THE EFFECT OF ASCARIS LUMBRICOIDES INFECTION ON IMMUNITY PRODUCTION BY LAPINIZED HOG CHOLERA VACCINES

by

KENNETH DUANE WEIDE

B. S., Kansas State College
of Agriculture and Applied Science, 1956
D. V. M., Kansas State College
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INTRODUCTION

Hog cholera losses in the United States alone amount to over 50 million dollars a year (5). The incidence of hog cholera, reported in 1956, was 2,756 outbreaks involving 56,316 animals in 42 states, Hawaii, and Puerto Rico (Mulhern, 89). It should be noted that these reported cases were from a limited number of practitioners and was therefore only an indication of the extent of the disease. Hog cholera is thought to cause greater economic losses than any other disease involving the swine industry (Hastings, 51).

Only about 50 per cent of hogs in the United States are vaccinated against hog cholera. Sixty to 70 per cent is considered sufficient to prevent any large epizootic (McNutt, 79). Hence, unless adequate steps can be taken to eradicate hog cholera, an epizootic might possibly occur even greater than those of 1913, 1926, and 1949-50 (Hutchings, 58).

In 1956, an estimated 72 per cent of all swine vaccinated against hog cholera were injected with modified virus. The use of serum and virulent virus had dropped from 96 per cent to 28 per cent (Aitken, 3 and Milligan, 86). The lapinized vaccines are hard to evaluate as there have been no general serious outbreaks of hog cholera since their introduction (58). Biester and Schwarte (13 and 14), in 1952, stated that the results of their tests indicated the same problems encountered with serum and virus could also be encountered with the new lapinized vaccines.

With new emphasis being placed by swine producers, livestock officials, veterinarians, and government agencies on the eradication

of hog cholera, it has become imperative that research be accelerated in an attempt to answer many of the perplexing problems confronting the swine industry. This project was undertaken because of the difficulty veterinarians sometimes encounter in vaccinating swine against hog cholera using attenuated vaccines. Field work indicated that frequently herds of swine in which losses occurred following vaccination had severe ascarid infection and considerable damage had been done to the liver by the migrating larvae. An attempt has been made here to investigate the effects of the lapinized hog cholera vaccines on the young pig, and to study the effects of ascarids and their migrating larvae on the growing pig and their resulting influence, if any, on the immunizing potential of such infected pigs.

REVIEW OF LITERATURE

Hog Cholera

> Swine fever (hog cholera, pestis suum) is a febrile infective disease caused by an ultra-virus (filterable virus) and characterized by an acute course with symptoms of septicemia, usually of a hemorrhagic character in the course of which croupous pneumonia and croupous diphtheroid processes in the gastro-intestinal tract may develop (Hutyra, et al., 59).

The disease was first observed in North America in Ohio in 1833 and gradually spread over the United States. The advent and growth of railroad transportation from 1875 to 1900 favored this spread. England reported its first outbreak in 1862, from which it spread to Sweden in 1887, and by 1895, the disease was prevalent throughout all of Europe (59).

In 1885, hog cholera was recognized as an independent disease by Salmon and Smith who attributed its cause to a short motile bacillus (Bacterium suipestifer). In 1903, de Schweinitz and Dorset put forth evidence to prove swine fever was caused by a filterable virus and that Bacterium suipestifer was a secondary organism. Almost at the same time Michigan workers reached the same conclusions. This was supported by European and African workers (59).

Classification and naming of the virus officially appeared in Bergey's Manual of Determinative Bacteriology, 1948 edition. It belongs to the family Charonaceae, the genus Tortor, and the species suis.

The incubation period of the virus is from five to ten days following contact with sick animals or their discharges. The first signs observed are usually a dullness and anorexia. The disease is generally accompanied by diarrhea, though constipation may occur during the early stages of symptoms. Body temperatures are 106° to 107° F. and purple hemorrhagic areas may appear on the abdomen. In acute forms, death may occur in a few hours. In less severe attacks the affected individuals may linger five to seven days (84). The morbidity and mortality are close to 100 per cent. If the course of the disease is prolonged more than seven days, bacterial complications may occur, shown chiefly by pneumonia and ulcerative enteritis (Hagan and Bruner, 48; Udall, 123).

