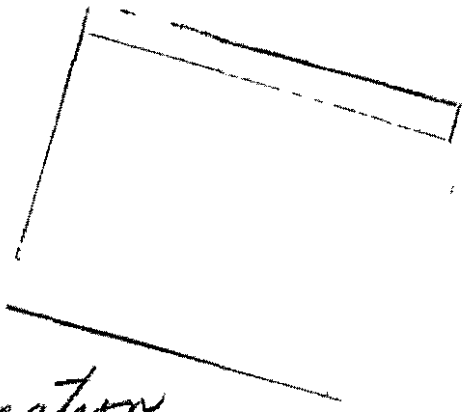


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THE PATHOGENESIS OF FOOT AND MOUTH DISEASE IN SWINE

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Even though foot and mouth disease (FMD) is a major economic threat to commercial pig farmers in endemic areas little information is available concerning the pathogenesis of the disease in swine. This paper describes histological observations made during both natural and experimental disease episodes in Colombia. A relationship is suggested between these findings and the epidemiology of the disease.

The natural disease A total of 19 swine necropsies were performed on three farms where FMD had been confirmed. The impact of the disease on these farms is shown by the following data. All were breeder-fattening operations.

Deaths from all causes in the first month of the first outbreak (Valley O subtype O1) were 82 of 270 pigs. In the second outbreak (also Vallee O subtype O1) deaths in the first two weeks numbered 36 of 200 pigs. Six sows aborted. The third outbreak (Vallee A subtype A27) was the most severe. In an operation totalling 1 093 animals 307 deaths from all causes occurred in the 4 months from the start of the outbreak and 105 fetuses were aborted. Twenty-four deaths (fatteners) occurred within 48 hours of the start of the outbreak.

Table 1 shows the age and sex of necropsied animals. Tissues from all organ systems were systematically collected for study in 10% isotonic buffered formalin, embedded in paraffin cut at 5 to 6  $\mu$  thickness and routinely stained with haematoxylin and eosin. Special stains such as van Gieson, PAS and von Kossa were used when necessary. Table 2 shows the order of prevalence of internal lesions following microscopic examination. Four of the animals that died acutely in the third outbreak had segmental necrotizing lesions involving the coronary artery. This vascular damage related to the FMDV could not be found described in the reviewed literature. Necrosis of the myocardium and somatic muscle was striking in most cases examined. Lesions in the liver were of the cordiac failure type. The pancreas of 4 of the animals examined showed regression of acinar cells, loss of the cord like arrangement and dropic degeneration of some islet cells. Notable in the series of necropsies performed in the third outbreak was the absence of external FMD lesions in two of the fatteners that died.

The experimental disease An experiment was designed to describe sequentially the pathological microscopic changes in pigs inoculated with a field strain of virus (Valley O subtype O1). In the field outbreak concerned muscle destruction was the most characteristic feature. A group of 15 animals (2 months old, average weight 20 pounds) was randomly divided into 5 subgroups of 3. The time interval for killing the subgroups was 1, 4, 7, 14 and 30 days post-infection. All animals were inoculated in the biceps femoris with approximately 1 000 mouse intraperitoneally LD<sub>50</sub>/ml of virulent virus. Five (5) pigs served as controls. Tissues collected for histological examination comprised all organ systems. They were treated and processed as described in previous cases. With few exceptions it can be said that the appearance of gross lesions coincided with the rise in body temperature. Most pigs showed clinical signs of FM three days post-infection. Histologically

myocardial lesions were found in 2 out of 15 inoculated animals (13.3%). Somatic muscle necrosis in 1 (6.6%).

The difference in prevalence of myocardial and somatic muscle lesions between the natural disease (84.2 and 63.2%) and the experimental disease (13.3 and 6.6%) requires explanation. An interesting possibility could be that the distribution of lesions between epithelial and muscle tissue could relate to size and perhaps frequency of the challenging dose of virulent virus. This in turn could relate to the epidemiological circumstances of an outbreak. Persons responsible for disease surveillance in countries free from foot and mouth should note that acute porcine cases need not show epithelial lesions.

The authors acknowledge the valuable help of Dr. E. D. Roberts.

Table 1. The age and sex distribution of 19 pigs necropsied following infection with Foot and Mouth Disease Virus

Age	Sex		Total
	Male	Female	
0 to 56 days	1	8	9
57 to 120 days	4	1	5
121 to 180 days	5	0	5
Total	10	9	19

Table 2. Distribution of internal lesion associated with Foot and Mouth Disease in 19 pigs necropsied

Site	Number of pigs in which lesions were found	Prevalence
		of lesions %
Myocardium	16	84.2
Gastrocnemius muscle	12	63.2
Liver	11	57.9
Masseter	7	36.8
Pancreas	4	21.0
Brain	2	10.5

Selected references: Garlunas P, Cottral G E and Seibold, H R (1964) Am J Vet Res 25 1062-1069; Henderson W M (1960) Adv Vet Sci 6 19-77; Platt H (1956) J Path Bact 72 299-312; Platt H (1958) J Path Bact 76 119-131; Seibold H R (1960) Am J Vet Res 21 870-877; Cunliffe H R (1962) Can J Comp Med Vet Sci 26 182-185; Graves J H and Cunliffe H R (1959) U S Livestock San Ass 63rd Annual Proceedings 340-345; Henderson W M (1952) J Hyg Camb 50 182-194.

