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Potential for residual contamination by *Streptococcus equi* subspp *equi* of endoscopes and twitches used in diagnosis of carriers of strangles

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Abstract

Background: Endoscopic examinations are essential for diagnosis and treatment of strangles (*S equi* infection) in horses. However, even after disinfection, endoscopes may retain viable bacteria or bacterial DNA. Twitches are commonly used during endoscopic examinations and can thus also potentially transmit the organism to other horses.

Objectives: To evaluate the efficacy of different disinfectant methods to eliminate *S equi* from experimentally contaminated endoscopes and twitches and the effectiveness of field disinfection of endoscopes used in sampling carriers of *S equi*.

Study design: Experimental contamination and observational field study.

Methods: One endoscope and 30 twitches were contaminated with standardised *S equi* broth solutions. The endoscope was disinfected following three protocols using various disinfectants for manual disinfection. A fourth protocol used an automated endoscope reprocessor (AER). The twitches (*n* = 30) were disinfected following eight different disinfecting protocols. Three endoscopes used in sampling for silent carriers were disinfected following a field-based protocol. After each protocol the endoscopes and twitches were sampled for *S equi* by culture and qPCR.

Results: Following experimental contamination all endoscope disinfection protocols, apart from 1/6 of the ethanol protocol were *S equi* culture negative. However, no endoscope disinfection protocol completely eliminated retention of *S equi* DNA. Field disinfection of endoscopes after sampling carriers yielded no culture positives and all but one (13/14) were qPCR negative. All twitches disinfected following experimental contamination were culture negative but sodium hypochlorite was the only disinfectant that completely eliminated detection of *S equi* DNA.

Main limitations: Experimental contamination may not reflect the numbers of *S equi* transferred to endoscopes or twitches during use on silent carriers and purulent secretions from infected horses may influence survival of *S equi*.

Conclusions: While most disinfection methods appear to ensure removal of cultivable *S equi*, residual DNA can remain on both endoscopes and twitches.

KEYWORDS

horse, cleaning, disinfection, equipment

1 | INTRODUCTION

Streptococcus equi subspecies *equi* (*S equi*) causes the highly contagious respiratory disease strangles in horses. A key reason for continuation of strangles outbreaks is the occurrence of long-term silent carriers, where viable *S equi* remains in the guttural pouches.[1] Guttural pouch sampling requires use of endoscope to collect the diagnostic samples for culture and/or qPCR.[2] However, after sampling silent carriers, endoscopes may remain contaminated with viable *S equi* bacteria or its DNA, with risk of the endoscope being a fomite for spread of strangles or contributing to false positive diagnosis for strangles when relying solely on qPCR. Additionally, since nose twitches are often used for restraint during guttural pouch endoscopy on horses, they are also potential fomites for retention of *S equi*.

Endoscopes are well-known potential fomites for transmission of infectious disease in human medicine, reportedly due to inadequate protocols or noncompliance for manual cleaning and disinfection of endoscopes, or improper use of automated endoscope reprocessors (AER).[3,4] However, even with use of reprocessing procedures more rigorous than the industry standards positive post disinfection samples are reported.[5] In veterinary medicine the potential for an endoscope to act as a fomite for infectious disease has received little attention, apart from its role in an outbreak of rhinitis due to equine herpes virus type 3.[6]

Guidelines for cleaning of endoscopes have been only scantly described in veterinary literature[7] and even lack coverage in more recent equine hospital literature.[8] It is likely that protocols to clean and disinfect equine endoscopes vary, depending on whether the procedure is performed in the field or within an equine hospital, and on availability of specialised equipment such as access to a commercial AER.

There are no studies that identify the effect of different cleaning protocols for equine endoscopes following diagnostic procedures on strangles affect the likelihood of these endoscopes retaining viable *S equi* or its DNA. This study aimed to assess whether commonly used cleaning and disinfection methods were effective in removing viable *S equi* and/or its DNA from endoscopes as well as twitches experimentally contaminated with live *S equi*. Secondly, we assessed whether endoscopes used on known *S equi* silent carriers and subjected to field-based cleaning and disinfection would have residual presence of either viable *S equi* or their DNA.