Pathological changes (Runnells, 105) usually seen are petechia of the kidneys, urinary bladder, epiglottis and larynx, heart, lungs; mucosa of the large and small intestine; gastric mucosa and

serosa; and the serosa of the large and small intestines. Hemorrhagic lymphadenitis and splenic infarction are equally prominent. Cutaneous hemorrhages, catarrhal cecitis and colitis, catarrhal gastritis, and an encephalitis are sometimes seen (Udall, 123).

Nervous symptoms occur commonly due to the encephalitis. This is manifested by locomotor disturbances, local paralysis, grinding of the teeth, and occasionally convulsions (Hagan and Bruner, 48). In a recent study (Jones and Doyle, 62), 90 per cent of hogs affected with field cases of hog cholera had an encephalitis, though not all exhibited such outward symptoms.

Diagnosis is chiefly based on history of exposure, high body temperature, high mortality, and post-mortem lesions (84). Differential diagnosis from erysipelas, Salmonellosis, and Pasturellosis is frequently difficult. Inoculation of suceptible swine with filtered and non-filtered tissue extracts is a more positive means of differentiating these conditions.

Ascaris Lumbricoides Var. Suum

Swine ascarids, the large intestinal roundworms of swine, are found in hogs perhaps more often than any other internal porcine parasite (Morgan and Hawkins, 88). They are readily visible, the males averaging 150 to 250 millimeters by 3 millimeters and the females up to 400 millimeters by 5 millimeters. The eggs are very thick shelled and measure 50 to 80 microns by 45 to 55 microns, have an irregularly mammilated border, and are yellowish in color (88). Estimates on the number of eggs laid by each female run from 250,000 (88) to 1,400,000 (Kelley, 64) per day or a total of

30,000,000 to 168,000,000 in a lifetime.

The eggs are unsegmented when they leave the host. Under favorable weather conditions they embryonate in 14 days; but a period of 40 days or more is needed after embryonation for the eggs to have a high degree of hatchability (88). Newly embryonated eggs hatch poorly. The eggs have been known to live over 4 years in soil under continuous cultivation. Direct sunlight is lethal in 3 to 15 hours, but other weather conditions have little effect. Common disinfectants do not destroy the eggs (88).

Upon ingestion by a susceptible host, the "mature" eggs hatch and penetrate the intestinal wall, enter the mesenteric blood vessels and lymph channels, and are carried to the liver (88). They may reach the liver as early as 24 hours following ingestion of the egg (LaPage, 73). After considerable migration through the liver, the larvae are carried by the blood stream to the heart and on to the lungs, reaching the lung capillaries in an average time of 10 days (88). The larvae, now 0.7 millimeters to 1.5 millimeters long, then escape from the capillaries into the alveoli of the lungs where they grow and molt (Monnig, 87). From 7 to 23 days following infection, they migrate up the trachea and are re-swallowed (87). Usually about 10 days are spent by the larvae within the lung (73).

Larvae reach the small intestine, especially the ileum, in 21 to 29 days after ingestion of the egg, but on occasion have been known to arrive in 4 to 5 days (73). The parasite matures and eggs are produced in 54 to 70 days (73) (Underdahl and Kelley, 124) to 2 1/2 months (88) following ingestion of the embryonated egg.

