing systems, and if they can be eluted in the fresh gas flow, then the potential for cross-contamination exists (Nielsen et al. 1980; Langevin et al. 1999). Previous reports have stated that there is a risk of bacterial transmission through anaesthetic equipment (Phillips & Spencer 1965; Beck & Zadeh 1968; Olds et al. 1972) but many failed to provide a proof of causation (Albrecht & Dryden 1974). In New South Wales, a cluster of five cases of hepatitis C in patients from the same theatre operating list has been reported (Chant et al. 1994). Although no specific mode of transmission was identified, the investigators suspected a viral cross-infection caused by contamination of the common anaesthetic breathing system in use for that theatre session.

Equivocal conclusions from several prospective trials with different experimental designs have, however, divided opinion; the presence of bacteria adsorbed on anaesthetic equipment may not significantly increase the risk of lung infection (Du Moulin & Saubermann 1977). In one study involving 293 patients, the routine use of sterile anaesthesia breathing systems with bacterial filters did not decrease the risk of postoperative respiratory infection when compared with breathing systems just washed and dried (Feeley et al. 1981). There is little evidence to implicate anaesthetic machines and breathing systems as either a source of, or a vector for, bacterial infection of patients undergoing general anaesthesia, with the exception of the intensive care settings (Hogarth 1996).

Anaesthetic breathing systems are classified as semi-critical equipment because they are in contact with mucous membranes but do not penetrate body surfaces. Medical anaesthesia guidelines for prevention of cross-infections have been revised after the New South Wales hepatitis C outbreak in 1993 (Chant et al. 1994). Until this date, cleaning and disinfection of anaesthesia systems following each use were not mandatory in the ASA guidelines, whereas the Association of Great Britain and Ireland Anaesthetists already recommended a disposable or sterilized system for each patient (Knoblanche 1996). This new policy in medical anaesthesia requires universal precautions, especially with the risk of HIV transmission. Sterilization is desirable for anaesthetic systems, but not easily possible; decontamination and high-level disinfection is acceptable (Dorsch & Dorsch 1998). Efficient disinfection of anaesthesia circuitry is difficult and time consuming because of the air trapped in the corrugations (George 1975; Murphy et al. 1991). The use of disposable breathing systems or specific bacterial filters for every patient has therefore been recommended (Knoblanche 1996). Hydrophobic bacterial filters are extremely efficient *in vitro* according to the manufacturer (Leijten et al. 1992; Rathgeber et al. 1997) but convincing evidence of their efficacy is lacking, particularly with regard to viruses (Knoblanche 1996; Demers 2001). Bacterial filters have not been proved to reduce the incidence of postoperative pneumonias (Garibaldi et al. 1981). They can lead to intraoperative complications, particularly occlusion (McEwan et al. 1993), and are expensive.

Currently, there are no official guidelines for veterinary anaesthesia. The cost–benefit ratio of using sterilized breathing systems or filters is questionable. The situation in veterinary anaesthesia is probably similar to that in medical hospitals in less developed countries (Richard et al. 2001). Owing to financial constraints, veterinary anaesthetists commonly re-use the same breathing system for a number of animals without knowing if this practice is safe for subsequent animals. The objective of the current study was to provide evidence to enable an informed decision to be made. The first part of the study (part 1) gave an overview of the bacterial population that

colonizes veterinary anaesthetic breathing systems after 1 day of normal use and compared the bacterial load between different parts of the breathing system. Part 2 reported the bacterial population trends in breathing systems over 2 months of clinical use. Finally, part 3 investigated the survival of natural oro-pharyngeal bacteria in anaesthetic breathing systems. The results of these investigations, and a review of the literature, may provide evidence on which suitable guidelines for veterinary breathing system processing could be based.

Materials and methods

This study was performed following the normal standard of care and treatment regarding the use of breathing systems in common usage in this hospital. No ethical approval was required.

