Characteristics of *Haemophilus pleuropneumoniae* Isolates and Some Epidemiological Findings on Porcine *Haemophilus* Pleuropneumonia in Saskatchewan

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SUMMARY

Thirty isolates of *Haemophilus pleuropneumoniae* from clinical and slautherhouse cases of porcine *Haemophilus* pleuropneumonia in Saskatchewan as well as six isolates from British Columbia and Ontario were subjected to cultural, biochemical, serological and antibiotic sensitivity tests. All strains were Gram-negative pleomorphic rods or coccobacilli which grew only in the presence of V factor and all produced porphyrin from delta-aminolaevulinic acid. Biochemically, the organism was positive for urease, O-nitrophenyl-β-D-galactopyranosidase and the fermentation of sucrose, mannitol, dextrose, lactose and xylose, but was usually negative for indole. Most strains of *H. pleuropneumoniae* were sensitive to chloramphenicol, furamazone, carbenicillin and ampicillin, but only about 50% were sensitive to tetracycline. Serotype 5 was more common than serotype 1 or the untyped strains among Saskatchewan isolates. In addition, serotype 3 was identified from British Columbia.

Retrospective epidemiological studies showed that *Haemophilus* pleuropneumonia occurred and recurred on farms in the Saskatoon and adjoining districts, serviced by the diagnostic laboratories of the Western College of Veterinary Medicine and that the disease was more common among three month old pigs during the fall-winter season.

RÉSUMÉ

Les caractéristiques des souches de *Haemophilus pleuropneumoniae* et quelques observations épidémiologiques sur la pleuro-pneumonie porcine à *Haemophilus*, en Saskatchewan

Cette expérience consistait à effectuer des études bactériologiques, biochimiques et sérologiques, ainsi que des antibiogrammes, sur 30 souches de *Haemophilus pleuropneumoniae* isolées de cas de pleuro-pneumonie porcine à *Haemophilus,* tant dans le champ qu'aux abattoirs; elle portait également sur six autres souches isolées en Colombie Britannique et en Ontario. Toutes ces souches correspondaient à des bâtonnets ou à des coccobacilles pléomorphes et gram-négatifs qui ne croissaient qu'en présence du facteur V et qui produisirent tous de la porphyrine, à partir de l'acide delta-aminolévulinique. Les tests biochimiques révélèrent que ces bactéries étaient positives à l'uréase et à l'O-nitrophényle-β-D-galactopyranosidase; elles dégradaient aussi le sucrose, le mannitol, le dextrose, le lactose et le xylose, mais elles ne réagissaient pas à l'indol. La plupart des souches de *H. pleuropneumoniae* s'avérèrent sensibles au chloramphénicol, au furamazone, à la carbénicilline à l'ampicilline, tandis que seulement 50% se révélèrent sensibles à la tétracycline. Le sérotype #5 se rencontra plus fréquemement que le sérotype #1 ou que les souches à sérotype indéterminé, parmi celles de la Saskatchewan. On identifia en plus le sérotype #3, en Colombie Britannique.

Une étude épidémiologique rétrospective révéla l'incidence et la récidive de la pleuro-pneumonie porcine à *Haemophilus,* dans les porcheries de la région de Saskatoon et des districts environnants, desservis par le laboratoire de diagnostic du Collège de Médecine Vétérinaire de l'Ouest. Cette étude démontra aussi que la maladie sévissait plus souvent chez les porcs âgés de trois mois, au cours de l'automne et de l'hiver.

INTRODUCTION

The first reported cases of porcine *Haemophilus* pleuropneumonia (PHP) in Canada were seen about ten years ago in Saskatchewan (1). The disease has recently been recognized as a problem in Ontario (2) and British Columbia (3).

With the exception of the latter report (3) there have been no detailed publications on the serotypes of *H. pleuropneumoniae* present in Canada, their distribution or their microbiological characteristics.

While the incidence of subclinical/chronic PHP is apparently not high in Saskatchwan (4) outbreaks of the acute form of the disease have occurred annually in at least five to eight herds in the area of the province serviced by the Western College of Veterinary Medicine (WCVM) (5).

The objectives of the present study were: (a) to determine the serotypes, the cultural/biochemical characteristics and antibiotic sensitivity patterns of isolates of *H. pleuropneumoniae* and (b) to outline some of the pertinent epidemiological features of PHP in Saskatchewan.

