

Comparison of Goat and Horse Blood as Culture Medium Supplements for Isolation and Identification of *Haemophilus influenzae* and *Streptococcus pneumoniae* from Upper Respiratory Tract Secretions

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Received 1 June 1994/Returned for modification 12 July 1994/Accepted 22 August 1994

The results of this study show that goat blood as a culture medium supplement is as supportive as horse blood for the isolation and identification of *Haemophilus influenzae* and *Streptococcus pneumoniae* from clinical material. Care is required in the preparation of goat blood chocolate agar to ensure that a thermolabile growth inhibitor of NAD-dependent *Haemophilus* species is eliminated.

Acute respiratory infections are now the principal cause of death in children under 5 years old in developing nations. Pneumonia is responsible for 70% of the deaths of about 8,000 children who die each day (1, 12). Etiological studies of community-acquired pediatric pneumonia have emphasized the importance of *Haemophilus influenzae* and *Streptococcus pneumoniae* as causative agents (11). Systemic infections due to *H. influenzae* and *S. pneumoniae* can be effectively treated with antibiotics, but recent reports have described a high prevalence of in vitro resistance of both organisms to commonly used drugs (9, 10). The World Health Organization Program for Control of Acute Respiratory Infections has sponsored the establishment of national drug resistance surveillance programs in several developing countries. A technical problem encountered has been the lack of a reliable source of either horse or sheep blood, which is required for supplementing isolation and susceptibility-testing media. Human blood, which is an inappropriate substitute, is frequently used. Goat blood, however, is more commonly available in many developing countries. This report compares the use of goat and horse blood-supplemented media for the isolation of *H. influenzae* and *S. pneumoniae* from clinical material.

One hundred thirty nasopharyngeal aspirates were collected by suction catheter from Australian Aboriginal children under 5 years old who were hospitalized with (90 subjects) or without (40 subjects) acute respiratory infections and stored at -70°C in skim milk-glucose-glycerol broth (5). The samples were cultured in parallel, in batches of 10, on Oxoid blood agar base no. 2 (Unipath Ltd., Hampshire, England) supplemented with 5% defibrinated goat and horse blood. For *S. pneumoniae*, goat and horse blood agars, each with and without 2.5 μg of gentamicin sulfate (John Bull Laboratories, Melbourne, Australia) per ml, were inoculated. Chocolatized (heated) goat and horse blood agars, each with and without 300 μg of bacitracin

(Sigma Chemical Co.) per ml, were inoculated for the isolation of *H. influenzae*. The order of inoculation of goat and horse blood-supplemented media with nasopharyngeal aspirates was alternated between batches.

Multiple type populations of *S. pneumoniae* and *H. influenzae* on both goat and horse blood-supplemented media were sought by subculturing four randomly selected colonies of each organism from selective plates. Differences in type specificity between subcultures were identified by sero- and biotyping (*H. influenzae*) and serotyping (*S. pneumoniae*).

The semiquantitative assessment of the density of growth, identification, and typing of encapsulated strains of *H. influenzae* and *S. pneumoniae* have been described previously (6, 7). In addition, nonencapsulated (nonserotypeable) *H. influenzae* isolates were confirmed by coagglutination with the use of the Phadebact *Haemophilus* test (Karo Bio Diagnostics AB, Huddinge, Sweden). Suspect *H. influenzae* isolates were tested for hemolysis on horse blood-supplemented Oxoid blood agar base no. 2 containing added hematin and NAD (Sigma Chemical Co.), each at a concentration of 15 $\mu\text{g}/\text{ml}$ of medium.

Differences in isolation rates were tested on paired samples with McNemar's tests (3). The sample size was chosen to enable the detection of differences of 10% or greater, with 99% power.