Pathology arising from the larvae is due to tissue reaction against their presence and migration (88). The main site of damage is the liver (6), (73), (87), (88). If large numbers of larvae are present, small hemorrhages appear under the capsule and around the intralobular veins. The liver becomes enlarged and congested. Degeneration of the liver cord cells, characterized by cloudy swelling, fatty degeneration, and necrosis, especially around the central veins, then occurs. Fibrosis follows, which may be local or general and is macroscopically seen as the characteristic "mottling." This is due to superficial diffuse fibrosis just under the liver capsule. Larvae that die while in the liver, become encapsulated, may caseate, and later calcify. Biester and Eveleth (12) found considerable fluctuation in blood sugar levels during larval migration and since no pancreatic pathology could be detected, attributed it to liver damage. Twenty-four hours following ingestion of large numbers of ascarid eggs, Biester and Eveleth (12) found only an evidence of red blood cells in the liver. No larvae could be seen and the amount of interlobular connective tissue was small. By 48 hours the liver was swollen with numerous yellow and blue mottled areas on the capsule. The anterior surface exhibited hemorrhagic foci. The hepatic epithelium was granular and hydropic, foci of a dense infiltration of eosinophils were seen, and the connective tissue increase was significant. Seventy-six hours after ingestion, the liver showed considerable mottling and swelling, the epithelium was still hydropic and granular, and the eosinophilic infiltration was more generalized. By six days the surface of the liver was

studded with numerous yellow-gray areas and larvae were seen histologically. There was an advanced diffuse proliferation of interlobular connective tissue replacing the eosinophils. The hydropic condition of the epithelium was lessened and fibroblasts were abundant. By the 14th day, the liver was undergoing repair, the organ was not enlarged but the capsule was covered with numerous, overlapping gray areas. The hepatic cells were normal, the connective tissue had increased outlining the lobules distinctly, and the eosinophils were gone except for a few trapped in the connective tissue.

Lung pathology (6), (73), (87), (88), occurs when the larvae break from the capillaries into the alveoli. Hemorrhages and edema may occur in heavy infections as early as 22 hours after infection, but sometime between 7 and 23 days is most common. Hemorrhages caused by the larvae may persist 6 days. Due to secondary invaders, a pneumonia and bronchitis usually results with desquamation of the alveolar epithelium. Eosinophilic infiltration of the pulmonary parenchyma is common. Difficult breathing and a harsh cough persisting for 4 or 5 days constitute the so-called "thumps." Verminous pneumonia results from large numbers of migrating larvae.

Erratic migration (LaPage, 73) of the larval stages may occur, larvae being found in the heart, brain, kidneys, and lymph glands. The pathology is similar to that exhibited by the liver.

Pathology of the adult location (Monnig, 87 and Morgan and Hawkins, 88), is characterized by digestive disturbances, blocked bile ducts, and superficial ulcers of the mucosa caused by gnawing of the worm and further aggravation by the toxicity of dead adults.

Symptoms (87), (88), (124) depend on the severity of the infection. Cough, retarded growth, lung exudation, and pneumonia are common. Diagnosis (87) is usually made on pulmonary symptoms ("thumps"), unthriftiness, and fecal demonstration of the non-embryonated egg. It has been observed that after the infected pigs reach 35 pounds in weight, the growth rate is similar to non-infected pigs (87). The author's own observations tend to support this.

Treatment is necessarily limited to the parasite after it reaches the intestine. Sodium fluoride, oil of chenopodium, cadmium and piperzine salts, and phenothiazine are used as anthelmintics. Purging with skim milk is effective (88). Prophylaxis is by strict sanitation, constant vigilance, and using the McLean County Plan of parasite control.

Antibody Formation

The classical types of antibodies are plasma proteins (16), (17), (26), (102). Carpenter (26) stated many reasons for considering antibodies as serum proteins and few dispute the further classification into globulins, particularly gamma globulins (16), (83), (102). Mathews and Buthala (77) reported their experiments indicated that gamma globulins are responsible for passive immunity obtained from hog cholera serum.

The site of antibody formation, however, is a topic of considerable speculation. Most authors (16), (17), (26), (78), (83), (93), (97), (102), agree though that the reticulo-endothelial

(R-E) system is involved in antibody production. They also recognized that plasma cells and lymphoid cells may play a part. The spleen, liver, lymph glands, and bone marrow may be involved. Carpenter (26) suggested that the origin of antibodies might differ according to the site, route of inoculation, and nature of the antigen.

According to Maximow and Bloom (78), macrophages cannot be sharply separated from lymphoid cells. Their theory is that new macrophages arise from hypertrophy and development of lymphocytes and monocytes. If this is true, there would be little difference as to the cellular origin of antibody.

