Evaluation of a Selective Medium for Isolation of *Haemophilus pleuropneumoniae*

K.A. Gilbride and S. Rosendal*

ABSTRACT

Crystal violet, lincomycin, spectinomycin and bacitracin were evaluated as selective agents in media for isolation of *Haemophilus pleuropneumoniae*. No single antimicrobial agent or combination of two or more inhibited all non-*Haemophilus* strains (*Escherichia coli, Pasteurella haemolytica, Pasteurella multocida, Streptococcus faecalis, Streptococcus equisimilis* and *Staphylococcus aureus*) without marked suppression of 16 *H. pleuropneumoniae* strains. A medium containing 1 μg/mL of crystal violet, 1 μg/mL of lincomycin, 8 μg/mL of spectinomycin and 128 μg/mL of bacitracin inhibited one *E. coli* strain and the Gram-positive strains while *H. pleuropneumoniae* strains were suppressed to a minor degree only.

Haemophilus pleuropneumoniae was isolated on the selective medium on three occasions from the nose or pharynx of two out of eight experimentally inoculated pigs. *Haemophilus pleuropneumoniae* was recovered from the nose of only two pigs at necropsy and from tonsil of one, whereas the lower airways in most pigs and the lung lesions in all pigs were positive. There was no advantage to using the selective medium for the recovery of *H. pleuropneumoniae* at necropsy from these eight experimentally infected pigs, probably because other bacteria were absent or present in very low numbers in the tissues with *H. pleuropneumoniae*.

The isolation rate on selective medium was higher than the rate on non-selective medium ($p \leqslant 0.1$; χ^2 test) when the airways of slaughtered pigs were cultured. This was likely due to a high degree of contamination.

Dry swabs placed in tryptone yeast extract with nicotinamide-adenine-dinucleotide gave a significantly higher recovery rate than commercial Culturette swabs in modified Stuart's transport medium. This difference was more marked after 24 hours storage at room temperature, suggesting that the Culturette swab is not suitable for transporting samples to diagnostic laboratories for *H. pleuropneumoniae* culture.

Fifty-one *Haemophilus* strains were isolated from 224 slaughtered pigs and identified as *H. pleuropneumoniae* serotype 1 (five strains), serotype 5 (nine strains), serotype 7 (18 strains), "minor group" identical with strain 202 (four strains) and "nontypeable" (15 strains).

Key Words: *Haemophilus pleuropneumoniae*, selective medium, pneumonia, swine, *Haemophilus*.

RÉSUMÉ

Cette expérience consistait à évaluer le cristal violet, la lincomycine, la spectinomycine et la bacitracine, à titre d'agents sélectifs, dans des milieux de culture destinés à isoler *Haemophilus pleuropneumoniae*. Aucun de ces agents ne réussit, seul ou en association avec deux autres ou plus, à inhiber toutes les bactéries autres que *Haemophilus*, à savoir: *Escherichia coli, Pasteurella haemolytica, Pasteurella multocida, Streptococcus faecalis, Streptococcus equisimilis* et *Staphylococcus aureus*, sans entraver sérieusement la croissance de 16 souches de *H. pleuropneumoniae*. Un milieu de culture contenant 1 μg/mL de cristal violet, 1 μg/mL de lincomycine, 8 μg/mL de spectinomycine et 128 μg/mL de bacitracine réussit à inhiber complètement une souche d'*E. coli* et celles des bactéries gram-positives, mais incomplètement celles de *H. pleuropneumoniae*.

À trois reprises, le milieu de culture sélectif précité permit d'isoler *H. pleuropneumoniae*, de la cavité nasale et du pharynx de deux des huit porcs infectés de façon expérimentale. Lors de la nécropsie, on recouvra *H. pleuropneumoniae* de la cavité nasale de seulement deux porcs et des amygdales d'un autre, mais on réussit à l'isoler des voies respiratoires inférieures de la plupart des porcs et des lésions pulmonaires de tous. On ne remarqua aucun avantage à utiliser le milieu sélectif précité pour l'isolement de *H. pleuropneumoniae*, lors de la nécropsie des huit porcs infectés de façon expérimentale, probablement à cause de l'absence ou de la présence d'une trop faible quan-

*Department of Veterinary Microbiology and Immunology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1.