Sampling technique

Sterile cotton-tipped swabs were moistened with 200 μL of Hanks Balanced Salts Solution (Life Technologies Ltd, Paisley, UK) before each sample. The swabs were rotated on the designated sampling area for a standard period of 1 minute. Each sample was collected by the same investigator in the same conditions (in a sterile area, wearing sterile gloves and a surgical mask). The samples were transported to the laboratory in a sterile tube without transport media and cultured within 1 hour.

Sampling material

Parallel-Lack breathing systems (Intersurgical Ltd, Workingham, Berkshire, UK), which are the most frequently used breathing systems in our hospital, were used as the sampling surface. Each breathing system was labelled for identification. Before any experiment, breathing systems were manually rinsed and disinfected in 1% Virkon (Antec International, Sudbury, Suffolk, UK), rinsed again, dried and then sterilized with ethylene oxide (Anprolene; H.W. Andersen Products Ltd, Essex, UK), They were kept in a heated airing cabinet for 24 hours at 20 °C.

Samples were taken from two sites, as shown in Fig. 1. The distal sampling site (DSS, Fig. 1a) was the internal surface of the breathing system just upstream from the adjustable pressure limiting valve. The proximal sampling site (PSS, Fig. 1b) was the internal surface of the Y-piece that connects inspiratory and expiratory hoses. The valve was removed to perform the sampling.

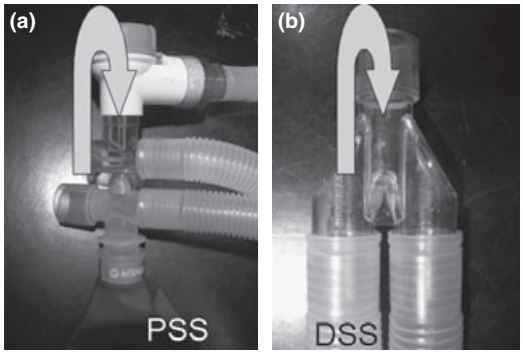

Figure 1 Sampling sites in part 1 (a) Distal sampling site (DSS). (b) Proximal sampling site (PSS): Y-piece. Thick arrow indicates sampling sites.

Sterility control samples

Sterility controls were performed at this stage to ensure that the sampling technique was reliable and that breathing systems were bacteria-free after sterilization. Control samples were taken from the PSS of 21 randomly chosen sterilized breathing systems immediately after opening of the pack.

Sampling protocols

This study was carried out in three parts.

Part 1: Breathing system colonization after 1 day of use

Sterile anaesthetic breathing systems were used, during a whole working day of anaesthesia, on a number of animals. At the end of the day, samples were collected from PSS and DSS, within 2 hours after the last use. Animals on the anaesthesia list were randomly allocated to a specific breathing system. The breathing systems studied during this phase of the experiment were identified and distinguished from other systems not included in the sampling protocol. The following parameters were recorded at each sampling site: number of connections, cumulative anaesthesia time during the day and culture result. If the culture result was positive, the number of colony-forming units (CFUs) and the number of different species of bacteria were recorded.

Part 2: Bacterial population follow-up during a 2-month use

Four breathing systems were assigned to four different areas in the hospital and used for 2 months under routine conditions (without cleaning or sterilization). PSS of each were swabbed once every 2 weeks on a Monday morning. The cumulative anaesthesia time and the number of connections were recorded during this period. Quantitative bacteriology (positive culture result, number of CFU, number of different species) and bacterial identification were performed for a total of five samples per breathing system over the 2-month period.

Part 3: Survival time of oro-pharyngeal bacteria in an anaesthesia breathing system

Four pairs of culture swabs were taken from the mouths of two different animals. The sampling time and the technique of collection (rolling the swab at the oro-pharyngeal junction) were standardized. The first swab of each pair was rolled against the internal surface of the Y-piece of four sterile breathing systems for inoculation. The identical swab of the pair was directly cultured as a parallel control. At 1 minute, 1 hour and 1 day post-deposition, samples were taken from three distinct and clearly identified areas of the same PSS. The number of CFU at these times, were compared to the parallel control colony count.