The functions of the liver in antibody production appear to be prominent. From a reticulo-endothelial standpoint, the liver is the largest organ of the body and is rich in fixed macrophages, the Kupffer cells, which are similar to other R-E macrophages and perform similar functions (47), (78). In addition, Popper and Schaffner (97) cited experiments which prove: (a) that antibodies against azodye proteins are formed by the Kupffer cells of the liver; (b) by partial blockage of the liver Kupffer cells, mild infections may become serious and resistance to certain infections is reduced; and (c) in many liver diseases of man and during antibody formation in rabbits, the cytoplasm of Kupffer cells is very basophilic, indicating the presence of nucleic acid, apparently as an indication of increased protein formation, some of which may be antibody. Physiologically speaking, amino acids are synthesized in the liver to plasma proteins (37), (56), (76), (97). Storage of

plasma proteins is largely in the liver (97). New plasma protein flows chiefly from the liver (76) and in hepatectomized animals, all plasma protein drops rapidly and drastically (97). Boyd (16) stated that it is reasonable to assume that antibodies are made in the same part of the body as other serum proteins and Merchant and Packer (83) stated that antibody formation takes place in those cells responsible for globulin production, i. e., liver cells.

From a standpoint of structure and physiology, the liver, as a part of the reticulo-endothelial system, can be assumed to be associated closely with antibody production.

Nutrition

The importance of protein to immunity production is cited by numerous authors (23), (24), (60), (69), (95), (98), (99), (100), (102), (113). Gell (43) and Schneider (107) stated that a severe and prolonged protein deficiency is necessary to interfere with antibody production. Rodabaugh and Elder (39 and 103), in a study of the role of protein in hog cholera immunization found that a ration grossly deficient in protein does not prevent immunization when vaccinated with serum and virus. Using modified vaccines, with or without serum, they found that losses occurred (when the immunity was challenged) only in pigs on a low protein diet. Only a small percentage of the low protein pigs were affected, however, most surviving with only a temporary body temperature rise. This indicated that some other factor was responsible for the break (or failure to become immunized). Whatever this factor may have been, it was aggravated by the deficient protein ration.

Vardiman, et al. (126) stated that a debilitating condition such as anemia did not alter passive resistance to hog cholera in suckling pigs.

Hog Cholera Vaccines

Prior to the development of prophylactic vaccines against the virus of hog cholera, outbreaks reached epizootic proportions about once each six to eight years in the corn belt of the United States. Since 1926, there has been no major epizootic of this disease (Hastings, 51).

The simultaneous method or serum and virus method of vaccination originated at the Iowa Experiment Station at Ames, Iowa, in 1905, by Dorset, Niles and McBryde (94). It was patented in 1906 and first used commercially in 1907 (81 and 114). For over 30 years it was the only means of combating the hog cholera virus and was widely and effectively used. Its many faults included the introduction of the infection into cholera-free areas, the maintenance of the live virus on the premises, the reaction of the host against the vaccine in spite of the use of serum thus tending to lower his resistance against other organisms, and the danger of contaminating the virus or serum with other swine pathogens (72 and 114).

In 1935 Boyton developed a vaccine from tissues of infected swine (Smith, 114). This was released for public use in 1940 (Merchant, 81) and had the advantage of leaving no local or systemic effects after vaccination, and could be used even in enteric-afflicted pigs (114). Though the vaccine has its

usefulness, its many disadvantages (82 and 114), included the need for two injections two weeks apart for solid immunity, two to three weeks were required before pigs were immune, the immunity lasted only 10 to 12 months, and serum could not be used simultaneously.

McBride, in 1942, developed the crystal violet vaccine for immunizing against hog cholera (Merchant, 81). The vaccine was of value at the time it was developed but had many disadvantages (83 and 114). Serum destroyed the immunizing ability of the vaccine. Pigs under seven weeks failed to develop immunity, two doses were required for solid immunity, and a period of 12 days elapsed before the animals were solidly immune. Its use was advocated after the 1949-50 breaks because it was a "dead" product and less likely to "light up" infections (Schneider, 108).