Submitted March 29, 1983.

tité d'autres bactéries dans les tissus desquels on isola *H. pleuropneumoniae*.

La culture d'échantillons des voies respiratoires de 224 porcs tués dans un abattoir donna un taux d'isolement plus élevé sur le milieu sélectif que sur milieu non sélectif ($p \leqslant 0,1$; χ^2 test). Ce résultat semblait dû à un degré de contamination élevé.

Des écouvillons secs, placés dans un bouillon à l'extrait de levure, tryptoné et enrichi par le facteur V, donnèrent un taux de recouvrement sensiblement plus élevé que le firent les écouvillons commerciaux "Culturette", pourvus du milieu de transport modifié de Stuart. Cette différence s'avéra plus accentuée après un séjour de 24 heures à la température de la pièce, indice que les écouvillons "Culturette" ne conviennent pas au transport d'échantillons jusqu'aux laboratoires de diagnostic où on procède à la culture de *H. pleuropneumoniae*.

On isola 51 souches d'*Haemophilus*, à partir d'échantillons des 224 porcs tués dans un abattoir. De ce nombre, cinq correspondaient à *H. pleuropneumoniae* du sérotype #1; neuf, à *H. pleuropneumoniae* du sérotype #5; 18, à *H. pleuropneumoniae* du sérotype #7; quatre, au "groupe mineur", identique à la souche #202; les 15 autres souches s'avérèrent cependant non typables.

Mots clés: *Haemophilus pleuropneumoniae*, milieu de culture sélectif, pneumonie, porcs, *Haemophilus*.

INTRODUCTION

Healthy carrier pigs play an important role in the spread of pleuropneumonia caused by *Haemophilus pleuropneumoniae* (1). However, the ecology of this organism is poorly understood. Isolation attempts from the nasooropharyngeal cavity are impeded by the less fastidious and rapidly growing normal bacterial flora. A selective medium would offer great advantages to studies on the rate of infection in the upper respiratory tract of pigs. One such medium was described by Little (2). It contained crystal violet (1:250,000) and bacitracin (1.6 μg/mL) and mainly suppressed Gram-positive bacteria. In a previous study, we found that the minimal inhibitory concentrations (MIC) of spectinomycin, lincomycin, bacitracin and crystal violet were relatively high when 51 *H. pleuropneumoniae* strains were tested (3). These drugs might be of potential use as selective principles in a medium for isolation of *H. pleuropneumoniae*. The purpose of this study was to develop and evaluate a selective medium based on laboratory experiments with known bacterial strains and isolation from experimentally infected pigs and slaughtered pigs of unknown carrier status.

MATERIALS AND METHODS

LABORATORY EXPERIMENT WITH KNOWN STRAINS

Trypticase soy agar (TSA) (Difco Laboratories Inc., Detroit, Michigan) with 5% calf blood and 0.1% nicotinamide-adenine-dinucleotide (NAD) was used as a base for the selective medium. Calf blood was chosen over sheep blood because of more distinct hemolysis around *H. pleuropneumoniae* colonies, even though it has higher NAD inhibitory activity (4). Crystal violet (Fisher Scientific Company, Fairlawn, New Jersey), lincomycin, spectinomycin (The Upjohn Company, Kalamazoo, Michigan) and bacitracin (Becton Dickinson Canada Inc., Mississauga, Ontario) were added to the base medium in concentrations and combinations as shown in Table I. The concentrations of the antimicrobial agents were chosen two to four times below the MICs determined previously (3).

The following bacteria were used in experiments to evaluate the selective media: 16 strains of *H. pleuropneumoniae* (five of serotype 1, three of serotype 2, one of serotype 4, five of serotype 5 and two of serotype 7), two strains of *Escherichia coli*, one *Pasteurella haemolytica*, one *Pasteurella multocida*, one *Streptococcus faecalis*, one *Streptococcus equisimilis* isolated from a pig and two *Staphylococcus aureus* strains.