2 | MATERIALS AND METHODS

2.1 | Experimental study endoscopes

One endoscope (VMEC-92, Genuine Medica Pvt. Ltd) was contaminated with a standardised *S equi* 24 hours broth solution with a concentration between 4.1×10^8 and 6.5×10^8 CFU/mL and subsequently cleaned and disinfected following four different protocols (Table 1) with six replicates in each group. Before each contamination a leak test as per manufacturer's instructions was performed to ensure the endoscope was watertight. The four chosen methods for cleaning and disinfection of the endoscope are common protocols used by practicing veterinarians and veterinary nurses in horse clinics in our region.

2.1.1 | Contamination

A 20 hours culture broth solutions of *S equi* was prepared by adding two colonies of *S equi* strain (CCUG 23255 *Streptococcus equi* subsp. *equi* ATCC 33398) into 250 mL brain heart infusion (BHIB, CM 1135, Thermo Fisher Scientific) and incubated at 37°C. The

TABLE 1 Culture and qPCR results from endoscopes subjected to experimental *S equi* broth culture contamination (4.1×10^8-6.5×10^8 CFU/mL) followed by four differing methods of cleaning and disinfection

Endoscope samples	Cleaning and disinfection method	Culture positive	qPCR positive
Group A	Ethanol[a]	1/6[a]	6/6[b]
Group B	2-aminoethanol and didecyldimethylammoniumchloride disinfectant[b]	0/6[a]	5/6[b]
Group C	Ortho-phthalaldehyde disinfectant[c]	0/6[a]	2/6[b]
Group D	Automatic Endoscope Reprocessor, acetic acid disinfectant	0/6[a]	1/6[b]

Note: Group A vs B vs C vs D: [a]Culture P_B = .25, [b]qPCR P_B = .003. Freeman-Halton extension of the Fisher exact probability test where P_B = probability that the null hypothesis holds.
[a]U-sprit 70%, CCS Healthcare A/S.
[b]Everbrite Super, NCH Europe Inc.
[c]Cidex® OPA, Johnson & Johnson AB.
[d]APERLAN Poka-Yoke Agent A and Agent B, Getinge Group.

bacterial growth in the broth solution was ranging from 4.1 to 6.5×10^8 colony forming units per mL. The distal 7 cm of the tip of the insertion tube of the endoscope was immersed for 10 minutes in a vial containing 10 mL of the *S equi* broth solution. After the contamination the tip of the endoscope was dried manually with clean paper and the working channel flushed with 50 mL of room air.

2.1.2 | Cleaning and disinfection

Group A

This protocol represents commonly used protocol in the field practice in Sweden for endoscope cleaning between patients when infectious disease is not suspected. The air/water and the suction valves were removed from the endoscope and cleaned with ethanol (U-sprit 70%, CCS Healthcare A/S) and clean paper. The external surface of the endoscope was first cleaned manually with soft paper moistened with ethanol, then 50 mL of ethanol was flushed through the biopsy channel followed by flushing with 100 mL of distilled H_2O and finally with 150 mL of room air. The external surface of the endoscope and the air/water and suction valves were then rinsed with distilled water and dried manually with clean paper.

Group B

This protocol represents commonly used protocol in the field practice in Sweden for endoscope cleaning between patients when infectious disease is suspected. The external surface of the endoscope and the air/water and suction valves were manually cleaned with 2-aminoethanole and didecyldimethylammoniumchloride disinfectant (Everbrite Super, NCH Europe Inc.) and clean paper, and 20 mL of this solution was flushed through the biopsy channel. A single use channel cleaning brush with bristles on both ends (Scope Glide Scope Brush, Scandivet AB) was passed through the biopsy channel. The water bottle connected to the endoscope for flushing of the optical lens was filled with 50 mL of didecyldimethylammoniumchloride disinfectant and the air/water valve was activated until all the solution was flushed through the channel, after which the procedure was repeated using 50 mL distilled water. The biopsy channel was then flushed with 100 mL of distilled water followed by 150 mL of room air. The external surface of the endoscope and the air/water and suction valves were rinsed with distilled water and then dried manually with clean paper.