MATERIALS AND METHODS

Bacterial Cultures

Thirty strains of *H. pleuropneumoniae* from Saskatchewan came from two sources, namely, PHP affected lungs collected at an abattoir[1] and from epidemics of acute PHP. In addi-

[1]Intercontinental Packers, Saskatoon, Saskatchewan.

tion, two Ontario isolates (serotypes 1 and 5) were obtained from the Animal Diseases Research Institute (ADRI), Ontario and four strains originated from the Veterinary Diagnostic Laboratory, Abbotsford, British Columbia. All the strains were passaged into the yolksac of seven day old embryonated eggs and 1.0 mL stocks of infected egg yolk stored at -70° C.

Cultural, Biochemical, Serological and Antibiotic Sensitivity Characterization of H. pleuropneumoniae

The requirements for X and V factor by 27 strains of *H. pleuropneumoniae* were determined on two agar media: (a) Pleuropneumonia-like-organism (PPLO) agar medium containing 6% horse serum, 5% fresh yeast extract and 0.1% dextrose and (b) 5% sheep blood agar (BA). The PPLO plates were inoculated in the usual way to ensure isolation of single colonies while the BA plates were streaked over their entire surface and then centrally cross-streaked with a loopful of a β-hemolytic *Staphylococcus aureus* strain (6). All the plates were incubated at 37°C (PPLO plates under 10% CO_2) for 18 hours after which patterns of hemolysis and colonial appearance of growth were recorded. All 36 strains were stained by the Gram's stain, but only 21 strains were stained by Anthony's procedure for capsules (7).

Twenty-five strains were tested for carbohydrate fermentation by the Minitek[2] method (8) using 0.025 mL of a thick suspension of the organisms. The test for conversion of delta-aminolaevulinic acid (ALA) to porphyrins was carried out according to Kilian's modification (9).

Thirty-six *H. pleuropneumoniae* isolates were serotyped by whole cell agglutination tests with antisera (to serotypes 1 to 5 and strain 202) supplied by Professor A. Gunnarsson[3] (10). Six cultures representing serotypes 1 to 5 and strain 202 obtained from the same source and two typed strains (serotypes 1 and 5) from Ontario served as reference strains. Cross-agglutination studies were performed with five untyped strains using rabbit antisera raised against one of the strains (10).

Antibiotic sensitivity tests were performed on a minimum of 15 to a maximum of 27 strains using PPLO agar plates (150 x 15 mm) inoculated with eight hour PPLO broth (containing 10% horse serum, 5% fresh yeast extract and 0.1% dextrose) cultures of the organism. The antibiotic discs[4] were then placed on the inoculated plates which were subsequently incubated as before. Two control sets of two PPLO plates that were inoculated with either *Escherichia coli* or *S. aureus* and had the same antibiotic discs applied were also incubated at 37° C with and without adding CO_2.

Retrospective Epidemiological Studies

Anamnestic data on herds in Saskatchewan that experienced confirmed outbreaks of PHP from 1975 to 1980 were obtained from necropsy and diagnostic bacteriology records at WCVM.

Histopathological Examinations

Haematoxylin and eosin stained sections of porcine lungs from cases of acute PHP were obtained from the Department of Veterinary Pathology, WCVM, for microscopic examination.

RESULTS

All the strains of *H. pleuropneumoniae* examined were Gram-negative pleomorphic rods or coccobacilli which grew only in the presence of V factor and were hemolytic. All 21 strains of the organism stained with Anthony's strain had varying amounts of capsular material (Figure 1).

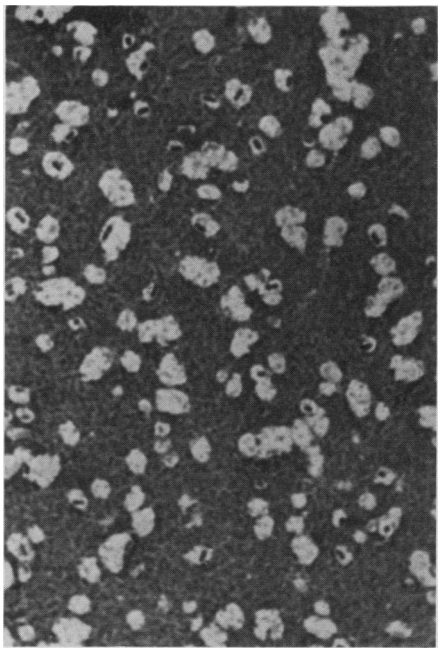

FIGURE 1. Capsular material present in a *Haemophilus pleuropneumoniae* strain isolated from acute PHP. Anthony's stain. X1280.