Five colonies were picked from an overnight plate culture on the noninhibitory base medium and suspended in saline to match a 0.5 McFarland nephelometer barium sulfate standard (approximately 5×10^6 CFU/mL). A loopful (0.02 mL) of this suspension was then streaked on each selective plate. The plates were examined after incubation for 18-24 hours at 37°C under normal atmospheric conditions. Growth was scored as follows: 0, no growth; 1, $<$ 25 colonies and $\leqslant$ 0.4 mm diameter; 2, 25-200 colonies and 0.5-1.0 mm diameter; 3, $>$ 200 colonies and $>$ 1.0 mm diameter.

RECOVERY OF *H. PLEUROPNEUMONIAE* FROM EXPERIMENTALLY INFECTED PIGS

Twelve 25-30 kg SPF pigs were divided into three groups of four (group A, B and C). Each group was housed in an enclosed isolation unit.

The animals in group A were inoculated intratracheally with 10 mL of sterile saline (control group). The animals in group B were inoculated with 10 mL of a saline suspension of the penicillin resistant *H. pleuropneumoniae*, strain WF83 (serotype 7) containing 10^6 CFU/mL. The pigs in group C received a similar inoculum of a nalidixic acid resistant substrain of *H. pleuropneumoniae*, Shope 4074 (serotype 1).

The nose and pharynx of each pig was swabbed before inoculation and three times a week thereafter. The pigs were killed by an overdose of phenobarbital (Winthrop Ltd., Aurora, Ontario) two weeks after inoculation and necropsied. At necropsy swabs were taken from the following tissues: nasal cavity, tonsil surface, tonsil cut surface, larynx, trachea, left and right main bronchus, left and right lung, pleural cavity,

bronchial lymph node and spleen. Other tissues were swabbed if changes were present. Each site was swabbed with four swabs. Two of the swabs were Culturettes (Marion Scientific Corp., Kansas City, Missouri) which are swab assemblies containing modified Stuart's transport medium. The other two swabs were dry polyester fiber-tipped wood applicators (Becton Dickinson Canada Inc., Mississauga, Ontario). The dry swabs were placed in tryptone yeast extract broth with 0.01% NAD immediately after swabbing. One set of plates was streaked with one of the Culturettes and another set with one of the dry swabs within two hours. Both Culturettes were then stored at room temperature for 24 hours to simulate transport after which each was streaked on a set of plates. The dry swabs were transferred to tryptone, yeast extract broth containing 0.01% NAD, 1 μg/mL of crystal violet, 1 μg/mL of lincomycin, 8 μg/mL of spectinomycin and 128 μg/mL of bacitracin (selective broth). They were incubated 24 hours at 37°C when each was streaked on a set of plates. A set of plates constituted: 1) TSA with 5% calf blood, 2) same with 0.1% NAD; 3) same with 0.1% NAD, 1 μg/mL crystal violet, 1 μg/mL lincomycin, 8 μg/mL spectinomycin and 128 μg/mL bacitracin (selective medium A); 4) same as selective medium A without spectinomycin (selective medium B). The choice of selective media was made on the basis of the results from the laboratory experiment with known strains.

RECOVERY OF *H. PLEUROPNEUMONIAE* FROM SLAUGHTERHOUSE PIGS

The nasal cavity, the tonsil surface, a fresh cut surface of the tonsil and the lung (dorsocranial part of right diaphragmatic lobe) of 224 pigs were swabbed during six visits to the slaughterhouse. The tissues were swabbed with the dry swabs which were then placed in tryptone yeast extract broth with 0.01% NAD during transport back to the laboratory. Within two hours the swabs were streaked on a set of plates (TSA + blood, TSA + NAD, selective medium A and B) and then placed in selective broth and incubated 24 hours at 37°C after which an additional set of plates was streaked.

CHARACTERIZATION OF ISOLATES

Suspect colonies were picked and streaked onto 5% calf blood agar with 0.1% NAD to test for hemolysis and provide bacteria for the Gram stain reaction. Growth factor requirements were tested in two ways: i) isolates were evenly inoculated on 5% calf blood agar with a diagonally streaked *S. aureus* strain, and ii) isolates were evenly inoculated onto TSA plates, to which were added filter paper strips (Becton Dickinson Canada Inc., Mississauga, Ontario) containing X or V factor. After 24 hours at 37°C in normal atmosphere, all plates were examined for growth around the staphylococcal streak and each factor strip. The urease test was performed by streaking a loopful of bacteria on TSA slopes containing 10% urea, 0.1% NAD and phenol red.