Group C

This protocol represents current cleaning and disinfection according to the University Equine Hospital in Uppsala protocol for endoscope cleaning between patients when infectious disease is suspected but in the absence of a commercial AER. The water bottle connected to the endoscope was filled with distilled water and the air/water valve was pressed until air and approximately 10 mL of the water in the bottle was passed through the flushing channel. The external surface of the endoscope and the air/water and suction valves were manually cleaned with isopropyl

alcohol containing surfactant (DAX Surface Disinfection Plus, CCS Healthcare A/S) and clean paper. The endoscope was then immersed in a water bath at 37°C, the air/water and the suction valves removed and two cleaning caps were connected to the endoscope. The flushing and suction channels were then flushed with 100 mL of an enzymatic cleaner diluted as per manufacturer´s instructions (5 mL of enzymatic cleaner (Cidezyme®, Johnson & Johnson AB) mixed with 500 mL of distilled water) and an additional 100 mL of this solution was flushed through the biopsy channel. A single used channel cleaning brush was passed through the biopsy channel and the suction channel twice. The endoscope was then removed from the water bath and the irrigation and suction channel and biopsy channel were each flushed with 100 mL of ortho-phthalaldehyde disinfectant (Cidex® OPA, Johnson & Johnson AB). After this the endoscope was immersed in a fluid bath containing ortho-phthalaldehyde disinfectant for 15 minutes after which it was removed. All channels were then flushed with 100 mL of distilled water followed by 150 mL of room air. The external surface of the endoscope and the air/water and suction valves were rinsed with distilled water and then dried manually with clean paper.

Group D

This protocol represents cleaning and disinfection with a commercial automated endoscope reprocessor (AER) when infectious disease *is* suspected. The external surface of the endoscope was precleaned manually with isopropyl alcohol with surfactant (DAX Surface Disinfection 45+, CCS Healthcare A/S) and clean paper after which 50 mL of distilled water was flushed through the biopsy channel. As for group C a single use channel cleaning brush was passed twice through biopsy channel and suction channel. The endoscope was placed in an AER (Poka Yoke AER, Getinge Group) and a standard cleaning and disinfection program was completed according to the manufacturer´s instructions with per acetic acid used as disinfectant (APERLAN Poka-Yoke Agent A and Agent B, Getinge Group). Each of the above four cleaning procedures were conducted with 6 replicates.

2.1.3 | Sampling

The sampling procedures were performed immediately after the disinfection without drying period. Endoscope wash samples were obtained by flushing 50 mL distilled water through the biopsy channel of the endoscope, the flush fluid collected in a sterile tube and 9.5 cm of the distal tip of the endoscope briefly immersed in the collected fluid. The sampling procedures were performed in the same room as the contamination of the endoscope but in a separate area with an approximately 100 cm high wall separating the different areas. The person performing the sampling was wearing disposable protective clothes and plastic gloves. For group D (cleaning and disinfection with AER) the sampling procedure was performed in the room where the AER

was located which was separated from the room where the contaminations were performed. All endoscope wash samples were analysed similarly by bacterial culture for detection of *S equi* in endoscopes after disinfection. In the laboratory, the fluid in the 50 mL plastic tubes was centrifuged at 3320 *g* for 15 minutes. One µl of the pellets was collected and plated on COBA plates (agar plates with 5% defibrinated horse blood, supplemented with colistin acid and oxalinic acid). All culture plates were then incubated in 5% CO_2 atmosphere in 37°C and resulting numbers of CFU of *S equi* quantified after 24 and 48 hours. One colony was selected from each group and analysed with matrix-assisted laser desorption ionisation-time of flight mass spectrometry MALDI-TOF (Bruker Nederland BV, Leiderdorp) for microbiological identification. In addition, all samples were analysed for the presence of *S equi* by real-time PCR using primers and probes developed by Båverud et al[9] and the same procedure for the PCR-analysis as Frosth et al.[10]

2.2 | Field study endoscopes

In the field study a group of 38 Icelandic horses were sampled after full clinical recovery from a strangles outbreak, in which 14 horses were identified as *S equi* carriers by nasopharyngeal lavage followed by endoscopic lavage of both guttural pouches.[11] Three endoscopes were used to examine 38 horses (8, 14 and 16 horses, respectively,

were examined with each scope). After each horse was examined and underwent guttural pouch lavage, the endoscope was manually cleaned externally with distilled water and clean paper followed by flushing the biopsy channel with distilled water (60 mL) and by immersion of the insertion tube in a shallow fluid bath containing ortho-phthalaldehyde disinfectant for at least 20 minutes with the working channel filled with the same solution. The endoscope was then removed from the fluid bath and rinsed with distilled water, followed by flushing of the working channel with 60 mL of distilled water. Next, samples for evaluation of the endoscopes were obtained by flushing the biopsy channel with 50 mL sterile distilled water and collected into a sterile plastic tube and stored at 4°C until analysis. The distal tip of the endoscope was not immersed in the sample tube during the sampling procedure but was in contact with the collected fluid. The sampling procedure was performed in the stable environment during which the ambient temperature was between 1 and 4°C, the procedure was performed immediately after the disinfection without drying period. The endoscope samples from those horses identified as carriers of *S equi*, were analysed the next day by bacterial culture as described earlier and qPCR[9] for detection of *S equi*.