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SWINE DYSENTERY CAUSED BY TREPONEMA HYODYSENTERIAE IN COLOMBIA

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Pathology and Microbiology Sections Animal Health Program CIAT  
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A large spirachaete Treponema hyodysenteriae was established by Harris, Glock and Christensen (1972) as an etiologic agent of swine dysentery in the United States. The disease was first recognized in Colombia in July 1974 in the Cauca Valley. Two farms were infected and detailed study was made on one.

A group of 378 pigs aged between 2 and 4 months developed a severe mucohemorrhagic diarrhoea. Their condition rapidly deteriorated in spite of treatments with broad spectrum antibiotics. Twelve animals died in the first week following the onset of dysentery.

Three animals were necropsied. Tissues for histological studies were fixed in 10% buffered formalin, processed by standard techniques and stained with haematoxylin and eosin. Sections of the large intestine were also stained with Warthin-Starry silver impregnation technique. Small amounts of mucosa and faeces from the colon were suspended in drops of physiologic saline and viewed by dark-field microscopy. Faecal smears from sick and necropsied animals were stained by Gram, Giemsa and carbol fuchsin. Colonic contents were submitted for cultivation of spirachaetes, Vibrio coli and Salmonella organisms.

Large highly motile spirachaetes were abundant in the mucosal scrapings and colonic contents examined. Fewer smaller tightly coiled organisms resembling Vibrio coli were also observed. Stained faecal smears showed numbers of faintly stained gram-negative large spirachaetes. The serpentine shape of the organisms was better defined when stained with Giemsa and carbol fuchsin. Balantidium coli was repeatedly observed in stained smears.

Histologic lesions in the colon and caecum were characterized by hyperemia and edema of the mucosa, with desquamated epithelial and red blood cells free in the lumen. Large spirachaetes could be seen intermingled with mucus and cellular debris on the surface of the mucosa and in the lumen of the crypts. Colon sections stained with Warthin-Starry revealed clearly defined large spirachaetes predominantly on the mucosal surface and inside the crypts. Balantidium coli was abundantly present on the surface and crypts of the intestine.

Treponema hyodysenteriae was isolated using the technique described by Harris, Kinyon, Mullin and Glock (1972). Vibrio coli was concurrently isolated.

All sick pigs were given Ronidazole\* in the drinking water and their condition was markedly improved in 24 hours. No diarrhoea was present on the fourth day of medication.

A second episode occurred on the same two farms in the same month a year later. Treponema hyodysenteriae was again isolated from sick and necropsied animals and Vibrio coli and Balantidium coli were again demonstrated. An additional

lesion observed was the presence of small circumscribed ulcers in the colonic mucosa. The outbreaks again responded dramatically to the same drug.

Attempt was made to transmit the Treponema artificially. The spirachaete was grown anaerobically for 72 hours on 5% equine blood agar plates. Two ml gelatin capsules were filled with washings from the plates using a phosphate buffer solution. Each of 8 SPF pigs aged 2 months were given 1 capsule orally and their faeces were checked daily by dark-field microscopy. Spirachaetes were detected in all animals 2-6 days post infection but clinical symptoms developed in only 4 within a 20 day period of observation. Symptoms were characterized by dark-grey diarrhoea, dehydration, weight loss and inappetence. All pigs were necropsied at the end of the period of observation. Macroscopic lesions were seen only in the sick pigs and consisted of colitis, typhlitis and regional lymphadenitis. Histologically pigs showed degeneration of the superficial epithelium and congestion and leucocytic infiltration of the lamina propria.

The field and experimental studies demonstrated the pathogenicity of Treponema hyodysenteriae. However the concurrent demonstration of one other organism known to be able to cause dysentery in swine (Balantidium coli) and the experimental demonstration of the spirachaete in the faeces of apparently healthy pigs leads to questions of the inter-relationship between the two organisms and the exciting cause for clinical symptoms.

Balantidium coli has been found as the sole pathogen responsible of outbreaks of dysentery in collaborating piggeries where malnutrition was rampant. Studies suggest that this usually non pathogenic protozoa can become pathogenic when an interaction of disease factors occurs. As spirachaete infected pigs are usually heavily infested with balantidium the possibility exists that the presence of the protozoa in diseased colons enhances the severity of lesions.

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\*Merck-Sharp and Dohme

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The following information is for the use of the Director of the Institute of Tropical Agriculture and Forestry (ITAP) and the Director of the Institute of Agricultural Sciences (IAS) of the University of the Pacific (UP) in Lima, Peru.