Haemophilus pleuropneumoniae strains were positive for urease O-nitrophenyl-β-D-galactopyranosidase (ONPG) and fermented sucrose, mannitol, dextrose, lactose and xylose (Table I). All 25 strains tested produced porphyrin from ALA.

TABLE I
BIOCHEMICAL REACTIONS BY THE MINITEK METHOD OF TWENTY-FIVE STRAINS OF *HAEMOPHILUS PLEUROPNEUMONIAE*

Test or Substrate	*H. pleuropneumoniae*	*E. coli*[a]	*Proteus*[a]	*Staphylococci*[a]
Dextrose	25/25[b]	+	+	+
Indole	4/25[c]	+	-	-
Lactose	25/25[d]	+	+	+
Mannitol	25/25	+	-	+
ONPG	25/25[e]	+	-	+
Sucrose	25/25	+	-	+
Urea	25/25	-	+	-
Xylose	25/25[f]	+	+	+

[a]Controls.
[b]Number positive/total.
[c]Only one strong positive belonging to serotype 3.
[d]Weak positive reactions.
[e]Three isolates showed weak positive reactions.
[f]Two isolates showed weak positive reactions.

[2]Bioquest, Cockeysville, Maryland.
[3]National Veterinary Institute, Uppsala, Sweden.
[4]BBL — Becton, Dickinson & Co., Mississauga, Ontario.

TABLE II
ANTIBIOTIC SENSITIVITY OF STRAINS OF *H. PLEUROPNEUMONIAE*

Antibiotic	No. Sensitive	No. Resistant	Total	% Sensitive
Ampicillin	23	3	26	88
Carbenicillin	22	2	24	92
Chloramphenicol	25	2	27	93
Furamazone	21	1	22	95
Gentamycin	1	20	21	5
Neomycin	0	26	26	0
Polymixin B	20	4	24	83
Streptomycin	0	27	27	0
Tetracycline	13	14	27	48[a]
Trimethoprim	6	9	15	40[b]

[a]Four strains had borderline resistance.
[b]Two strains had borderline resistance.

The antibiotic sensitivity pattern of *H. pleuropneumoniae* strains is shown in Table II. More than 80% of strains were sensitive to furamazone, chloramphenicol, carbenicillin, ampicillin and Polymixin B, but only 40 to 50% were sensitive to tetracycline and trimethoprim. All the strains tested were resistant to streptomycin and neomycin. Results of the comparative antibiotic sensitivity studies with *E. coli* and *S. aureus* showed that CO_2 incubation did not interfere with or enhance the inhibitory capabilities of the various antibiotics tested.

Two-thirds of the 30 Saskatchewan isolates belonged to serotype 5 while the remaining ten were divided equally between serotype 1 and the untyped strains (Table III). In Saskatchewan epidemics one serotype appeared to be involved in a particular herd with one exception where both serotypes 1 and 5 were involved. Three strains, all of which came from British Columbia, were identified as serotype 3. Cross-agglutination studies with untyped strains gave no cross-reaction.

Retrospective epidemiological studies indicated that PHP was more common in three month old pigs (Figure 2) during the fall-winter months (October to December) (Figure 3). However, the disease occurred throughout the year (Figure 3) with the exception of the month of June. The location of herds that experienced outbreaks of PHP confirmed at WCVM from 1975 to 1980 is shown in the diagram of the Province of Saskatchewan arranged by crop districts (Figure 4). Figure 4 also indicates the farm locations of earlier (1975-1978) and recent (1979-1980) recurring endemics of PHP. Porcine *Haemophilus* pleuropneumonia has been diagnosed in six crop districts, No. 6,8,9,5,3 and 7, which generally also had the highest number of pigs from 1975 to 1979 in that order (Table IV).

TABLE III
RESULTS OF SEROTYPING OF *HAEMOPHILUS PLEUROPNEUMONIAE* ISOLATES

Province	Serotype # and No. of Isolates				
	1	3	5	Untyped	Total
British Columbia	1	3	-	-	4
Ontario[a]	1	-	1	-	2
Saskatchewan	5	-	20	5	30
Total	7	3	21	5	36

[a]Originally typed and supplied by Animal Diseases Research Institute, Ottawa, Ontario.

