# Improving rate of success in isolating *Haemophilus parasuis* from clinical samples

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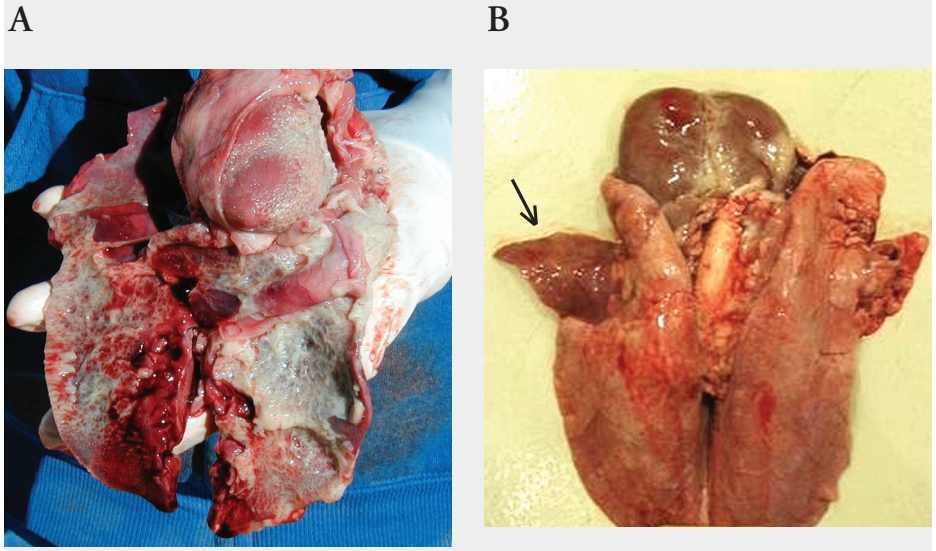
Isolation of *Haemophilus parasuis* from clinical samples is still a challenge for many veterinarians. Although this organism may be detected in clinical samples by polymerase chain reaction (PCR),[1] isolation is necessary for further characterization of isolates by serotyping[2–4] and genotyping.[5] Factors that may influence *H parasuis* isolation include selection of animals for sampling and handling of samples prior to submission to a diagnostic laboratory.[6,7] This article will describe in detail some important measures that may improve the chances of isolating *H parasuis* from clinical samples.

**Figure 1**: Systemic infection with *Haemophilus parasuis* is usually characterized by development of fibrinous polyserositis. However, some animals may develop only pneumonia. A: Fibrinous pleuritis and pericarditis in a field case; B: Pneumonia (arrow) after experimental infection.




## Selection of animals for sampling

*Haemophilus parasuis* is rarely isolated from dead pigs. In order to improve chances of isolation, untreated pigs showing clinical signs characteristic of acute infection should be euthanized and sampled. Although pigs showing respiratory distress (abdominal breathing, coughing) and swollen joints are the best candidates for sampling, pigs with central nervous system signs may also be considered.[6,7] It is important to differentiate acute from chronic lesions. Pigs that survive a nursery outbreak may develop clinical signs early in the finisher. These clinical signs may be associated with development of fibrosis in the thoracic and abdominal cavities. Isolation of *H parasuis* from chronically affected animals is usually unsuccessful.

## Sampling sites

Non-respiratory sites are preferred for *H parasuis* isolation. *Haemophilus parasuis* is a commensal organism of the upper respiratory tract and may be isolated from the nasal cavity, tonsil, and trachea of healthy animals.[8,9] Ideal sites for isolation are brain (meninges), pericardium, pleura, peritoneum, and joints. Lung tissue may be submitted when fibrinous pleuritis is observed (Figure 1A). In some cases, *H parasuis* induces only pneumonia (Figure 1B). Isolates recovered from pneumonic lungs may or may not represent the "problem" strain affecting the herd.[5,7] Although *H parasuis* causes septicemia and is expected to be present in the blood during acute infection,[10] isolation from blood samples collected from field cases is infrequent.

## Sampling procedures

Samples for *H parasuis* isolation may be collected with sterile swabs placed in transport systems containing Stuart or Amies media. The Amies system maintains viability of *H parasuis* better than the Stuart system.[11] Swabs should be collected from organs with fibrinous exudate on the surface (Figure 2A). Immunohistochemical studies have demonstrated that free *H parasuis* cells are usually concentrated in the fibrinous exudate.[12] Fluids from joints, peritoneum, pericardium, and thoracic cavity may be collected using sterile syringes (Figure 2B). *Haemophilus parasuis* survives longer in whole tissues than in swabs. Because different *H parasuis* strains may be isolated from different body sites in the same pig (Figure 3), tissue samples should be submitted in separate bags. Live animals may be sampled without the need for euthanasia. Synovial fluid, for example, may be collected from swollen joints by using a sterile syringe and needle. Samples should be refrigerated immediately after collection.