Quantitative/qualitative microbiological techniques

Culture swabs were inoculated onto nonselective media (Columbia Sheep Blood Agar), and selective media: Mannitol Salt Agar and MacConkey media (Oxoid Ltd, Basingstoke, Hampshire, UK). The deposit was streaked over the agar surface of each media with a sterile loop following the classical quadrant method (Barrow & Feltham 1993). The agar plates were incubated aerobically overnight at 37 °C. Bacteria were identified using standard techniques (Barrow & Feltham 1993). A sample was considered as negative if the agar plates were free of colonies after 48 hours' incubation. Possible fungal and mycobacterial growth were not investigated, nor were patients for respiratory tract infection.

Statistical analysis

Continuous data were reported as mean ± standard deviation (SD) for parametric data, and nonparametric data were reported as median and interquartile range. A normal distribution was verified with a Kolmogorov–Smirnov test for the number of connections (quantitative discrete variable). Categorical data were reported as proportions. When appropriate, continuous data were analysed with a *t*-test for independent samples (parametric) or a Wilcoxon test for paired samples (nonparametric). Cumulative anaesthesia time or number of connections was categorized for statistical analysis (see Table 1), respectively, in '0–55 minutes' and 'more than 55 minutes' and '1–3 connections' and 'more than three connections'. Chi square or Fischer's exact test was used for comparison of categorical data. Incidence of colonization (categorical data) at PSS and DSS was compared as paired observations for the same breathing system, therefore McNemar's *t*-test was used in this case. $p \leq 0.05$ was considered significant. SPSS software (SPSS for Windows, Version 12.0.1; SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Table 1 Culture results at proximal sampling site (PSS) according to cumulative anaesthesia time and number of connections

	Result of cultures at PSS			
	Negative (−)	**Positive (+)**	**Odds ratio (95% CI)**	***p*-value**
Cumulative anaesthesia time (minutes)				
0–55	11	10	0.66[a] (0.17–2.48)	0.54*
56–160	10	6		
Global	55.4 ± 34	57.9 ± 24		0.8**
Number of connections (times)				
1–3	11	9	0.86[b] (0.21–3.16)	0.81***
4–8	10	7		
Global	3.8 ± 1.4	3.6 ± 1.0		0.75 ****

[a]Odds ratio of positive result with longer anaesthesia time. [b]Odds ratio for positive result with higher number of connection. *Chi-square after categorization, no difference of result within different time groups. **Independent sample *t*-test, no difference in anaesthesia time within different culture groups. ***Chi-square after categorization, no difference of result within different connection groups. ****Independent sample *t*-test, no difference in number of connection within different culture groups.

Results

Sterility controls

No organisms were cultured from any of the PSS of the 21 control breathing systems after sterilization.

Part 1: Breathing system colonization after 1 day of use

Swabs were collected from the PSS and DSS of 37 different breathing systems. Culture results at PSS are summarized in Table 1 according to cumulative anaesthesia time and number of connections. Table 2 compares positive culture results, number of CFUs, cumulative anaesthesia time and number of connections at proximal and DSS. Culture results were positive in 19 of 74 samples.

Bacterial species identified

Between one and six CFUs were identified for each positive sample, except for one with 30 colonies. In all but two cultures, colonies were identified as Gram-positive, catalase-positive cocci. They were either coagulase-negative staphylococci (CNS) or *Micrococcus* sp. Large aerobic Gram-positive spore-forming rods were found on two samples (*Bacillus* sp.).

Importance of sampling site

Sixteen positive cultures were obtained from PSS compared with only three at DSS. Two breathing systems had positive cultures at both sampling sites, while one system was positive at DSS only. These results are listed in Table 3. The frequency of positive culture at PSS and DSS was significantly different ($p < 0.001$). The relative difference between proximal and distal contamination was 35% (95% CI 14–55). Odds ratio for colonization at PSS *versus* DSS was 14 (95% CI 2.64–74.33).