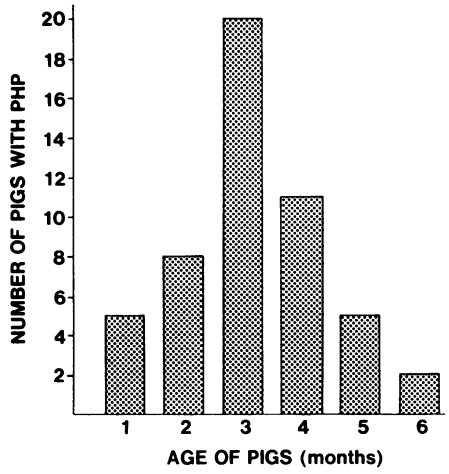

FIGURE 2. Age distribution of confirmed cases of porcine *Haemophilus* pleuropneumonia (PHP) compiled from the diagnostic records of the Western College of Veterinary Medicine in the period 1975-80.

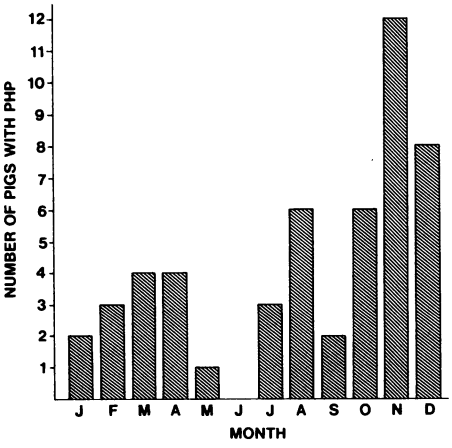

FIGURE 3. Monthly occurrence of confirmed cases of porcine *Haemophilus* pleuropneumonia (PHP) compiled from diagnostic records of the Western College of Veterinary Medicine in the period 1975-80.

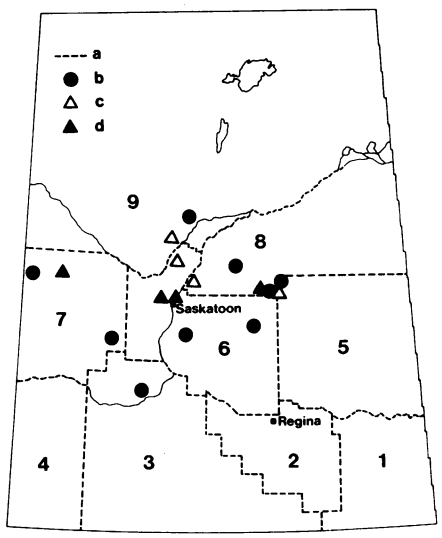

FIGURE 4. Location in Saskatchewan of herds that experienced epidemics of porcine *Haemophilus* pleuropneumonia (PHP), confirmed by the Western College of Veterinary Medicine in the period 1975-80.
a = crop district boundary
b = nonrecurring outbreaks of PHP 1975-80
c = recurring outbreaks 1975-80
d = recurring outbreaks 1979-80

Histopathological Findings

Microscopically, the lungs of pigs that died from acute PHP showed a

TABLE IV
PIGS ON FARMS, SASKATCHEWAN, BY CROP DISTRICT

Crop District	Total Pigs (thousand head)				
	1975	1976	1977	1978	1979
1	37.0	35.2	34.0	32.0	36.8
2	22.0	22.5	23.0	20.0	23.1
3	49.5	48.7	49.0	52.0	59.8
4	29.5	32.9	34.0	40.0	45.3
5	54.0	53.7	53.5	58.0	67.5
6	86.0	89.7	100.0	118.0	134.5
7	34.5	34.7	33.0	30.0	33.8
8	118.0	118.1	112.5	108.0	127.6
9	61.5	61.8	61.0	62.0	71.6
Province	492.0	497.3	500.0	520.0	600.0

Source: Agriculture Statistics, 1979. Saskatchewan Agriculture, Regina, Saskatchewan.

necrotizing fibrinous pleuropneumonia. Prominent features of these lesions were: (a) an overlying fibrinous pleuritis and (b) mononuclear cell infiltrations and distension of the interlobular septa by edema fluid and fibrin.