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FIRST OUTBREAK OF TRANSMISSIBLE GASTROENTERITIS (TGE) IN COLOMBIA

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Transmissible gastroenteritis of swine (TGE) was not known to exist in Colombia before an outbreak occurred in the Cauca Valley involving 6 commercial piggeries in January 1973

A group of 80 pigs was imported from 6 farms in Ohio and Indiana (USA) to Cali, Colombia. They were quarantined for 16 days prior to embarkation. There was no quarantine at the port of entry in Colombia and the animals were distributed to nine farms the day after arrival.

Seven days later a report was received of an epidemic of diarrhoea at one piggery, closely followed by reports the following day of a similar condition at two others. By the fifth day of the initial report the mortality rate in sucklings and weaned pigs was approaching 100 per cent and three more piggeries had been affected making the situation acute. Clinical signs were characterized by profuse yellowish diarrhoea, vomiting, weight loss, dehydration and death in pigs less than 3 months of age. Adult pigs presented similar but less severe symptoms with depression, agalactia in lactating sows and weight loss as characteristic features.

Ten animals were necropsied. Tissues for histological examination were fixed in 10 per cent neutral buffered formalin, processed by standard procedures and stained with haematoxylin and eosin. Small pieces of stomach, small and large intestine were placed in glass vials (5 ml each) containing Hank's solution for viral isolation. Sick pigs were bled for antibody determination. Liver, kidney, spleen, stomach and intestinal contents were taken for toxicological studies.

Carcasses were dehydrated. Main lesions at necropsy were confined to the stomach, small intestine and kidneys. They consisted of severe focal necrosis of the gastric mucosa, dilation of the small intestine which contained a copious yellowish-dark fluid and hyperaemia of the kidneys. The wall of the jejunum was almost translucent in most cases examined.

Microscopic lesions in the majority of stomachs examined were confined mainly to the fundic region. They were characterized by a severe necrosis of the mucosa involving the epithelium and lamina propria with complete destruction of fundic glands. Lesions in the small intestine were characterized by marked atrophy of the villi.

The crypt-villus ratio was nearly one to one and the columnar epithelium had been replaced by flattened cuboidal cells. Islands of denuded villi were swollen by congestion and oedema. The epithelial cells of the proximal convoluted tubules of the kidneys were swollen and the distal tubules had proteinaceous casts and crystalloid deposits.

After the microscopic examination of the tissues a diagnosis of TGE was made based on symptomatology, epidemiology and characteristic histological findings. The possibility of an intoxication was discarded.

Tissues and sera were sent to the Instituto Colombiano Agropecuario (ICA) Veterinary Research Laboratory (LIMV)

in Bogota and to the USDA Animal Disease Laboratory, Plum Island. The diagnosis was confirmed by virus isolation in Bogota and serologically by Plum Island. The range of antibody level in the sera against 100 TCID<sub>50</sub> of TGE virus was 1:4 - 1:126 which was considered in the positive range.

The outbreak occurred in six out of nine piggeries that had received imported pigs 2 weeks before. Taking advantage of fortuitous sera in the CIAT Animal Health serum bank, samples stored were studied by ICA comparing sera taken before and after the outbreak. No antibodies for TGE virus were detected in sera that had been taken before the outbreak in all six affected piggeries. Post-outbreak sera were TGE positive giving circumstantial evidence that the disease had been introduced by the imported animals.

Although the impact of the disease was devastating the majority of sucklings and weaners (1:141) being lost in affected piggeries, no evidence exists at present that the disease spread to other localities or appeared in the same piggeries after the initial episode. The suggestion has been made that the movement of pigs from problem to free zones could start a new outbreak indicating the possibility of carriers of the virus. Although this is quite feasible, recovered pigs from the outbreak in this country have been in contact in repeated occasions with swine from other areas with no signs of TGE appearing in susceptible animals. Continuous farrowing is said to be also an important contributing factor for the perpetuation of the disease in affected piggeries, but this is a common management practice here in the tropics.

Ecological factors may be significant in the epidemiology of the disease in tropical environments. Studies should be made on the epizootiology of TGE in non temperate zones to devise control measures accordingly. It is possible that the disease although devastating when first introduced (direct losses were calculated at US\$1 600 600) is of no subsequent importance in the tropics.

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DESCRIPTION  
This paper describes the first two outbreaks recognized in Colombia of porcine encephalomyelitis. Both occurred in the Cauca Valley near Cali.

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PORCINE ENCEPHALOMYELITIS IN COLOMBIA  
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Pathology and Microbiology Sections Animal Health  
Program CIAT apartado aereo 67-13 Cali Colombia (S. A.)

This paper describes the first two outbreaks recognized in Colombia of porcine encephalomyelitis. Both occurred in the Cauca Valley near Cali.