Table 3 Pairing between culture results at proximal sampling site (PSS) and distal sampling site (DSS)

	Culture result at DSS		
	Negative	**Positive**	**Total**
Culture result at PSS			
Negative	20	1*	21
Positive	14*	2	16
Total	34	3	

*McNemar's test on discordant pairs, $p < 0.001$, difference in proportion 35 ± 20.5% (95% CI).

Importance of connection time and number of connections

Cumulative anaesthesia time was not significantly different in the positive culture group (57.9 ± 24.37 minutes) compared with the negative culture group (55.4 ± 34.53 minutes) ($p = 0.8$). No association was found between positive bacterial result and cumulative anaesthesia time (odds ratio 0.66, 95% CI 0.17–2.48, $p = 0.54$).

The mean number of connections was not significantly different between positive culture (3.6 ± 1.0) and negative culture (3.8 ± 1.4) ($p = 0.75$). Similarly, no association was found between positive bacterial result and number of connections (OR 0.86, 95% CI 0.2–3.2, $p = 0.81$).

Association between other factors

Table 4 represents the distribution of number of CFUs according to cumulative anaesthesia time

Table 2 Comparison of results at proximal sampling site and distal sampling site

	Sampling site		
	Proximal	**Distal**	**Odds ratio (95% CI)**
Positive culture results	16/37	3/37	14* (2.6–74.3)
Number of CFUs, median (interquartile range)	2.5 (1–6)	1 for each	
Cumulative anaesthesia time (minutes)	56.5 ± 30.2		Common to both sampling sites
Number of connections	3.7 ± 1.3		

CFU, colony-forming unit. *Odds ratio of proximal site *versus* distal site contamination.

Table 4 Comparison of number of colony-forming units (CFUs) at proximal sampling site according to anaesthesia time and number of connections

	Number of CFUs		
	1–3	**4–8**	***p*-value**
Cumulative anaesthesia time (min)			
0–55	4	6	0.14*
56–160	5	1	
Number of connections			
1–3	4	5	0.36**

*Fisher's exact test, no association between number of CFUs and longer anaesthesia time. **Fisher's exact test, no association between number of CFUs and higher number of connections.

and number of categories of connections. No association was identified between number of CFUs and number of connections (Fisher's exact test 0.358) or cumulative anaesthesia time (Fisher's exact test 0.145).

Distal sampling site

Three of the 37 samples collected at DSS were positive. Their cumulative anaesthesia time was 79, 85, and 89 minutes, *versus* a mean time of 54.1 ± 30.2 minutes for the 34 negative cultures. Their number of connections were 3, 7 and 7. However, the sample size was too small to allow statistical analysis.

Part 2: Bacterial population follow-up during a 2-month use

Table 5 summarizes the cumulative anaesthesia time and the total number of connections over 2 months. Table 5 also indicates the week when a sample culture was positive and the result of the bacterial identification. Figure 2 represents the number of CFUs collected from the breathing systems during the 2-month trial period.

There was no association between bacterial populations isolated consecutively from the same breathing system (Fig. 2). A macroscopic contamination of the inner surface of the Y-piece of 'system 3' was noticed during the sample at week 4. One colony of oxidase-positive Gram-negative cocci and one colony of oxidase-negative Gram-negative cocci were identified from this sample. The breathing system continued in routine use for the rest of the study and no further colonies were isolated.