DISCUSSION

The results of the cultural characterization studies on *H. pleuropneumoniae* are in agreement with previous findings on this organism (6,11). All the strains tested were able to metabolize ALA. The action of *Haemophilus* strains on ALA has been studied and a perfect correlation exists between the absence of hemin requirement and the ability to convert ALA to porphyrins (9,12). The porphyrin test is now regarded as indispensable in taxonomical studies of the genus *Haemophilus* (11,13). The biochemical results obtained by the Minitek method are very similar to those existing (11) with the exception that lactose tended to give weak positive reactions in our tests while the other workers generally obtained negative reactions. In the absence of standardized procedures and appropriate media, the Minitek system devised for identification of *Neisseria* (8,14) could be a suitable alternative system for the identification of *H. pleuropneumoniae*.

The presence of capsular material of *H. pleuropneumoniae* cells may be an important factor in the pathogenesis of PHP. Capsules are known to play a role in the pathogenicity of microorganisms either through interference with humoral defences (15,16) or with mobilization of phagocytes (16). Some capsulated bacteria resist ingestion by phagocytes yet others survive intracellular digestion (17). To what extent surface components of *H. pleuropneumoniae* are involved in any of the mechanisms mentioned has not yet been determined.

The lack of susceptibility of many strains of *H. pleuropneumoniae* to the broad spectrum tetracycline antibiotics used commonly in the swine industry should be noted (Table II). This indicates the need to routinely carry out such tests on *H. pleuropneumoniae* isolates before instituting treatment in epidemics of PHP.

Serotyping of *H. pleuropneumoniae* has proven useful in the tracing of origins of herd outbreaks of disease (3). Although serotype 5 is more common than serotype 1 or the untyped strains, the latter group, because of its serological heterogeneity, will likely cause greater problems in the immunoprophylactic control of the disease than either serotype 1 or 5. There is great need, therefore, to serologically characterize the untyped strains more definitely.

Retrospective epidemiological studies revealed that PHP occurred most frequently in three month old pigs (Figure 2) and although the disease persisted throughout the year (Figure 3), it occurred most frequently from October through December. These findings are similar to those in previous studies in Ontario (2) where PHP affected mainly feeder pigs in the intensive pig rearing areas of that province. Stress conditions such as extreme weather conditions and recent transportation were suspected of playing a major role in those outbreaks. The role of these factors in PHP outbreaks in Saskatchewan was not revealed by these retrospective studies.

The present studies indicate that PHP was and, in spite of antibiotic chemotherapy, is still endemic in some herds in an area with the highest density of pigs in Saskatchewan. These findings seem to indicate that PHP will remain an endemically important disease in the swine industry until better immunoprophylactic procedures or improved methods of detection and elimination of carrier pigs (herds) are available.

ACKNOWLEDGMENTS

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ADDENDUM

Since submission of this manuscript, another paper (Libal MC, Gates CE. Antimicrobial sensitivity patterns of *Haemophilus pleuropneumoniae* isolates from pigs. J Am Vet Med Assoc

1982; 180: 399) has been published on U.S.A. isolates. A major difference from our study was the greatly increased percentage of strains sensitive to gentamycin (100%) and neomycin (60%). These discrepancies could possibly be attributed to differences in media and conditions of incubation used.

LETTERS TO THE EDITOR

Aortic Valvular Dysplasia with Subvalvular Aortic Stenosis

DEAR SIR:

Congenital cardiac disease is reported in approximately 10% of all canine cardiac cases (8). Aortic stenosis, which usually occurs as a subvalvular aortic ring, is one of the more common congenital cardiac defects especially in the Newfoundland, German Shepherd and Boxer breeds (4). Valvular stenosis appears as a dome-shaped diaphragm with its valve commissures incompletely fused (4). The occurrence of subaortic stenosis and aortic insufficiency due to malformation of the aortic valve apparatus has been described (11). This letter reports one such case and describes the macroscopic and microscopic lesions of what has been termed incomplete differentiation of the cardiac valves (6).