The shortcomings of the simultaneous, Boyton, and crystal violet products for immunizing swine led investigators to search for new and better methods. This search was aided by information obtained from experiments conducted during World War II. Many of these experiments were with the rinderpest virus in an attempt to develop a method of producing tissue vaccines and modifying the virus by animal passage, so that in case of biological warfare, large amounts of vaccine could be produced rapidly, and large numbers of animals vaccinated (Smith, 114). It was noted that sometimes when a virus is transferred from one animal to another of a different species, it becomes less virulent when injected back into the original species (Schmidt, 106). If this process is repeated in series, the virus then becomes non-pathogenic for the

original animal but still produces strong immunizing power (Smith, 114).

In 1949, and again in 1950, the need for new vaccines was emphasized by severe outbreaks of hog cholera and the finding in some of a variant virus (27), (98), (125).

The first reported evidence of actual transmission of hog cholera virus in other hosts was in September, 1939. After the 11th passage in a rabbit, the virus could no longer be detected by its pathogenicity for pigs following inoculation with rabbit blood (Zickis, 128).

Within the last decade, three types of modified vaccines utilizing animal passage or tissue culture have been developed, one of rabbit origin, one of porcine origin, and one of tissue origin (Gray, 46). Their advantages lie chiefly in that the animal is freed from a drastic leucopenia and the possibility of contaminating farm premises with live virus is omitted (119).

The tissue culture vaccine (114) was first offered for sale in February, 1953. It is a live modified virus vaccine developed by serial passage through tissue culture. The modifying culture consists of nutrient fluid containing swine serum and sterile bone marrow from hog cholera suspects.

In October, 1946, Koprowski, et al. (71) successfully carried the hog cholera virus for 12 consecutive passages in rabbits, using infected rabbit spleen. Also, after eight passages, the virus was passed back through one pig and then on to rabbits for eight further passages, using infected blood each time. Baker (8) reported in December, 1947, that if hog cholera virus, passed 10 to 15 times

through rabbits, was injected into susceptible pigs, only a thermal response took place and the pigs were then immune to unattenuated hog cholera virus. Hence, the virus had been attenuated for the pig but still retained an ability to produce immunity. This provided the basis for lapinized hog cholera vaccines.

In 1951, further experiments by Koprowski, et al. (72) using serially-passed virus in rabbits proved the so-attenuated vaccine to be non-pathogenic; it was not observed to gain virulence by hog to hog passage; and being of other species origin, it could not contain other pathogens for swine. The protection was found to be induced as early as the third or fourth day. This is probably due to an interference phenomenon which tends to blend into the growing antibody production or level. Protection was still solid at 12 months and the product was stable when desiccated.

In April, 1952, Koprowski (70) reported that following 295 serial passages in rabbits, the attenuated virus was still capable of immunizing swine. Field trials by Harvey, et al. (49) reported, in 1951, the use of lapinized vaccine in 74 herds, involving 10,147 pigs, 2,105 of which were in poor condition and considered poor risks for any immunizing procedure. No deaths and no reactions occurred and those non-vaccinated animals in the same herd remained healthy. Samplings from each herd, a total of 852 head, were challenged with the virulent virus in 14 to 90 days after vaccination. Ninety-seven and one-half per cent survived the challenge.

In 1951, Killinger, et al. (68) announced the development of a rabbit modified, swine propagated, live virus with high immunizing properties and low pathogenicity. Since it was of porcine origin, serum was used with the modified virus. In a field test involving 6,578 pigs, no post-vaccinal trouble was noticed and following challenge of 126 of the pigs, 96.8 per cent were immune. Over 95 per cent of 24 sows were still immune after 18 months.

Public announcement of the new vaccine in popular magazines took place in May, 1951 (Keilholz, 63). In August, 1951, three firms were licensed to produce modified hog cholera vaccines of animal origin. Two were of rabbit and one was of porcine origin (Smith, 114).