Part 3: Survival time of oro-pharyngeal bacteria in an anaesthesia breathing system

The number of CFUs isolated in the parallel positive controls and over time is represented in Fig. 3. The bacterial population found on oral sampling was typical of normal aerobic canine oro-pharyngeal flora, predominantly *Pasteurella* sp., coagulase-positive and -negative staphylococci, alpha-haemolytic *Streptococcus* sp. and *Moraxella* sp. Bacteria that survived at 1 minute or more in the breathing system were not recognized pathogens except in one case: 20

Table 5 Results for different breathing systems during part 2 of the experiment

	Cumulative anaesthesia time (minutes)	**Number of connections**	**Time of positive sample**	**Identification**
System 1	911	21	Week 8	1 colony CNS
System 2	615	24	Week 4	1 colony CNS
			Week 10	1 colony CNS
System 3	1087	24	Week 2	1 colony CNS + 1 colony diphteroid
			Week 4	1 colony oxidase + Gram-negative cocci + 1 colony oxidase – Gram-negative cocci
System 4	1065	37	Week 6	4 colonies CNS
			Week 10	4 colonies CNS

CNS, coagulase-negative staphylococci.

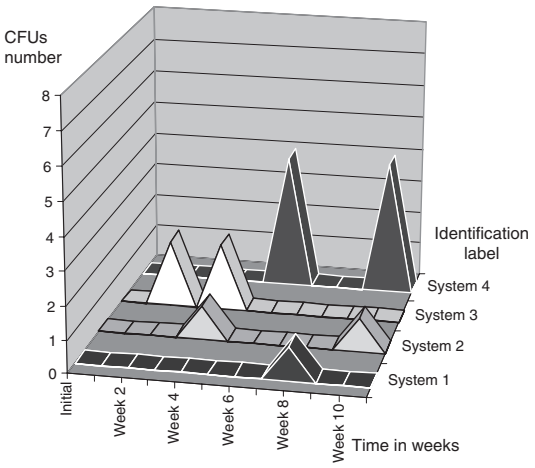

Figure 2 Number of colony-forming units (CFUs) isolated every 2 weeks, over 2 months in part 2 of the experiment. *x*-axis: time in week; *y*-axis: number of CFUs; *z*-axis: identification label of individual breathing system.

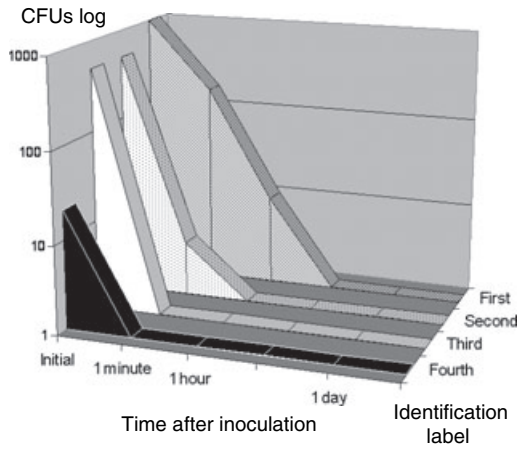

Figure 3 Number of colony-forming units (CFUs) isolated on positive control and over the time (in part 3 of the experiment).

colonies confirmed as *Staphylococcus intermedius* were cultured on the control sample of the first breathing system. Only one colony of *S. intermedius* was identified from the same breathing system at 1 minute, but none at 1 hour. The number of CFUs isolated from the breathing system after 1 minute was significantly lower than that in the parallel control sample ($p = 0.042$). Between 83% and 100% of the colonies of the positive control lost their viability at 1 minute. Bacteria were cultured from only one breathing system at 1 hour and from none at 1 day.

Discussion

Collection technique

In part 1 and part 2 of the study, the growth of bacteria isolated from anaesthesia apparatus was minimal. Swab collection, as described in previous protocols (Du Moulin & Saubermann 1977; Bengtson et al. 1989; Rathgeber et al. 1997) is considered to be less sensitive than a broth extraction technique (George 1975). In the latter, a nutrient broth is poured into the tubing, collected and directly cultured. One possible criticism of this study is that colonization might have been underestimated due to the low sensitivity of the swab collection technique on a dry surface. The use of a broth extraction technique would have made more likely detection of bacteria present in low number, 'detaching' them from possible biofilm formation.