Although positive serological results were obtained of the presence of Teschen disease final confirmation must wait virus isolation and characterization

The first outbreak was seen in September 1973. In a total of 118 pigs there was a 27.1% morbidity and 8.4% mortality. Symptoms were characterized by depression, mild ataxia and muscle tremor. Coma and death occurred in animals less than two months of age. Irritability was not seen in affected animals.

The second episode of encephalomyelitis occurred in March 1975. In a total of 250 pigs several sucklings died with signs of central nervous system disorder. Symptoms were characterized by paralysis, prostration, clonic movements, nystagmus and death. Morbidity and mortality were impossible to evaluate as the piggery was going through an outbreak of leptospirosis that caused numerous abortions and neonatal mortality.

Two animals 15 days of age were taken to the laboratory and necropsied. The macroscopic examination of the tissues did not reveal significant changes. Tissues taken for histopathology comprised all organ systems. They were collected and fixed in 10% buffered formalin and processed by standard techniques. Tissues taken for virology were brain, spinal cord, liver, spleen, small intestine and kidneys. They were collected in 5 ml vials containing Hank's balanced salt solution pH 7.4. Porcine and rabbit kidney primaries were used as culture media.

Three piglets 2 days of age were taken to the laboratory and necropsied. The macroscopic examination of the tissues did not reveal significant changes. Tissues for histopathology and virology were taken and treated in the same manner as previously described in the first episode. Twelve (12) serum samples from sick and in-contact animals were taken for serologic studies.

Serum samples were taken from sick and in-contact animals for serologic studies.

Histologic findings Microscopic lesions in the animals examined were focalized in the brain and spinal cord. They consisted of a mild lymphocytic meningitis. No other significant histologic abnormalities were observed in the rest of tissues examined.

Histologic findings Microscopic lesions in both animals were localized in the brain and spinal cord. They consisted of mild lymphocytic meningitis at the base of the brain, over the cerebellum and to a lesser extent in the spinal cord.

Virology Primary attention was given again to the brain and spinal cord. Tissues from the three animals were treated the same way as previously described in the first episode. After 3 blind passages of brain tissue of animal 75C286 CPE was seen in rabbit kidney cells. Further work revealed the virus to have a titer of  $10^{6.5}$  ID<sub>50</sub> x ml. It produces clear plaques in tissue culture and is resistant to ether and chloroform. It is stable to heat in the presence of MgCl<sub>2</sub> and does not affect suckling mice intracerebrally. Further characterization of the agent isolated is being carried out by U.S.D.A. Plum Island Animal Disease Center.

Changes found in the grey matter of the spinal cord consisted of gliosis, predominantly composed of microglia. Lesions were localized in the ventral horns. Cerebellar changes were intense and consisted of focal destruction of Purkinje cells and molecular layers with perivascular infiltrations. Cerebral changes were mild and consisted of scattered glial nodules in the basal nuclei, olfactory bulbs, hippocampus and pons. Perivascular infiltrations or glial nodules were absent in other regions of the CNS.

Porcine encephalomyelitis may be widespread in South America than presently realized. There is need to study the possible importance of the disease to the swine industry.

Virology Primary attention was given to the brain and spinal cord. Tissues were ground with mortar and pestle and diluted to a 10% suspension with Hank's balanced salt solution as diluent. After centrifugation the supernatant fluid was used for tissue culture inoculation. After 10 blind passages in porcine and rabbit kidney primaries the possibility of virus isolation was discarded. The same procedure applied for the other tissues collected. No virus could be isolated.

The authors acknowledge the valuable help of Dr. A. H. Dardiri, Leader Diagnostic Investigations, PIADC.

Serology Thirty-two (32) serum samples for test for antibodies against Teschen disease were sent to the U.S.D.A. Plum Island Animal Disease Center. Thirty-one of them had significant titers against Teschen disease virus. Virus neutralization index (VNI) went from 2 to >5 in sick and in-contact animals. One serum sample taken 2 months before the outbreak had a VNI of >5. This points out to the possibility that the disease syndrome had been present in the piggery before the condition was actually noted.

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PROLIFERATIVE GLOMERULONEPHRITIS IN YOUNG PIGS

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Glomerulonephritis preceded by a history of streptococcal infection is commonly reported from human patients. The structural changes seen in the kidneys suggest that the tissue damage is related to an immunologic mechanism. The same condition has been suspected for some time in domestic animals. This paper presents clinical and histological observations from 25 Yorkshire pigs submitted for necropsy at various times from a farm where the buildings are heavily contaminated with a Group A Streptococcus

Four to 6 months old pigs commonly showed cutaneous abscesses from which could be cultured Streptococcus pyogenes Group A as the principal pathogen. Some animals showed a transient posterior paralysis and some assumed a dog sitting position. Recovery could be spontaneous without treatment others could become recumbent in spite of antibiotic therapy.

Table 1 lists the presence or absence of internal abscesses and kidney lesions. Abscesses could occur in the lungs, heart or deep in the muscles and the kidneys could appear enlarged, pale or mottled.