Significant protection against hog cholera is reported to take place if the animal is exposed to the live virus 24 hours prior to vaccination and if the virus and vaccine are given simultaneously (Harvey and Cooper, 50). Highly significant protection occurs one day after vaccination and thereafter. However, not all animals show resistance before the second day and therefore serum is recommended if exposure takes place before, during, or just following vaccination (Percival, et al., 96). A research report in 1954 (85) stated that only a slight thermal response occurred in swine vaccinated with modified hog cholera vaccines when challenged by virulent viruses from five different sources.

Smith (114) reported losses from rabbit vaccines at about one per cent and quoted Aitken whose survey found 0.57 to 2.72 per cent of pigs vaccinated with modified vaccines sickened and 0.1 to 1.0 per cent died. Herl (54) reported the use of modified vaccines in

2,433 herds involving 178,615 swine and found no losses in 2,077 herds (89 per cent) having 137,538 pigs. Deaths were reported in 356 herds (11 per cent) involving 41,077 head. An average of 4.9 per cent (1,932 animals) in the infected herds were lost. Losses on the total vaccinated was 1.1 per cent. Length of immunity has been found to be four years and two months in some cases (91).

In 1956, an estimated 72 per cent of all vaccinated hogs were injected with modified virus (Milligan, 86).

Hematology

A number of variations in the normal hematology of porcine have been reported and are summarized in Table 1. It appears that the erythrocyte and leucocyte counts are lower in the young than in the old. The lymphocyte count increased with age at the expense of the neutrophils. The eosinophil count has been reported by some to increase with age (Craft and Moe, 32) and by others to remain nearly the same (Gardiner, et al., 42). Basophils appeared to remain the same. Palmer (92) reported no sex differences in differential or erythrocyte counts but that leucocytes tended to be higher in male animals.

Kernkamp (67) credited Dinwiddie as first calling attention to the leucopenia that occurs during hog cholera. Cahill (20), Shu (111), Thorp and Graham (121), Cole (29), Kernkamp (67), and Lewis and Shope (74 and 75), all described a leucopenia as the most striking cellular change. The red cell count dropped gradually as the disease progressed (67), (74), (75), (111), (112). Some authors (Kernkamp, 67 and Shu, 111) reported a decrease in

Table 1. Summary of reported blood studies of swine of various ages.

Author	Age	RBC 10^6/ mm^3	WBC 10^3/ mm^3	Lym. %	Mono. %	Neut. %	Eos. %	Bas. %	Hem. %	Sed. mm/hr
Albritton (4)	Adult	6.93	7.0-20.0						30-53	3-8
	1-12 hours	5.51-5.91							39-40	
	1-10 days	2.62-5.26							18-36	
Coffin (28)		5.0-9.0	8.6-20.0	40-60	1-10	30-50	1-10	0-4	32-47	1-14
Coles (30)	Adult	5.0-8.0	9.0-16.0	48-50	1-4	38-43	1-5	0	32-55	
Craft (32)	Birth			44.8		54.9	0.1			
	30 days			62.3		34.1	3.7			
	60 days			63.4		27.3	9.2			
	90 days			61.0		34.4	4.5			
	120 days			55.1		41.1	3.6			
	180 days			64.9		30.6	4.4			
Dinwiddie (35)	3-5 mos.	6.33	11.83	59.0		35.0	3.0	0.5		
Dukes (36),(37)		7.40	17.11	47.0	8	41.0	2.5	1.0	41.5	1-14
Gardiner (42)	1 day		6.7-16.0	12-43		54-87			33-44	

Table 1 (concl.).