2.3 | Experimental study twitches

Plastic handles of twitches were contaminated with standardised *S equi* broth solution and cleaned and disinfected following

TABLE 2 Culture and qPCR results for twitches subjected to experimental contamination of broth culture of *S equi* followed by differing cleaning and disinfection protocols

	Contaminating S equi solution (CFU/mL)	Cleaning method	Disinfection method	Culture positive samples	qPCR positive samples
Disinfection					
Plastic handles	1.1×10^9	Surfactant-based cleaner[a]	Potassium monopersulphate[c]	0/6[a]	5/6[b]
Plastic handles	1.1×10^9	Surfactant-based cleaner[a]	Potassium peroxymonosulphate[d]	0/6[a]	5/6[b]
Plastic handles	2.5×10^8	Surfactant-based cleaner[a]	Boiling water, 10 min	0/6[a]	6/6[b]
Plastic handles	2.5×10^8	Enzymatic cleaner[b]	Ortho-phthalaldehyde[e]	0/6[a]	6/6[b]
Plastic handles	Not analysed	Surfactant-based cleaner[a]	Sodium hypochlorite solution[f]	0/6	0/6
Cleaning					
Plastic handles	1.1×10^9	Surfactant-based cleaner[a]	None	4/6	2/2
Cotton ropes	1.1×10^9	Surfactant-based cleaner[a]	None	6/6	nd
Controls					
Plastic handles	1.1×10^9	None	None	6/6	nd
Cotton ropes	1.1×10^9	None	None	6/6	nd

Note: Plastic handle cleaning and disinfection methods (excluding sodium hypochlorite): [a]Culture $P_B = 1.0$, [b]qPCR $P_B = .35$, Freeman-Halton extension of the Fisher exact probability test where P_B = probability that the null hypothesis holds.

[a]Yes Original, Procter & Gamble, Sweden AB.

[b]Cidezyme®, Johnson & Johnson AB.

[c]DesiDos™, SeptiChem Asp.

[d]Virkon® S, DuPont/Antec Int. Ltd.

[e]Cidex® OPA, Johnson & Johnson AB.

[f]Klorin™, Colgate-Palmolive Company.

protocols that likely represent various common cleaning practices (Table 2). Plastic handles and cotton ropes from twitches were also cleaned without disinfection and sampled after contamination without neither cleaning nor disinfection. The plastic handles of the twitches were used in veterinary field practices prior to the experiment thus ensuring clinically relevant surface wear. Before each contamination the plastic handles and the cotton ropes were cleaned with a tensid-based detergent (Yes Original, Procter & Gamble) and disinfected in a bath with a disinfectant containing potassium monopersulphate (DesiDos[TM], SeptiChem) for 10 minutes. The plastic handles were used for one or several contamination trials but the cotton ropes were only used for one contamination trial each.

2.3.1 | Contamination

Each plastic handle and/or cotton rope was immersed in 5 mL of *S equi* solution (as described earlier, see also Table 2) in a plastic bag for 30 minutes.

2.3.2 | Cleaning and disinfection

The plastic handles were cleaned and disinfected following different protocols with six plastic handles in each group, plastic handles and cotton ropes were also cleaned without disinfection (Table 2). In total 30 plastic handles were used in the study, some of which were reused if tested *S equi* negative. In the protocols where cleaning with surfactant-based detergent were included the twitches were cleaned manually with the detergent and lukewarm water. When enzymatic cleaner was used for cleaning, the twitches were immersed briefly in a plastic bucket containing enzymatic cleaner diluted as per manufacturer´s instructions (5 mL of enzymatic cleaner (Cidezyme[®], Johnson & Johnson AB) mixed in 500 mL of distilled water), followed by 10 minutes immersion in the various disinfection solutions or in boiling water. For detergents and disinfectants used, see Table 2.