Kidneys for histologic examination were collected in 10% buffered formalin embedded in paraffin and sectioned at 3 to 5 μ. These sections were stained with haematoxylin and eosin (H and E) and periodic acid-Schiff (PAS).

Samples for routine bacteriological studies were taken from kidneys and abscesses seen at necropsy.

Sera were checked for the presence of antistreptolysin O titers (ASO) \*\*

Histologic findings A summary of the histologic features of the kidney sections studied is given in Table 2. The primary changes were glomerular in nature. Several types of glomerular lesions were observed: endothelial cell proliferation and exudation were the most characteristic. Epithelial cell crescents and adhesions of the visceral and parietal layers of Bowman's capsule were also present. Basement membrane thickening was absent in the sections examined.

Bacteriologic findings No bacteria could be isolated from affected kidneys. Streptococcus pyogenes Group A was constantly isolated from the abscesses.

Antistreptolysin O (ASO) titers No ASO titers were found in the sera tested in spite of all 18 animals showing glomerulonephritis histologically.

The clinical, histological and bacteriological findings suggested that the proliferative glomerulonephritis in this group of Yorkshire pigs was related to the streptococcal

infections. The similarity of lesions with those described in human cases also suggested the immunological nature of the tissue damage.

Although the results of the ASO tests were negative, titers of 12 to 125 Todd's unit had previously been detected on the same farm in piglets with acute streptococcal dermatitis. These are not as high as in comparable human cases. It is possible that antistreptolysin O antibodies are not formed as readily in pigs as in man.

Table 1 Major internal macroscopic findings in necropsied animals

Animal condition	No of pigs	Abscesses		Kidney lesions	
		Present	Absent	Present	Absent
Recumbent	13	8	5	13	0
Paralyzed	5	4	1	4	1
Dead	7	4	3	7	0
Total	25	16	9	24	1
Percentage		64%	36%	97%	3%

Table 2 Histologic features of affected kidneys

No animals examined	Glomerulonephritis				
	Endothelial proliferation	Exudation	Crescents	Adhesions	basement membrane thickening
25	25	23	19	23	0
Percentage	100%	92%	76%	92%	0%

Selected references: Earle D P and Jennings R B (1961) J Clin Invest 40: 1525-1595; Lerner R A and Dixon F J (1966) Lab Invest 15: 1279-1289; Lerner R A, Dixon F J and Sun Lee (1968) Amer J Path 53: 501-512; Woolcock J B (1973) Aust Vet J 49: 85-90; Editorial (1968) Amer J Med 44: 493-498

\*\*GIBCO Laboratories

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ISOLATION OF LEPTOSPIRA SPP FROM THE KIDNEYS OF BROWN RATS (RATTUS NORVEGICUS)

Guzmán TRAPPED ON INFECTED PIG FARMS  
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Program CIAT apartado aereo 67-13 Cali Colombia (S A)

Rodents have long been incriminated as maintenance hosts of Leptospira spp. pathogenic to domestic and farm animals. In the Cauca valley of Colombia serotype pomona has been isolated from aborted fetuses on pig farms and serologically positive reactions have been obtained for 6 serotypes with pomona, autumnalis and icterohaemorrhagiae predominating. All infected farms where leptospirosis constitutes an important reproductive constraint have heavy infestations with the brown rat (Rattus norvegicus). This study was made to determine the role of this rat in the epidemiology of the disease.

Four farms were initially chosen all having histories of leptospirotic abortions. Six traps were set each night on each farm and collected the following morning for 7 consecutive days. The live rats were taken directly to the laboratory, bled, killed by ether inhalation and necropsied. After a general examination the kidneys of each rat were removed aseptically, one being used for bacteriological culture and the other for histological examination.

**Bacteriology** Kidneys were ground in a mortar and pestle and then diluted to a 10% suspension using Sorensen's phosphate buffer (pH 7.5) as diluent. Serial dilutions were made on a ten-fold basis to  $10^{-3}$ . From the dilutions of each kidney 0.5 ml of the  $10^{-1}$  dilution was inoculated IP into a hamster and 0.5 ml of the  $10^{-2}$  and  $10^{-3}$  dilutions were inoculated each into 1 tube of semi solid Fletcher's medium and 1 tube of Korthof's liquid medium both containing 10% rabbit serum. Inoculated hamsters were routinely bled at 4-7 days and 9-11 days and blood was inoculated into the same media. All inoculated culture tubes were incubated at 29°C for 4 weeks and examined for growth at 7-10 day intervals. Subcultures were made from positive tubes and sent for identification to the Panamerican Zoonosis Center, Buenos Aires. To date 8 isolations have been made from 111 kidneys cultured (Table 1). Seven corresponded to serotype icterohaemorrhagiae and one to pomona.

**Histology of rat kidneys** Kidneys collected for microscopic examination were fixed in 10% formalin and processed by standard techniques. Lesions compatible with sub-acute and chronic leptospirosis were found in 48.6% of specimens examined (Table 2). Microscopic abnormalities were characterized by intertubular focal infiltration of lymphocytes, plasma cells, histiocytes and few neutrophils. Focal fibroplasia was seen in some of the kidneys examined. The fibrous tissue was replacing groups of proximal convoluted tubules. The glomeruli in affected kidneys escaped damage.