A ten week old German Shepherd puppy, in apparently good body condition, was referred to the Ontario Veterinary College for evaluation of a heart murmur. A grade III/V holosystolic murmur was loudest on the the left hemithorax in the area of the third intercoastal space at the level of the costochondral junction. A less intense murmur, grade II/V radiated anteriorly over the point of the sternum and to the right. Abnormalities of the heart rate, rhythm or pulse, and clinical signs of cardiovascular disease were not noted. The dog, a cryptorchid, was one of seven in a litter. All other pups were free of apparent congenital defects. There was no history of congenital defects in the sire's or dam's ancestry. On the basis of the location of the murmur at the left heart base and the systolic timing, the most common differential diagnos would include aortic or pulmonic outflow tract stenosis. Radiography and electrocardiography were suggested to help delineate the lesion. Because of the suspected hereditary nature of the lesion, and the costs that would be incurred by further investigative procedures, the breeder requested euthanasia and necropsy.

Grossly, the lungs were reddish brown and edematous. The left ventricle was concentrically hypertrophied. The aortic cusps were thickened, short and malformed and there was a constrictive subvalvular aortic band producing some stenosis of the left ventrical outflow tract (Figure 1). There was generalized, endematous lymphadenopathy.

The malformed aortic cusps were composed of loose myxomatous connective tissue, sparse collagen fibres and abundant ground substance. Sections staining lightly basophilic on hematoxylin and eosin stain and positive with alcian blue and colloidal iron stains confirmed the presence of glycosaminoglycans. Some focal areas of recent hemorrhages were present in the aortic valves. Hemosiderin-laden macrophages in areas of capillary proliferation and denser collagen deposition indicated hemorrhage of longer duration with attempted repair. From the base of the aortic valve leaflets, fibrous connective tissue extended into the left ventricular myocardium, dividing muscle bundles for a short distance. Early thrombi were present in many pulmonary and hepatic venules and capillaries. The liver was also edematous and congested.

Congenital subaortic stenosis and aortic insufficiency due to valvular malformation and aortic valve ring dilation has been seen in a four month old male Basset Hound (11). Other abnormalities found in that dog included anomalous origin of the coronary arteries, left ventricular hypertrophy with dilation of the left atrium and ventricle, mitral valvular insufficiency and tracheal stenosis. Clinically, one should expect to auscultate a systolic murmur due to the subvalvular aortic stenosis and a diastolic murmur due to the aortic valvular malformation and associated insufficiency (5,11). The diastolic murmur was not appreciated in this dog. Its intensity, if present, may not have been

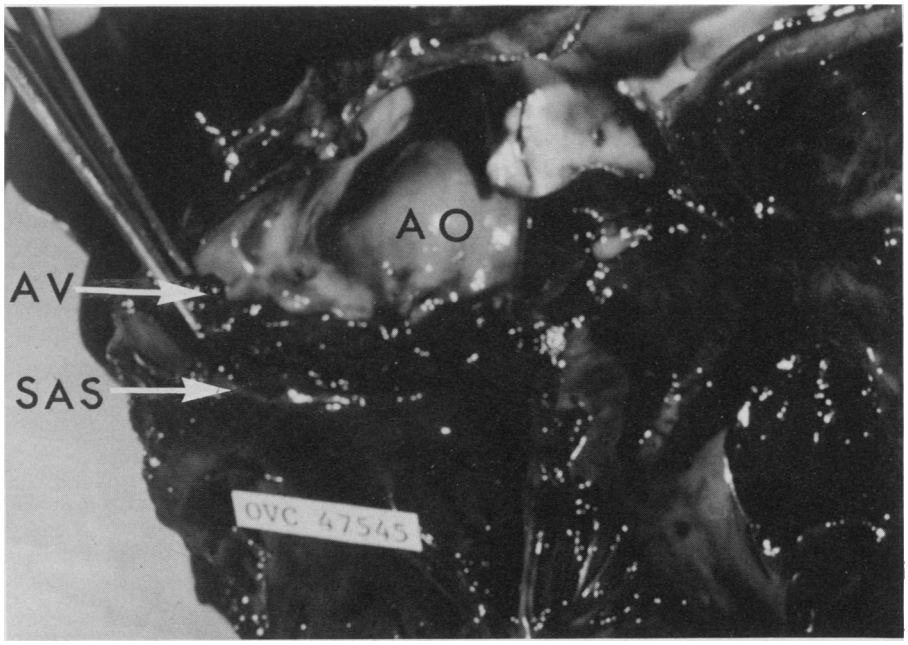

FIGURE 1. Aorta (AO), aortic valve (AV) and subvalvular aortic stenosis (SAS) is shown.