THE SURVIVAL OF *HAEMOPHILUS SOMNUS* ON TRANSPORT SWABS

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Introduction

Since its initial description (4), *Haemophilus somnus* has been implicated as an important opportunist pathogen of cattle and of particular relevance is its role in bovine respiratory disease (5). Recovery of the organism from field specimens is often difficult and a number of selective media have been described (1,2) which can enhance it, but the manner in which the specimen is taken and subsequently treated can significantly affect the likelihood of successful recovery of this bacterium. It has been shown experimentally that the length of survival of *H. somnus* following inoculation into both bovine secretions (3) and alginate swabs (1) is temperature dependent. Since swabs taken from field specimens may need to be sent some distance to a laboratory before primary culture we report here the effect of storage temperature and composition of the transport medium on the rate of recovery of *H. somnus* from experimentally inoculated laryngeal swabs.

Materials and Methods

Bacterial strains

Eleven field strains of *H. somnus* isolated from cases of bovine respiratory disease were used for this study, and grown on Brain Heart Infusion agar (Difco: code 0418-01-5), supplemented with 70 ml/l defibrinated bovine blood (BBL: code 12379) and 10 ml/l IsoVitalex (BBL: code 11876). Following overnight incubation at 37°C, in a 5% (vol/vol) CO_2 in-air atmosphere, growth of *H. somnus* was visible as small, grey, butyric colonies, with a distinct lemon-yellow pigment when picked from the agar surface with an inoculating loop.

Preparation of inoculum

Using a sterile cotton tipped swab, 3-5 discrete colonies were removed from an overnight agar culture of *H. somnus*. These were emulsified in 4 ml of sterile distilled water and homogenized using a vortex mixer. The cell density was then adjusted to approximate a 0.5 MacFarland standard, using a nephelometer. One ml of the suspension was inoculated into 9 ml of sterile distilled water and homogenized using a vortex mixer.

Ten µl of the resulting cell suspension (equivalent to approximately 10^5 cfu) were then inoculated onto the cotton tip of each laryngeal swab. Thirty-two swabs were inoculated from each standardized cell suspension.

Swabs and transport media

Sterile laryngeal swabs were obtained from Medical Wire & Equipment Company (code MW128). They were of 12 inch flexible wire shank, tipped with a small bud of absorbent cotton wool and are well suited from sampling the bovine nasopharynx. The shank and tip were covered with a removable plastic guard tube. Following inoculation, the laryngeal swabs were cut short, the guard removed and the swab placed in a tube of Amies transport medium either with charcoal (Difco: code 9345-27-4), or without charcoal (Difco: code 9343-26-7). Inoculated swabs stored in transport medium, were either examined immediately or incubated at 4°C or 18°C for 1, 2, 3, 4, 5 and 7 days.

Determination of viability

Immediately after inoculation, and at each of the time points, an inoculated swab was removed from transport medium and streaked onto one quarter of a supplemented Brain Heart Infusion agar plate. Using a sterile disposable inoculating loop, the inoculum was spread onto the other three quarters of the plate using a standard dilution streaking pattern.

Plates were incubated at 37°C in a 5% (vol/vol) CO_2 in-air atmosphere for 24 hours, then examined. Successful survival of the organism was recorded from those plates where the growth of bacterial colonies typical of *H. somnus* was observed on at least one quarter of the plate.

Results

Table 1 shows the effect of storage temperature and composition of the transport medium on the survival time of *H. somnus*.

Greatest survival of *H. somnus* was obtained from those swabs stored at 4°C in Amies medium containing charcoal, with all of the 11 strains tested being successfully recovered after three days of storage, and six of the 11 strains still surviving after seven days of storage. Poorest survival of *H. somnus* was obtained from swabs stored at 18°C in Amies medium without charcoal. No bacteria were viable after 24 hours of storage under these conditions. When stored in Amies medium without charcoal at 4°C, ten of 11 strains were recovered after 24 hours of storage, but none after that. The use of Amies medium with charcoal also enhanced the recovery rate of strains stored at 18°C, with complete viability being maintained for two days, but not subsequently.