Tissue sections from bacteriologically positive kidneys were stained with Warthin Starry and examined microscopically. Numerous Leptospira microcolonies were observed in the bordering epithelium of proximal convoluted tubules as well as in the lumen.

National Live Trap Corp - 6 x 6 x 12 with roll trigger

The isolation of the serotype pomona is considered to be of epidemiological importance in relation to the abortions, infertility and stillbirths in sows on infected premises as well as neonatal mortality in piglets.

The serotype icterohaemorrhagiae has been responsible for acute leptospirosis in piglets in other countries. Its importance in the swine population of the Cauca valley has yet to be assessed.

The authors acknowledge the valuable help of Dr. E. A. Wells.

Table 1 Isolation of two leptospira types from kidneys of Rattus norvegicus trapped on infected pig farms

Pig farm	No. kidneys cultured	No. isolations	<u>Leptospira</u> type
A	8	1	L <u>pomona</u>
B	27	0	—
C	30	2	L <u>icterohaemorrhagiae</u>
D	46	5	L <u>icterohaemorrhagiae</u>
Totals	111	8	

\*Media used Fletcher and Korthof

Table 2 Kidney histological lesions indicative of leptospirosis found in Rattus norvegicus trapped on infected pig farms

Pig farms	No. examined	No. with lesions	% with lesions
A	8	0	0
B	27	11	40.7
C	30	13	43.3
D	46	30	60.5
Totals	111	54	48.6

Selected references: Michna S. W. and Ellis W. (1974) Res. Vet. Sci. 16: 263-264. Reis R., Ryn E. y Pena C. M. (1973) Arq. Esc. Vet. Minas Gerais 25: 11-14. Sullivan N. D. (1974) Aust. Vet. J. 50: 216-223. Center of Disease Control. Leptospirosis Surveillance Annual Summary 1974. Issued September 1975. WHO Scientific Group on Research in Leptospirosis. Memoranda. Geneva 1962.

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LEPTOSPIROSIS IN SWINE IN THE CAUCA VALLEY OF COLOMBIA

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Studies are under way to determine the prevalence and incidence of leptospirosis in pig farms collaborating with CIAT in the Cauca Valley. Following the isolation of the serotype Pomona from aborted fetuses, serum samples from various piggeries have been submitted for analysis to the Panamerican Center of Zoonosis in Argentina. Serologically positive reactions have been obtained representing 6 serotypes with Pomona, Autumnalis and Icterohaemorrhagiae predominating.

Leptospirae isolations have been made at 4 different locations to date. Three strains corresponded to the serotype Pomona and one to the serotype Kennewicki, serogroup Pomona.

Leptospirosis in infected piggeries has been clinically characterized by epidemic abortions occurring at any time during gestation. Fetuses are usually found decomposed, making it of importance to examine each one of them to select the best for culturing and histological examination.

Routinely aborted fetuses are taken to the Laboratory and bacterial isolation is attempted from the kidneys. They are ground in a mortar and pestle and diluted to a 10% suspension using Sorensen's phosphate buffer (pH 7.5) as diluent. Serial dilutions on a ten-fold basis are then made to 10<sup>-3</sup>. From the dilutions of each kidney 0.5 ml of the 10<sup>-2</sup> and 10<sup>-3</sup> dilutions is inoculated each into 1 tube of Korthof's liquid medium and 1 tube of semisolid Fletcher's medium both containing 10% rabbit serum. Culture tubes are then incubated at 29°C for 4 weeks and examine for growth at 7-10 days interval.

Histological examination has been useful in most cases for making a rapid diagnosis of the condition. Kidneys and livers are the organs of choice to examine. Diagnostic lesions seen histologically in these two organs can be listed as subcapsular haemorrhage, tubular necrosis and intertubular haemorrhage and oedema in the kidneys and focal necrosis in the livers with dissociation of hepatocytes seen in fetuses aborted at the end of the gestation period.

Tissue sections from suspected kidneys are stained with Worthing Starry and examined microscopically. If positive leptospirae are observed in the bordering epithelium of proximal convoluted tubules, as well as in the lumen.

Rodents trapped in infected piggeries have been found acting as carriers of two serotypes. Seven strains of the serotype Icterohaemorrhagiae and one of the serotype Pomona have been isolated from the kidneys of the brown rat (Rattus norvegicus).

Table 1 gives an account of approximate monetary losses due to abortions in an infected piggery. As it can be seen, losses

in 1974-1975 were in the order of US\$17,000 and US\$7,442 respectively. Streptomycin given IM at the recommended dose of 25 mg/kg body weight to all sows in epidemic situations has been the treatment of choice. In infected piggeries a single antibiotic injection has helped to bring the disease under control. A second injection given to all breeding stock is recommended at 2 weeks interval.

