A simple technique for tracheal culture to detect respiratory pathogens in live pigs

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Isolation of respiratory colonizers such as *Streptococcus suis* and *Haemophilus parasuis* from the upper respiratory tract of naturally infected animals is very difficult, because oropharyngeal swabs usually get contaminated with common inhabitants of the normal flora. The fastidious growth requirements of bacteria from the *Haemophilus* spp. underscores the importance of performing a careful bacteriological study. Selective substances in the growth media used for isolation are usually helpful, but it has been shown that overgrowth of contaminants can completely prevent isolation of *Haemophilus* spp.[1]

In our experience, a successful bacteriological identification of respiratory colonizers depends on obtaining a good-quality sample after the piglet has been immobilized. A very convenient approach is to administer an anesthetic to relax the animal and facilitate swabbing the tonsil or upper trachea. In an effort to follow the dynamics of mucosal colonization by *H. parasuis*, we developed a technique that allows the isolation of organisms that colonize the tonsil and upper respiratory tract of swine.[2]

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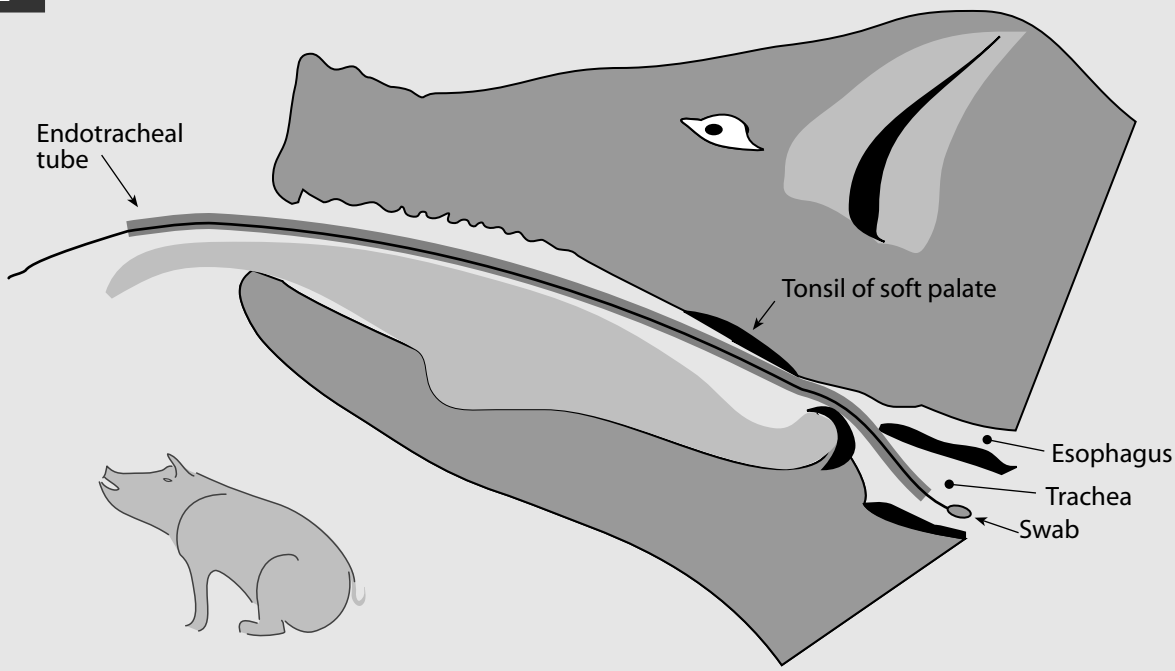
Materials and methods

The piglets from which samples have been taken included animals from different ages, as early as 1 day old. Administer a combination of 2 mL Xylazine (Rompum® 100 mg per mL) mixed in a 10-mL bottle of Ketamine hydrochloride (Ketaset® 100 mg per mL) intramuscularly to small piglets (1–15 days old) at a dose between 0.1 mL to 0.3 mL, according to their size. After 10 minutes, place the pig in a sitting position, extending the neck toward the front in order to facilitate visualization of the internal structures. After gently retracting the tongue, introduce a laryngoscope (Jorvet 80 mm blade) into the oropharyngeal cavity. Position the tube above the tip of the epiglottis as the reference point for the intubation. Introduce a noncuffed endotracheal tube (Sheridan® 3.0 mm) containing a flexible aluminum swab approximately 1 inch (3 cm) into the trachea (Figure 1). Once the endotracheal tube is secured, unsheath the swab into the tracheal lumen and gently swab the wall of the trachea. In order to avoid contamination, immediately pull the swab pack into its original sheathed position (inside the tube) before withdrawing the endotracheal tube. Once outside the animal, remove the swab and introduce it directly into a tube with culture media (e.g., Frey *Mycoplasma* broth supplemented with

Figure 1



Insertion of swab into trachea.

20% horse serum and nicotinamide adenine dinucleotide [0.16 mg per mL]). Grow the cultures overnight at 37°C in 5% CO_2 after performing five serial ten-fold dilutions from the original sample. Then plate the highest dilution tube where growth is present onto an appropriate selective media.

For most practitioners, when trying to isolate a pathogen such as *H. parasuis*, it has been found that samples taken from animals that have not been previously immobilized are usually contaminated. The effect of Xylazine and Ketamine combination at a low dose is adequate for performing simple diagnostic procedures. Recovery from this anesthetic combination is usually rapid, allowing the animals to be returned to their crates soon after the swab is taken. Most piglets are able to attain a standing position after 30 minutes. However, FDA approval of these anesthetics is currently under discussion.

References

1. Pijoan C, Morrison R, Hilley H. Dilution technique for isolation of *Haemophilus* from swine lungs collected at slaughter. *J Clin Microbiol*. 1983; 18:143–145.

2. Solano G, Rapp-Gabrielson V, Carvalho L, Collins J, Winkelman N, Pijoan C. The role of maternal immunity in *Haemophilus parasuis* infection. *Proc AASP Annual Meeting*. 1996; Nashville, Tennessee, pp. 433–436.