Difference in collection sites

In part 1 of the study, PSS was found to be significantly more colonized than DSS ($p < 0.001$) and this result confirms the finding of Leijten et al. (1992) in which *Serratia marescens* was intentionally nebulized in anaesthesia breathing systems. The components that were the closest to the nebulizer showed the highest degree of contamination. The same trend was observed in clinical studies when volunteers with respiratory infection were asked to cough into sterile anaesthesia apparatus (Pandit et al. 1967).

In part 1, PSS and DSS were both colonized in 2 of 37 samples, but bacteria were only identified as CNS and were not recognized pathogens. The bacteria isolated could have been the same at both sites, suggesting possible airborne transport within the breathing system by the dry carrier gas. Nevertheless, in previous studies in which dogs' lungs were experimentally infected with *Pseudomonas aeruginosa* (Christopher et al. 1983), these animals expelled the organism into the breathing system after 24 hours of ventilation, but the migration distance was much shorter (not >60 cm) with dry gas than with continuous heated aerosol therapy (up to 450 cm).

Origin and pathogenicity of bacteria found

Bacterial flora identified in part 1 included *Bacillus* sp., coagulase-negative *Staphylococcus* sp. or *Micro-*

coccus sp. These organisms are environmental and skin saprophytes (Hogarth 1996). Due to their low pathogenicity, they are not likely to be causal agents of postoperative pneumonia in immunocompetent patients. In part 1 of the study, neither longer daily connection time, nor a higher number of patient connections increased the incidence of PSS colonization. This suggests that PSS colonization is independent of exposure to the patients' airways and supports the environmental origin of the flora isolated in breathing systems. Ibrahim & Perceval (1992) identified the same organisms whether breathing systems were connected during 4 hours to a patient or to the fresh gas only. Du Moulin & Saubermann (1977) isolated the same low-pathogenicity flora in small numbers from breathing systems exposed to healthy patients or exposed to other patients with Gram-negative bacillus pneumonia. The authors concluded that patients rarely contaminate anaesthetic apparatus with significant levels of bacteria, regardless of prior airway contamination and duration of anaesthesia.

Bacterial survival in anaesthesia breathing systems

Biofilms are bacterial populations enclosed in an extracellular matrix that adheres to a porous medium. The biofilm protects nonsporing bacteria from environmental changes, antibiotic or macrophage actions, and might contribute to the occurrence of ventilator-associated pneumonia (Inglis et al. 1989; Bauer et al. 2002). The second part of the study showed that the bacterial population does not build up in breathing systems over time, and no correlation between bacterial cultures isolated from sample $week_n$ to $week_{n+2}$ could be identified. Furthermore, the bacterial population isolated from a previously colonized apparatus was not found on the next sample, suggesting that these bacterial populations became nonviable between the two sampling times. The bacteria were environmental commensals except two Gram-negative cocci isolated from a visible deposit found in the Y-piece of 'system 3'. Oxidase-positive Gram-negative cocci (*Neisseria* sp. or *Moraxella* sp.) are potential respiratory nosocomial pathogens in the human respiratory tract (Boyce et al. 1985). Oxidase-negative Gram-negative cocci (as cultured from 'system 3') are generally not recognized pathogens, except *Acinetobacter* sp. (Cefai et al. 1990). Hirai (1991) demonstrated that some strains of *Acinetobacter* sp. were highly resistant to dry conditions and that presence of an animal protein substrate could prolong the survival of some enterobacteria already highly resistant to dry conditions. As this particular breathing system was used for bronchoscopy, the deposit observed might have come from some expectorated sputum containing bacteria of pulmonary origin and its presence might have promoted their survival. Following this incident, anaesthesia breathing systems were regularly inspected to detect the presence of mucoid material in the Y-piece or in the corrugations.