Table 1: Effect of temperature and composition of transport medium on the survival time of *H. somnus*.

Storage Temperature	Amies Medium	Number of strains successfully recovered following storage time in days: 0	1	2	3	4	5	7
4°C	With charcoal	11	11	11	11	10	8	6
	Without charcoal	11	10	0	0	0	0	0
18°C	With charcoal	11	11	2	0	0	0	0
	Without charcoal	11	0	0	0	0	0	0

Discussion

These results are in line with those previously published (1,3) which show that refrigeration can significantly improve the recovery of *H. somnus* from experimentally inoculated bovine secretions, or alginate swabs. No attempts were made during this study to mimic either the detrimental effect of contaminating commensal bacteria shown by Brewer *et al* (1), or the protective effect of bovine secretions shown by Dewey & Little (3).

The cotton tipped laryngeal swabs used for this study offer a number of advantages for sampling the bovine nasopharynx. The presence of the guard tube minimizes contamination of the swab tip as it passes through the external nares, and the long flexible shank and small tip aids penetration but minimizes damage to the turbinates and mucosal surfaces.

When considered in the context of field investigations, this study clearly demonstrates that *H. somnus*, sampled using guarded laryngeal swabs, could be expected to survive under the environmental conditions typically associated with overnight carrier services, when protected by Amies medium containing charcoal. In addition, survival times of *H. somnus* can be significantly increased by the use of refrigerated storage.

References

1. Brewer, R.A., Corbel, M.J., Stuart, F.A. 1985 Development of improved methods for the transport and isolation of *Haemophilus somnus*. *Research in Veterinary Science* 39: 299-306. 2. Corboz, L. 1986 Selective medium for isolation of *Haemophilus somnus*: antibiogram of 115 isolates from various sources in cattle and comparison of the selectivity of different media. In *Abstracts of the IVth International Symposium of Veterinary Laboratory Diagnosticians*: 576-579. 3. Dewey, K.J., Little, P.B. 1984 Environmental survival of *Haemophilus somnus* and influence of secretions and excretions. *Canadian Journal of Comparative Medicine* 48: 23-26. 4. Griner, L.A., Jensen, R., Brown, W.W. 1956 Infectious embolic meningoencephalitis in cattle. *Journal of the American Veterinary Medical Association* 129: 417-421. 5. Jackson, J.A., Andrews, J.J., Hargis, J.W., 1987 Experimental *Haemophilus somnus* pneumonia in calves. *Veterinary Pathology* 24: 129-134.

Summary

Isolation of *H. somnus* from field specimens is often difficult, and the manner in which they are taken can critically affect recovery of this pathogen. Laryngeal swabs are particularly suitable for sampling the bovine nasopharynx, but it has been documented that storage conditions can significantly affect survival of bacteria while in transit to the diagnostic laboratory. The object of this study was to assess survival of *H. somnus* artificially inoculated onto laryngeal swabs. Approximately 10^5 cfu of each of 11 field isolates was inoculated onto cotton tipped laryngeal swabs which were then immersed in Amies transport medium either with or without charcoal, and stored at 18°C or 4°C. At intervals up to seven days, swabs were removed from storage, inoculated onto agar, and incubated. When swabs were stored at 4°C in Amies charcoal medium, all *H. somnus* isolates could be recovered up to three days post inoculation, and six out of 11, at seven days. In Amies without charcoal stored at 18°C none of the isolates survived to 24 hours. In the context of field investigations, the study demonstrates that *H. somnus*, sampled using laryngeal swabs could be expected to survive under the conditions typically associated with overnight carrier services, when protected by Amies medium containing charcoal but that refrigerated storage is preferable for longer transit times.