TABLE I. Assessment of Growth of *H. pleuropneumoniae* and Other Gram-Negative and Gram-Positive Bacteria on Media with Selective Agents

Antimicrobial agents in TSA + 5% calf blood +0.1% NAD	*H. pleuropneumoniae*[a]	*E. coli* ATCC 25922	*E. coli* RIP 69	*P. haemolytica*, PH	*P. multocida*, PM	*S. faecalis* ATCC 29212	*S. equisimilis* B-HS	*S. aureus* ATCC 29213	*S. aureus*, SA
None	3.0	3	3	3	3	3	3	3	3
CV 1	2.7	3	3	3	3	3	2	0	1
Linc 1	3.0	3	3	3	3	3	0	0	1
Spec 16	2.7	3	0	3	3	3	1	3	3
Bac 320	2.1	0	3	0	3	0	0	0	0
CV1 + Linc 1	2.6	3	3	3	3	3	0	0	0
CV1 + Spec 16	2.2	3	1	1	1	2	0	0	0
CV1 + Spec 8	2.6	3	3	3	3	2	1	0	0
CV1 + Bac 320	1.5	0	3	1	0	0	0	0	0
CV1 + Bac 128	2.6	0	3	3	3	0	0	0	0
CV1 + Linc 1 + Spec 16	2.6	3	3	3	3	3	3	0	0
CV1 + Linc 1 + Spec 8	2.6	3	3	3	3	3	3	0	0
CV1 + Linc 1 + Bac 320	2.5	0	3	3	3	0	0	0	0
CV1 + Linc 1 + Bac 128	2.5	0	3	3	3	0	0	0	0
CV1 + Spec 16 + Bac 320	1.1	0	1	1	0	0	0	0	0
CV1 + Spec 16 + Bac 128	2.5	0	3	3	3	0	0	0	0
CV1 + Linc 1 + Spec 16 + Bac 320	0.3	0	1	0	0	0	0	0	0
CV1 + Linc 1 + Spec 8 + Bac 128	2.5	0	3	3	3	0	0	0	0

Note:
CV1: 1 μg of crystal violet/mL
Linc 1: 1 μg of lincomycin/mL
Spec 16: 16 μg of spectinomycin/mL
Bac 320: 320 μg of bacitracin/mL
[a]The average score of 16 strains

The Christie, Atkins and Munch-Petersen (CAMP) reaction was tested by streaking isolates perpendicular to a β-toxin producing *S. aureus* streak on 5% calf blood agar. A positive reaction was an enhanced zone of haemolysis in the incomplete β-toxin zone around the *Haemophilus* streak.

Isolates obtained from experimentally infected pigs were tested for growth on plates containing either 50 μg/mL of nalidixic acid or 50 μg/mL of penicillin.

Serotyping of the isolates was carried out by the indirect fluorescent antibody technique (IFAT) as described (5). The isolates were tested with antisera prepared against serotype 1, 2, 5, 7 and strain 202 (6). When the isolates did not react with these, they were further tested with antisera prepared against serotype 3, 4, 6 and *H. parasuis*. If they did not react with these, they were categorized as nontypeable.

RESULTS

Laboratory Experiments with Known Strains — The average growth score of 16 *H. pleuropneumoniae* strains on media with antimicrobial agents is shown in Table I together with the score for each of eight strains representing Gram-positive and Gram-negative bacteria which ideally a selective medium should inhibit. No single antimicrobial agent or combination of two or more agents inhibited all non-*Haemophilus* strains without marked suppression of *H. pleuropneumoniae* strains. Good inhibition of the Gram-positive bacteria was accomplished with bacitracin, but inhibition of *P. hemolytica*, *P. multocida* and one of the *E. coli* strains was generally not possible.