2.3.3 | Sampling

Each plastic handle or cotton rope was placed in a plastic bag after disinfection and/or cleaning, the plastic bag was then filled with 50 mL of tap water. A bacterial swab (ESwab[®], Copan), was immersed in the water and rolled against the surface of the plastic handle or cotton rope for one minute. Plastic handles and cotton ropes from twitches were also sampled in the same way after contamination without either cleaning nor disinfection as controls. The sampling procedures were performed in the same room as the contamination with use of disposable protective clothing and single use plastic gloves. Fluid from the bacterial swab was plated on COBA plates and the samples were analysed similarly by bacterial culture as

described earlier and qPCR[9] for detection of *S equi* on twitches after cleaning and disinfection.

3 | RESULTS

3.1 | Experimental study endoscopes

After cleaning and disinfection only one single endoscope sample in the cleaning group representing field-based cleaning (group A) was *S equi* culture positive. However, while there were significant differences among cleaning and disinfection methods (P_B = .003), some or all of the endoscope samples post cleaning and disinfection were qPCR positive to *S equi*, including 6/6 in group A, 5/6 in group B, 2/6 in group C and 1/6 in group D (Table 1).

3.2 | Field study endoscopes

In the field study, of the 14 horses identified as *S equi* carriers, seven horses were qPCR positive only in guttural pouch lavages, five only in nasopharyngeal lavages and two horses were positive in both nasopharyngeal and guttural pouch lavages (data not shown). Bacterial cultures were positive for *S equi* in five horses in guttural pouch lavage samples (all also qPCR positive). There were no positive bacterial cultures from the endoscopes after disinfection (0/14) but one sample obtained from endoscopes used to obtain guttural pouch lavage samples from the 14 silent carriers was positive to *S equi* DNA on qPCR. The positive qPCR endoscope sample was obtained from one of the horses which were positive for both bacterial culture and qPCR.

3.3 | Experimental study twitches

Viable *S equi* was isolated from plastic handles and cotton ropes from twitches that were contaminated but not cleaned nor disinfected and after cleaning without disinfection (plastic handles and cotton ropes) but from the plastic handles that underwent cleaning and disinfection there were no positive bacterial cultures. However, except with sodium hypochlorite, the plastic handles of twitches remained positive to *S equi* on qPCR for all other disinfectants, which did not differ in eliminating residual DNA (P_B = .35; Table 2).

4 | DISCUSSION

In the experimental study the efficacy of four different disinfectant methods to eliminate *S. equi* bacteria from an endoscope demonstrated that three of the four disinfection methods were effective to eliminate live bacteria from the endoscope, which means the risk of spreading viable bacteria to new horses is low. Even in a field study with examination of naturally infected horses

no live bacteria were found after cleaning and disinfection of the endoscope. The only method where viable bacteria were detected was from a single endoscope in the experimental contamination study after disinfection using only ethanol. This method is used in predominately clinical situations where an infectious disease is not suspected. However, silent carriers of strangles can be intermittently culture positive for *S equi* without any clinical signs of strangles or visual purulence in pharynx or guttural pouches[11] and choice of disinfection protocol is selected without prior knowledge of strangles status. It is also possible that some of the bacterial culture from the endoscopes or twitches were falsely negative and viable bacteria were present but the cultivability impaired. Nonetheless, silent carriers likely shed viable *S equi* in far fewer numbers than in our experimental contamination protocol.

On the other hand, none of the four methods in the experimental study nor the field method ensured complete elimination of bacterial DNA as analysed by qPCR. Most effective in experimental study was reprocessing in the AER where five of six samples were qPCR negative. Special automated reprocessors used for cleaning and disinfection of endoscopes are standard in human medicine but are still uncommon in veterinary medicine. Even if an AER is used the manual precleaning procedure is still necessary and the AER cannot practically replace the manual cleaning and disinfection of endoscopes under field conditions, which makes the knowledge of effective methods important. The second most effective method in the experimental study was the method used for group C (pre cleaning of the endoscope with isopropyl alcohol, cleaning with enzymatic cleaning and disinfection with ortho-phtalaldehyde). This method was the most time consuming of the manual cleaning methods but is used by horse clinics in this region and is feasible under field conditions. All of the tested disinfection protocols were effective to eliminate cultivable bacteria from the plastic handles from twitches. However, cleaning with the surfactant-based cleaner only without disinfection failed to eliminate viable *S equi* from plastic handles or cotton ropes. On the other hand, only disinfection of the plastic handles with sodium hypochlorite effectively eliminated *S equi* DNA.