## Submission of samples for isolation

*Haemophilus parasuis* is temperature sensitive. Viable *H parasuis* organisms become

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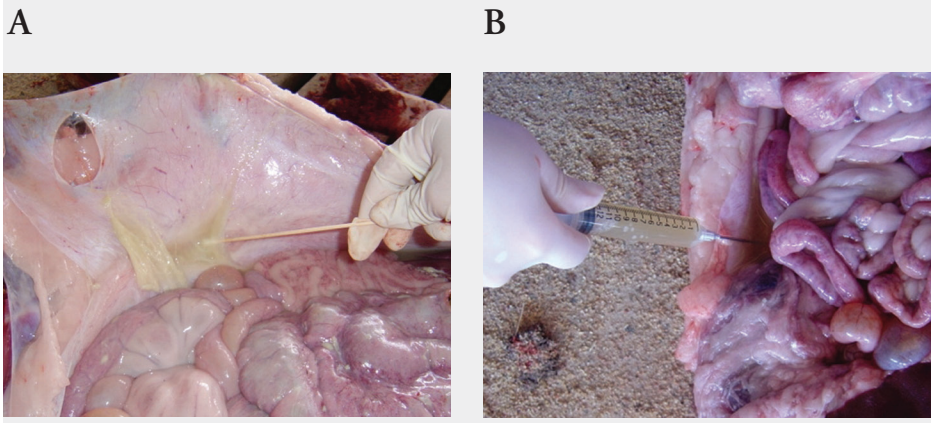
**Figure 2:** Samples for isolation of *Haemophilus parasuis* should be collected from organs showing fibrinous exudate on the surface. A: Sampling fibrinous exudate on the peritoneum using a sterile swab (field case); B: Collecting peritoneal fluid using a sterile syringe (field case).




undetectable in physiological saline at 42°C within 1 hour, at 37°C within 2 hours, and at 25°C within 8 hours.[13] At refrigeration temperature (4°C), however, *H parasuis* can survive for a relatively long time.[13,14] Samples collected for *H parasuis* isolation should be submitted to the diagnostic laboratory as soon as possible (1 to 2 days). Swabs, tissues samples, or syringes containing body fluids should be submitted in a Styrofoam container with ice packs. Tissues should be submitted fresh: the use of formalin is not necessary.

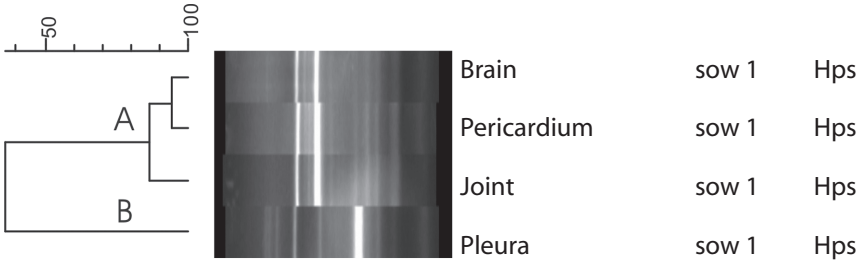
## Diagnostic tests

Isolation of *H parasuis* is important for accurate characterization of the prevalent strains causing disease in the herd. After biochemical identification, *H parasuis* isolates may be further characterized by serotyping[2–4] and genotyping[5] (Figure 3). Both tests provide relevant information for disease control. Genotyping by means of enterobacterial repetitive intergenic consensus-PCR identifies the prevalent strains causing disease in affected herds.[5] Euthanasia of 10 to 20 clinically affected nursery pigs allows for accurate identification of the prevalent *H parasuis* strains affecting the herd.[14] When isolation attempts are unsuccessful, detection of *H parasuis* in clinical samples by PCR[1] may be useful to define the role of this agent in mortality.

## Summary

The rate of success in isolating *H parasuis* from clinical samples may be improved by following a few critical procedures. As this organism is very sensitive to high temperatures, samples should always be maintained under refrigeration. Transport systems containing Amies medium or submission of whole tissue tend to improve viability of *H parasuis* during transportation. It is important to collect samples from untreated, acutely infected pigs. *Haemophilus parasuis* is more likely to be isolated from pigs that have been euthanized than from dead pigs.

**Figure 3:** Dendrogram based on *Haemophilus parasuis* genotyping by means of enterobacterial repetitive intergenic consensus-polymerase chain reaction. Two different strains were isolated from Sow 1. Strain A was isolated from the brain, pericardium, and joint samples, and Strain B from the pleura. Tissue samples from each body site were collected in separate bags.

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* Non-refereed reference.