Initial testing revealed that goat blood contained a thermolabile growth inhibitor for NAD-dependent haemophili, including *H. influenzae*, *H. parainfluenzae* and *H. parahaemolyticus*. The inhibitor was inactivated by more prolonged exposure to heat during the preparation of chocolate agar, by immersion of 250-ml volumes of goat blood-supplemented medium in water at 100°C for 15 min instead of the 5 min required for horse blood chocolate agar. The inhibitory effect of goat blood was not neutralized by adding a standard quantity of NAD (15 $\mu\text{g}/\text{ml}$ of medium) to unheated goat blood agar.

No statistically significant differences in the overall isolation rates, type frequency, and distribution for either *H. influenzae* or *S. pneumoniae* on goat or horse blood-supplemented selective and nonselective media were observed. Blood from neither animal significantly favored different type-specific recovery

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TABLE 1. Isolates of *H. influenzae* and *S. pneumoniae* from 130 nasopharyngeal aspirates cultured on media supplemented with defibrinated goat and horse blood

Isolate	No. of isolates cultured with:	
	Goat blood	Horse blood
<i>H. influenzae</i>		
Encapsulated (serotyped) strains	36	37
Nonencapsulated (biotyped) strains	162	163
<i>S. pneumoniae</i> encapsulated (serotyped) strains	127	126
Totals	325	326

rates for either organism. The comparative isolation rates for encapsulated and nonencapsulated *H. influenzae* and for encapsulated *S. pneumoniae* cultured from goat and horse blood-supplemented media are given in Table 1. The prevalence of multiple type-specific populations of *H. influenzae* and *S. pneumoniae* was similar on each substrate. The density of growth of each organism recovered from positive samples on primary isolation media varied only minimally with each animal supplement, and differences between samples were independent of the order of inoculation. Goat blood-supplemented agar, both heated and unheated, supported the characteristic colony morphologies of *H. influenzae* and *S. pneumoniae*, respectively, at primary isolation and in subculture. The presumptive identification of pneumococci by optochin susceptibility testing on nonselective goat and horse blood agar did not produce significantly different zones of inhibition on either medium.

It is probable that the thermolabile inhibitor in goat blood is an NAD-inactivating enzyme which prevents the growth of NAD-dependent bacteria by depleting available pyridine nucleotides. Earlier work has shown that goat, bovine, and sheep erythrocytes, all of which inhibit the growth of NAD-requiring *Haemophilus* species, possess high NADase activity. Conversely, the erythrocytes of horse, guinea pig, and rat support *Haemophilus* species and lack measurable NADase (2, 8). A level of about 40 times the concentration of NAD present in 5% chocolate sheep blood agar is needed to propagate *Haemophilus* species on unheated sheep blood agar. Because the NADase contents of sheep and goat erythrocytes are similar (2), a comparable concentration of NAD would probably be needed to ensure the growth of *Haemophilus* species on unheated goat blood medium.

Apart from the study by Krumwiede and Kuttner (8), that of Feinsod and Kim is the only other investigation, of which we are aware, that has evaluated goat blood as an alternative animal blood supplement for bacteriological isolation media (4). In that study both heated and unheated goat blood agars were compared with sheep blood-supplemented media for the recovery of respiratory bacteria from sputum samples. Goat blood chocolate agar was prepared by gentle heating and

further supplemented with NAD from broth cultures of *Staphylococcus aureus* or *Candida albicans*. The final concentration of NAD per ml of medium was not given, and no inhibition of *Haemophilus* species by goat blood media was reported. The growth of respiratory bacteria from just 11 sputum samples was studied, and comparative isolation rates were not given.

Our results indicate that goat blood is just as supportive as horse blood in culture media for the isolation and identification of *H. influenzae* and *S. pneumoniae* from clinical material. Care should be taken in the preparation of goat blood-supplemented chocolate agar to ensure that a naturally occurring thermolabile inhibitor of NAD-dependent *Haemophilus* species is eradicated.

This study was funded by the Program for the Control of Acute Respiratory Infections, World Health Organization, Geneva, Switzerland. J.D. and K.M. were supported by the National Health and Medical Research Council, Australia.

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