Leptospirosis in commercial piggeries in the Cauca Valley is possibly more widespread than presently thought. As studies are still in progress and relatively few serum samples have been tested to date, no figures are given yet relating to prevalence and incidence of the disease in the region.

The isolation of Leptospira interrogans serotype Kennewicki from aborted fetuses is of importance as it is the first isolation made in the Country and possibly the first written report of its isolation from aborted fetuses in Latin America.

The serotype Kennewicki has been responsible for one of the largest water-borne epidemics of leptospirosis in human beings in the United States.

Table 1 Abortions and direct economic losses due to Leptospirosis in an infected pig farm

Year	No sows	No abortions	No animals lost	Approximate monetary losses
1974 2nd half	490	76	514	552,746 Col \$ (US\$17,000)
1975 1st half	580	41	277	241,874 Col \$ (US\$7,442)

CONTROL PROGRAM

1975 2nd half 620

Streptomycin 25 mg/kg IM given to all sows in the piggery. Rat control.

Selected references: Dobson K. J. (1974) Aust. Vet. J. 50: 471. Michna S. W. and Ellis W. (1974) Res. Vet. Sci. 16: 263-264. Sullivan N. D. (1974) Aust. Vet. J. 50: 216-223. Center of Disease Control, Leptospirosis Surveillance Annual Summary, 1974, issue September 1975. WHO Scientific Group on Research, Leptospirosis Memoranda Geneva 1962.

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BRUCellosis A MAJOR REPRODUCTIVE RESTRAINT IN COMMERCIAL PIGGERIES

IN THE CAUCA VALLEY OF COLOMBIA

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Twenty of the commercial piggeries collaborating with CIAT were found by serological testing and culture to be infected with *Brucella suis*, biotype 1. As a result, a program was instituted in 1973 to determine the methods of control and eradication most acceptable to farmers. This paper gives a general account of the development of such a program to date.

Table 1 lists the main characteristics of 8 piggeries chosen to initiate the study. Administration, animal handling, types of buildings and health status were grouped under general management and graded as good (G), fair (F) and bad (B). Prior knowledge and improvement of these factors were thought to be essential if a control and eradication scheme was to succeed.

The baseline information needed for developing the methodology of approach was taken, from the 'Brucellosis Eradication', pamphlet published by the ARS-U.S. Department of Agriculture.

Plan 1 that recommends slaughtering of the infected herd and restocking was discarded as no validated brucellosis free herds existed in the Country at that time.

Plan 2 with few modifications was successfully applied in 3 of the infected piggeries (E, F, H - Table 1), where immediate elimination of all reactors was not feasible due to economic reasons. Basically the plan consisted of: a) testing and elimination of all positive boars. Animals with titers of 1/25 or more to both the slide and tube agglutination tests were considered positive; b) elimination of all non-pregnant serologically positive gilts and sows. Titters of 1/25 or more to both agglutination tests were considered positive; c) strict isolation of positive pregnant females and separation - isolation of offsprings at 56 days of age; d) testing of weaners at 6 and 7 months of age and if females 1 and then 2 months following parturition; e) elimination of infected females 56 days after parturition; f) as dogs that ate aborted fetuses in two of the infected piggeries were found with titers of 1/50 and 1/100, their elimination was recommended from all farms under study.

Plan 3 although not generally recommended was successfully applied in the remaining piggeries (A, B, C, D, G - Table 1). It consisted of: a) immediate elimination of all serologically positive reactors to both agglutination tests. Titters of 1/25 or more in both agglutination tests were considered positive; b) after the elimination of all reactors, all groups of breeding stock were tested at 60 days interval until 2 consecutive negative tests were obtained.

Three of the infected piggeries (E, F, G - Table 1) experienced foot and mouth disease (FMD) in 1972, shortly after the first brucellosis testing of breeding stock had been completed. The FMD outbreak lasted three months during which many positive gestating females aborted, because the FMD virus contaminating clean in-contact animals. Table 2 shows results of the agglutination tests conducted in the same

groups of breeding stock before and after the FMD outbreak. The percentage of brucellosis positive animals rose sharply in the three infected herds. Workers in countries where both diseases occur in swine should be aware of this fortuitous relationship to take measures accordingly.

Brucellosis in the Cauca Valley of Colombia still constitutes the most important economic threat to the swine industry. With the existence now of validated brucellosis free-herds, an effective campaign could be started by national authorities with the cooperation of farmers to put the disease into prospective for adequate control and eradication.

An economic analysis of the overall impact of brucellosis in infected piggeries, the cost of eradication, and the benefits resulting from its control is in progress.