TABLE II. Frequency of Isolation of *Haemophilus pleuropneumoniae* from Selected Tissues of Eight Experimentally Infected Pigs

Site	Number of Isolates				
	Blood agar	Blood agar +0.1% NAD	Selective medium A	Selective medium B	Total
Tonsil surface	0	0	0	1	1
Cut tonsil	0	0	0	1	1
Nasal cavity	0	0	1	2	2
Larynx	0	3	4	4	4
Trachea	3	5	6	7	7
Bronchus, left	2	3	3	3	3
Bronchus, right	3	7	7	6	7
Lung, left	1	1	1	1	1
Lung, right	4	8	8	7	8
Lymph node	0	2	2	2	2
Spleen	0	0	0	0	0
Pleura	0	0	0	0	0
Total number of isolates	13	29	32	34	36

Recovery of H. pleuropneumoniae from Experimentally Infected Pigs — Twenty-four hours after inoculation the eight infected pigs were lying down, anorectic and dyspneic. All pigs recovered clinically and resumed eating by the fourth day. The control pigs appeared normal throughout the experiment. None of the control pigs had pulmonary lesions at necropsy. Pulmonary necrosis or abscesses with overlying fibrous pleuritis was consistently found in the right diaphragmatic lobes of the experimental animals (detailed description will be published elsewhere).

Haemophilus pleuropneumoniae was not isolated from the nose or pharynx before inoculation or from any of the control pigs throughout the experiment. *H. pleuropneumoniae* was isolated on the selective medium B from the pharynx of one of the infected pigs in group C ten days after inoculation. *Haemophilus pleuropneumoniae* was isolated twice on both selective medium A and B from the nose of another pig in group C on day 10 and day 12 postinoculation.

Bacteria interpreted as normal flora grew from the larynx, tonsil surface, tonsil cut surface and nasal cavity of the control pigs (group A). No growth was obtained from other tissues of these animals, except for *Actinomyces* (formerly *Corynebacterium*) *pyogenes* which was isolated from a small abscess at the tracheal injection site of one of the control pigs.

Haemophilus pleuropneumoniae was recovered from tissues of the experimental pigs in groups B and C as shown in Table II. Pulmonary lesions were consistently positive. The recovery rate was highest in the deeper airways, whereas only two of the eight pigs had *H. pleuropneumoniae* in the nasal cavity. Two pigs had *H. pleuropneumoniae* in the bronchial lymph node. The pleura and the spleen were consistently negative.

TABLE III. Comparison of Media and Isolation Procedures for Recovery of *H. pleuropneumoniae* at Necropsy from 96 Tissues of Eight Experimentally Infected Pigs

Medium	Streaked after:	Culturette 1		Culturette 2	Dry swab 1		Dry swab 2
		2 hours	24 hours[c]	24 hours[c]	2 hours	24 hours[d]	24 hours[d]
TSA + 5% calf blood		3[a]	4	4	9	11	11
TSA + 5% calf blood + 0.1% NAD		17	8	8	21	24	24
Selective medium A		17	6	7	24	22	24
Selective medium B		19[b]	8	8	32[b]	22	22

[a]Number of isolates obtained
[b]Significantly different at $p \leq 0.05$ (χ_2 test)
[c]Left at room temperature for 24 hours to simulate transport
[d]Swab incubated in selective broth for 24 hours

Higher recovery rates were obtained with the dry swabs than with the Culturettes (Table III). There was poor preservation of *H. pleuropneumoniae* in the Culturette medium during 24 hours storage at room temperature. This was independent of whether the swab had been used to streak a set of plates or not. The recovery rate was not improved after incubating the dry swabs for 24 hours in a selective broth. The rates of recovery were not significantly different between the nonselective TSA blood agar with NAD and the selective media A and B. Trypticase soy blood agar without NAD supported growth of *H. pleuropneumoniae*; but the isolation rates were considerably higher on the media with NAD.

All isolates from the pigs in group B were identified as *H. pleuropneumoniae* serotype 7 and were penicillin resistant. The isolates from group C were identified as *H. pleuropneumoniae* serotype 1 and nalidixic acid resistant.