In part 3 of the current study, the number of colonies isolated from the breathing system after 1 minute was significantly lower than that for the parallel control sample ($p = 0.042$). CFUs were found in only one of the four breathing systems after 1 hour, and none after 1 day. Although our experimental population was limited, this result indicates that this breathing system is probably hostile to bacterial survival, although pathogens such as *S. intermedius* may still be viable for a short period of time.

When *S. aureus* aerosol was nebulized in circle anaesthetic circuits, some bacteria were still washed out from the circuit 30 minutes after gas flow interruption, but not after 1 hour (Langevin et al. 1999). In another experiment, *Enterobacter cloacae* were disappearing from the original inoculation point over a 3-hour period, yet could not be recovered downstream within the system during that time (Du Moulin & Saubermann 1977). This could have resulted from death or destruction of the organisms.

A combination of effects is probably responsible for bacterial death in anaesthetic apparatus. After disconnection from the patient, evaporation occurs because of changes in humidity, and this is generally accompanied by alterations in temperature. Sudden environmental changes severely affect bacterial metabolism, and Gram-negative bacteria are particularly sensitive to these changes (Du Moulin & Hedley-Whyte 1982). Furthermore, the bactericidal effect is magnified in the presence of high oxygen concentrations, especially for bacteria suspended in aerosols (Du Moulin & Hedley-Whyte 1982).

Rapid loss of bacterial viability under dry conditions could partially explain the low number of colonies isolated in part 1 of the study. Although the time between the last disconnection and sample collection was limited to 2 hours, bacterial load present in the initial inoculum might have been significantly reduced. After discontinuation of the flow of gases in a parallel-Lack circuit and

disconnection from the patient, there is likely to be a rapid change in the temperature and moisture content within the tubing. In contrast, in a circle system, there may be a more stable environment in the tubing for up to 1 hour post-disconnection (Langevin et al. 1999). The luminal surface of the tubing of a circle circuit may therefore provide a more suitable sampling site for the bacterial culture.

Conclusion

The findings of this study, coupled with those reported in the medical literature, suggest that sterile anaesthetic apparatus become colonized by environmental micro-organisms of low pathogenicity. The colonization flora that may be cultured from the apparatus after exposure to a patient or the environment is unlikely to be dangerous for the subsequent patient. Long-term survival of recognized pathogens in a dry environment is possible, although unlikely in the absence of biofilm.

Although low-virulence bacteria might be clinically relevant for the increasing population of immunosuppressed dogs and cats undergoing medical treatment and anaesthesia, a definite relationship between use of contaminated anaesthetic equipment and subsequent pulmonary infection remains to be established. Little is known as to whether bacteria or viruses expelled into the breathing system could easily be translocated from the wall of the tube to the respiratory tract of the patient. The potential for anaesthesia-related cross-contamination exists, but nosocomial infections rely on several other conditions (load of organisms expelled by one or several patients in the same breathing system, fresh gas flow, time between cases, virulence and survival capacity of the organism and susceptibility of the next patient). These results suggest that the routine use of a bacterial filter or a sterilized breathing system for veterinary anaesthesia cannot be supported by current evidence.

The use of bacterial filters may be justified between animals with potential respiratory infection, especially if the time between the two animals being attached to the same breathing system is less than an hour. Should a risk of viral cross-contamination risk exist (FIP, influenza), the breathing system should be discarded in the absence of reliable viral filters. The same policy should be applied if *Bordetella bronchiseptica*, *Cryptococcus neoformans* or *Mycobacterium bovis* infections are possible diagnoses.

A more realistic option would be to clean the parts directly exposed to the animal. The isolated Y-piece, as well as any equipment intercalated between the latter and the endotracheal tube (i.e. capnograph connector) could easily be individually sterilized once detached. As internal corrugations are extremely difficult to clean efficiently, the use of smooth bore tubing would enable efficient decontamination and improve the flow dynamics within the breathing system at the same time.

Acknowledgements

The authors wish to thank David Brodbelt for statistical advice and Maggie Bushnell for laboratory assistance.

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Received 21 March 2006; accepted 15 August 2006.