Table 1 Characteristics of 8 farms chosen to initiate the brucellosis control and eradication program

Farms	No. breeding stock	Reactors %	General management			
			Adm	Animal handling	Build types	Health status
A	110	1	G	G	G	G
B	118	5	F	G	F	F
C	602	2	F	F	G	F
D	106	11	F	B	B	B
E	165	29	F	B	B	B
F	108	51	F	B	B	B
G	83	62	B	B	F	B
H	264	83	B	B	F	B
Good	G - Fair	F - Bad	B			

Table 2 Effects of a foot and mouth disease (FMD) outbreak in 3 brucellosis positive farms

Farms	% reactors in breeding stock before FMD	% reactors in breeding stock after FMD
E	18	29
F	20	51
G	21	62

Selected references: Duee, J. P., Gaumont, R. (1974) Bulletin de L'Office International des Epizooties, 82: 123-142. Marschang, F. and Cioloca, T. (1970) Vet Med Rev, Leverkusen 2: 116-130. Brucellosis Eradication Recommended Uniform Methods and Rules (1969) ARS U.S. Department of Agriculture.

FOOD INTOXICATION ASSOCIATED WITH AEROMONAS SHIGELLOIDES

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The gram-negative rod Aeromonas shigelloides also known as Plesiomonas shigelloides has been associated on several occasions with outbreaks of gastroenteritis in human beings. Reports of the isolation of this bacteria from animals are scanty. It would seem that as in man this organism can account for infections in some species of animals. The present paper documents the isolation of A. shigelloides from the faeces of diarrhoeic swine fed a contaminated concentrate in one of the CIAT collaborating piggeries.

**History** In July 30, 1974 a severe mucohaemorrhagic diarrhoea developed in 8 animals of a group of 30 York-Shire gilts maintained in confinement. Symptoms were characterized by depression, anorexia and intermittent bloody diarrhoea. The rectal temperature of affected animals was within normal limits. On the second day of the episode 7 more animals got sick and 3 died in spite of palliative treatment. Symptoms were similar as those of previous cases although an extreme paleness of the mucous membranes was noted in recumbent animals that died. Necropsy findings were characterized by ulceration of the pars aesophagea and fibrinous clots filling the gastric cavities of all 3 animals. The small and large intestines contained variable amounts of blood. Other organs looked pale but otherwise normal.

As an outbreak of gastric ulcers was suspected following necropsy findings an immediate search was then made of possible inciting causes. The source and quality of each one of the feed components was thoroughly checked. Samples of the complete ration as well as of each one of its components were cultured in thioglycollate fluid medium, tetrathionate and brain heart infusion broths. Faeces were cultured in SS and MacConkey agar. Management practices were revised.

Search of the source and quality of feed components revealed an altered bone meal. Shortage of the product in the market had obliged the manager to use a lot that had been improperly stored for 4 years. The meal had a deep rancid smell and small pieces of decomposed meat. He had used it in the proportion of 40 kg per ton of food.

Aerobic and anaerobic cultures of samples of the complete ration, bone meal and faeces grew out a gram-negative rod identified as Aeromonas shigelloides. Biochemical characteristics of the isolate included fermentation with dextrose in OF medium, acid but no gas from 1% dextrose and maltose and positive reactions for  $\beta$ -D galactosidase, catalase, oxidase, indole, lysine and ornithine decarboxylase. Lactose, sucrose and mannitol were unchanged and negative tests were obtained with citrate,  $H_2S$ , urea, gelatin hydrolysis and phenylalanine desaminase. Further confirmation of the isolate was done by the Enterobacteriology Unit, Faculty of Medicine, Universidad del Valle. Antibiotic susceptibility showed the

organisms to be susceptible to kanamycin, chloramphenicol, neomycin and nitrofurans, and resistant to penicillin, streptomycin, tetracyclins and ampicillin.

Two hamsters were inoculated intraperitoneally with 2 ml of a light A. shigelloides suspension. The animals died 1 and 3 days post-inoculation. Lesions at necropsy were characterized by petechiae in serosal surfaces, gastritis and enteritis. The gram-negative rod was recovered from intestinal contents in both cases.

The outbreak was controlled one week after the first symptoms appeared. The concentrate as well as the contaminated bone meal were immediately withdrawn from consumption. Sick animals seemed to respond well to the palliative treatment instituted for all the group the first day.

Fifteen gilts out of 30 developed haemorrhagic diarrhoea and 3 died of acute haemorrhagic anaemia caused by bleeding ulcers. Haemorrhagic diarrhoea as seen in all affected animals is not a known feature of A. shigelloides infections in man. Taking into account clinical signs and necropsy findings, the possibility exists that this group of York-Shire gilts were developing acute gastric ulcers as a consequence of being fed an altered concentrate. If that is the case the presence of the potentially pathogenic gram-negative rod could contribute to the exacerbation of the ulcerating processes leading to acute haemorrhage and death from anaemia in a few animals.

**Acknowledgements** Dr Pedro Gracian, Enterobacteriology Unit, Faculty of Medicine, Universidad del Valle, confirmed the identification of the strain of Aeromonas shigelloides isolated.

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- 1/ Calcium carbonate
  - Kaolin
  - Charcoal
  - Nitrofurazone
  - Vitamin F
- 09
- IM