Recovery of H. pleuropneumoniae from Slaughterhouse Pigs — The recovery rate of *H. pleuropneumoniae* from slaughterhouse pigs is shown in Table IV. Most isolates were obtained from the tonsil surface, although the rate of isolation was not significantly different between the four sites cultured. *Haemophilus pleuropneumoniae* was occasionally isolated on TSA blood agar together with bacteria interpreted as normal flora. There was a definite advantage to the use of selective medium. The rate of recovery on selective medium A was higher than the recovery on nonselective medium with NAD ($p \leq 0.1$). The rate of isolation was not different between selective medium A and B. On only six occasions (four tonsils and two lungs) was *H. pleuropneumoniae* isolated on different media from the same sample. *Haemophilus pleuropneumoniae* was not isolated from swabs incubated 24 hours at 37°C in selective broth mainly due to overgrowth by *Proteus* or other rapidly growing enteric bacteria.

The isolated *Haemophilus* strains were identified by biochemical and serological procedures. The distribution of serotypes in relation to anatomical site of isolation is shown in Table V. All isolates were V-factor dependent and X-factor independent. All serotypes 1, 5 and 7 were hemolytic, urea- and CAMP-positive. Three of the strains identical with strain 202 were urea-positive, but nonhemolytic and CAMP-negative. One strain reacting with strain 202 serum was weakly hemolytic and CAMP-positive, but urea-negative. The strains not typeable with the available sera were nonhemolytic, urea- and CAMP-negative.

TABLE IV. Comparison of the Rate of Isolation of *H. pleuropneumoniae* from 224 Slaughterhouse Pigs Using Selective Media A and B and Nonselective Media

Site	Number of Isolates				
	TSA Blood agar	TSA blood agar + 0.1% NAD	Sel. A	Sel. B[d]	Total Isolates
Nasal cavity	1	3	5	1	10
Tonsil surface	3	4	6	2	15
Tonsil cut surface[a]	1	2	10	4	13
Lung[b]	1	6	5	3	13
Total	6	15[c]	26[c]	10	51

[a]Four isolates from the tonsil were recovered on both selective plates
[b]Two isolates from the lung were recovered on both selective plates
[c]Significantly different at $p \leq 0.1$ (χ_2 test)
[d]Used on 80 pigs only

DISCUSSION

Lincomycin, bacitracin and crystal violet have all been used as selective agents for *Hemophilus* bacteria (2, 7, 8). They are mainly effective against Gram-positive bacteria (9). Spectinomycin gave relatively high MICs against *H. pleuropneumoniae* (3) and was included in the selective media because of its effect against Gram-negative bacteria (9). Complete suppression of non-*Hemophilus pleuropneumoniae* bacteria without concomitant effect on *H. pleuropneumoniae* was not achieved. Of the media with spectinomycin the one containing 1 μg crystal violet, 1 μg lincomycin, 8 μg spectinomycin and 128 μg bacitracin was chosen for further studies as it seemed to have the best possible selectivity of the media studied. A similar medium without spectinomycin had virtually the same selective capacity and was also chosen for evaluation in the subsequent studies.

It was possible to isolate *H. pleuropneumoniae* from the nose and pharynx of two experimentally infected pigs on three occasions on selective agar. The suppression of some of the bacteria of the normal flora on the selective plates allowed easier detection of *H. pleuropneumoniae* colonies. These results suggest that selective media are helpful in the recovery of *H. pleuropneumoniae* from the upper airways of carrier pigs; but this should be confirmed. Although all the experimentally infected pigs had lung lesions the low re-

TABLE V. Distribution of *Haemophilus* Serotypes from Slaughterhouse Pigs in Relation to Site of Isolation

Site	Number of Isolates in Each Serotype and Group				
	Sero 1	Sero 5	Sero 7	202[a]	N.T.[b]
Nasal cavity	0	0	5	1	4
Tonsil surface	2	2	1	1	8
Tonsil cut surface	1	4	8	1	1
Lung	2	3	4	1	2
Total	5	9	18	4	15

[a]Identical with strain 202
[b]Nontypeable

covery rate from the nose indicates that some chronically infected pigs never shed or shed *H. pleuropneumoniae* in very low numbers. This is surprising since the organism was recovered from the pulmonary lesions of all pigs and with one exception from the trachea as well. It is possible that colonization of the naso-oropharyngeal cavity with *H. pleuropneumoniae* is impeded by the competitive normal bacterial flora. The low recovery rate from the nose may also be a result of dilution of the spillover of *H. pleuropneumoniae* to the airways from the primary lung focus. The recovery rates from nose, tonsil and lung of slaughtered pigs were similar, however; but these data may not reflect the true ecology of the bacterium since the entire airway may be contaminated during carcass processing.