A concentrated bacterial solution was used to contaminate the endoscope and the twitches in the experimental study. We expect the concentration of *S equi* in this solution was far higher than in purulent secretions from horses with strangles. This can suggest that the cleaning and disinfection protocols used in this study could be more effective in a clinical situation with examination of horses with strangles. On the other hand, purulent secretions from a horse is more viscous than the thin solution used for contamination and therefore likely more difficult to eliminate from a surface. The endoscope used in the experimental study was new, and thus free from small injuries and biofilm from earlier examinations that potentially could make it easier to sanitise. However, the three endoscopes used in the field study were older and had been used in several examinations previously and the twitches had been used in clinical situations prior to the use in this experiment.

The endoscopes and the twitches were sampled just once after each cleaning and disinfection procedure which is a risk for false-negative results. Serial sampling after each procedure would be ideal to avoid this risk. There is also a risk for false positives due to re-contamination after disinfection or contamination in the sampling procedure. To minimise this risk in the experimental study the sampling of the endoscope was performed in an area separate from the area where the contamination and the cleaning of the endoscope were performed and the person performing the sampling wore protective clothing and plastic gloves. In the field study endoscope sampling was performed in the stable environment where the horses were examined.

The method used for cleaning the endoscopes in the field study was similar to the method in group C for the experimental study with the exceptions that enzymatic cleaner was not used and no channel cleaning brush was used. The time for the endoscopes exposed for the disinfectant was slightly longer in the field study than in the experimental study (20 minutes vs 15 minutes). Nonetheless only one of 14 endoscope samples was qPCR positive for *S equi* and none was positive at culture. This further supports the thesis that the challenge in the experimental study was tougher than in a true field situation with *S equi* positive convalescent horses. Many silent carriers of strangles have negative bacterial cultures even if they are qPCR positive for *S equi*.[11] As such, in the 14 horses in this study identified as *S equi* carriers only five had a positive bacterial culture, the other nine horses were only qPCR positive. Thus the risk for culture positive endoscope samples was only based on a small number of proven carriers. Indeed, the risk for contamination of the endoscopes from environment after reprocessing may have been greater in the field study because the sampling was performed in the stable in close proximity to known carriers. It would thus be of interest to perform a field study with the cleaning and disinfection method used in group C, with ortho-phtalaldehyde as disinfectant and cleaning with enzymatic cleaner and channel brush, to investigate whether that specific protocol effectively eliminates *S equi* DNA in a field situation with naturally infected horses. Alternatively, purulent secretions from infected horses may be a more appropriate challenge instead of the bacterial solution used for contamination of endoscopes prior to cleaning and disinfection in our experimental study.

To minimise the risk of transmission of live bacteria between animals, it is essential to both clean and also disinfect twitch handles and to use a new twitch rope for each animal. In this study, sections of twitch ropes contaminated with *S equi* solution were positive for bacterial culture even after cleaning with surfactant-based cleaner. If a nasopharyngeal lavage or endoscopy is performed for a sampling procedure, sodium hypochlorite is shown to be effective to eliminate DNA from the twitch. However, this disinfectant has the disadvantage that it is cytotoxic and toxic to aquatic organisms.[12-14] Other options to minimise the risk of twitches contaminating samples are to perform the nasal lavage and endoscopic examination without the use of a twitch and instead use sedatives if necessary, or to use a new twitch for each horse for those occasions. It appears that even routine cleaning and disinfection of endoscopes and twitch handles can effectively eliminate cultivable *S equi* from their surfaces. However, when using qPCR as a method for detecting silent carriers

of strangles both endoscopes and twitches can be a source of a false positive for the presence of *S equi* if attention is not given to appropriate cleaning and disinfection methods.

ETHICAL ANIMAL RESEARCH

The study was approved by Uppsala Animal Ethical Committee, Uppsala, Sweden, diary nr C 36/14.

OWNER INFORMED CONSENT

Not applicable.

DATA ACCESSIBILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

E. Svonni, M. Andreasson, A. Rydén, M. Riihimaki and J. Pringle contributed to the study design, study execution, data analysis and interpretation and to the preparation of the manuscript. L.-L. Fernström contributed to the data analysis and interpretation, and the preparation of the manuscript. All authors approved the final manuscript.

CONFLICT OF INTEREST

No competing interests have been declared.

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