There was no advantage to using selective medium for the recovery of *H. pleuropneumoniae* from the lower airways of the experimentally infected pigs, probably because other bacteria were absent or present in very low numbers. In the slaughtered pigs, however, where the degree of contamination was high in the airways, there was a definite advantage to using selective media. In the study on the experimentally infected pigs the selective medium without spectinomycin (medium B) seemed to give more isolates than the selective medium with this drug (medium A). In the slaughtered pigs this difference was not observed. Occasionally *H. pleuropneumoniae* was isolated on TSA blood agar without NAD. This may occur when other bacteria in the sample provide growth factors or when growth factors from the tissue are carried over onto the plate.

The recovery of *H. pleuropneumoniae* from Culturette swabs was lower than the recovery from dry swabs. This difference became more marked after 24 hours incubation, suggesting that the Culturette swab would not be commendable for isolation of *H. pleuropneumoniae* particularly in cases where it can not be plated out immediately because of transport. There was no benefit to incubating the dry swab in selective enrichment broth. It did not provide additional isolates in the study on the experimentally infected pigs, most likely because of low contamination, and in the study on the slaughtered pigs the broth was not able to suppress contaminants.

The pigs sampled at the slaughterhouse came from many different herds. However, during one visit to the slaughterhouse 13 pigs from one herd were all positive for serotype 7 which would explain why this serotype was predominant. The four isolates identical with the "minor group" were all from different pigs. The role of these *Haemophilus* bacteria in pneumonia of pigs is not known. This is the first isolate reported in Canada. There were a surprising number of nontypeable *Haemophilus* strains isolated from the slaughtered pigs. Future studies should evaluate their taxonomical position and their role in respiratory tract diseases of pigs.

ACKNOWLEDGMENTS

Grants from the Ontario Ministry of Agriculture and Food, the Ontario Pork Producers Marketing Board and Agriculture Canada Food Production and Inspection Branch supported this research. The technical assistance of Miss D. Boyd, Mrs. S. Watson and Mr. P. Sinclair is much appreciated.

REFERENCES

1. **NIELSEN R, MANDRUP M.** Pleuropneumonia in swine caused by *Haemophilus parahaemolyticus*. A study of the epidemiology of the infection. Nord Vet Med 1977; 29: 465-473.
2. **LITTLE TWA.** *Haemophilus* infection in pigs. Vet Rec 1970; 87: 399-402.
3. **GILBRIDE KA, ROSENDAL S.** Antimicrobial susceptibility of 51 strains of *Haemophilus pleuropneumoniae*. Can J Comp Med 1984 (in press).
4. **KILIAN M.** The haemolytic activity of Haemophilus species. Acta Pathol Microbiol Scand (B) 1976; 84: 339-341.
5. **ROSENDAL S, LOMBIN L, DEMOOR J.** Serotyping and detection of *Haemophilus pleuropneumoniae* by indirect fluorescent antibody technique. Can J Comp Med 1981; 45: 271-274.
6. **ROSENDAL S, BOYD DA.** *Haemophilus pleuropneumoniae* serotyping. J Clin Microbiol 1982; 16: 840-843.
7. **CSUKAS Z.** New selective medium for the isolation of *Haemophilus* species. Acta Microbiol Acad Sci Hung 1980; 27: 141-145.
8. **LITTLE TWA, HARDING JD.** The comparative pathogenicity of two porcine *Haemophilus* species. Vet Rec 1971; 88: 540-545.
9. **HARVEY SC.** Antimicrobial drugs. In: Remington's pharmaceutical sciences, 5th ed. Easton, Pennsylvania: Mack Publishing Co., 1975.