Author	Age	RBC 10^6/ mm^3	WBC 10^3/ mm^3	Lym. %	Mono. %	Neut. %	Eos. %	Bas. %	Hem. %	Sed. mm/hr
Gardiner (42)	8 days		5.0-16.5	51-61		34-48			27-35	
	15 days		7.5-13.2	57-76		22-44			28-47	
Giltner (45)	2.5-6 mos.	6.8-8.8	9.5-25.0	30-79.8	0.8-10	13-60	1.2-11			
Habel and Biberstein (47)		5.7 ±0.7	14.7 ±4.5	38 ±13	4 ±2	53 ±11	4 ±2			
Kernkamp (65)	Birth	5.5-7.0	9.0-16.0							
	7 days	3.0-4.5	9.0-16.0							
	14 days	5.0-6.5	9.0-16.0	69		28				
	3-6 wks.	5.5-7.0	9.0 16.0							
	7-12 wks.	6.0-7.0	14.0-20.0							
Palmer (92)	2-42 days	3.86	13.5	63.3	2.6	32.1	1.3	0.3		
Runnels (105)		6.70	15.9	52	3	39	5	1.2		
Shu (111)		6.14-8.12	17.3-26.2	34-58	0-2	25-55	4-22	0-2.5		
Swenson, et al. (120)	36 hours	5.10 ±1.13	7.36 ±1.81						33.9 ±4.9	

basophils, eosinophils, and lymphocytes with a neutrophil increase while others (Lewis and Shope, 74) reported a neutrophil decrease and only a slight decrease in lymphocytes. Runnells (105) stated that the leucopenia is really a lymphocytopenia. The sedimentation rate generally increased as the disease progressed as shown by Kernkamp (67) who reported a 10-fold increase in sedimentation rate per hour.

In a typical outbreak of hog cholera, lowered total leucocyte counts may be evident within 48 hours after infection occurs (74), and decline to one to two thousand per cubic millimeter (67), (112), at the height of the disease. This leucopenia may precede any temperature rise or any clinical manifestation, and the extreme leucopenia reached will fluctuate only slightly for 8 to 13 days (if the animal lives) and then will rise but never reach the preinfective level (74), (111), except in the case of secondary invaders in which a leucocytosis may occur (111), (112). In addition to this rapid and severe leucopenia, a slow progressive anemia of moderate degree with a showering of compensatory, nucleated erythrocytes, accompanies hog cholera (35), (67), (74), (111).

That eosinophilia occurs with ascarid infection is fairly well established (11), (16), (26), (37), (57), (105). They have been found to concentrate around invading parasites and were noted to remain at the periphery of an inflamed area and play only a minor role in phagocytosis of foreign material (116). It is now believed that the eosinophil plays some part in dealing with toxic materials, this material probably being derived from protein.

Campbell (22) stated that the eosinophilia accompanying parasitic infection is due to the fact that such organisms have large amounts of keratin and that the keratin is responsible for an antigen-antibody reaction which causes the eosinophil increase. The antibody-antigen reaction has large numbers of combining sites due to the wide distribution of the parasite throughout the body. Eosinophilia then, is intimately associated with hypersensitivity. Campbell, et al. (21) further stated that foreign proteins in themselves do not attract eosinophils but that one must have an allergic reaction before hand, and that this is what stimulates the eosinophilia. The histamine released by antigen-antibody reactions is thought by some (11), (127), to be the specific cause of eosinophil increases, and this is somewhat substantiated by the fact that histamine injections cause an eosinophilia similar to that caused by ascaris extract injections.

Most of the study involving eosinophils and ascarids has been made using ascarid extracts on guinea pigs. This ascarid extract is an insoluble keratin-polysaccharide of high molecular weight (22), (127). Campbell (22) found that intra-abdominal injections of 5 milligrams per 100 grams body weight of the extract elicited a rise to two and one-half per cent circulating-blood-eosinophils and that 20 to 25 milligrams per 100 grams body weight caused a 25 per cent eosinophil count. In the latter case, the total leucocyte increase was one to five thousand per cubic millimeter, this being due to increases in eosinophils and monocytes. Basophils increased in some animals. The eosinophil rise was noted in 2 to 4 days after initial injection and reached its maximum in 5 to

10 days. Antibodies against the extract were detected at the height of the reaction and for some time afterward.

Vaughn (127) reported an increase in blood eosinophils as early as six hours after injection of ascaris extract into guinea pigs. He also noted early pathological changes in the lung. The alveolar walls thickened by swelling of the alveolar epithelium. This began at the larger vessels and bronchi, and spread to involve large areas of the lung. This was followed by emigration of eosinophils from the blood vessels into the perivascular connective tissue. Later eosinophils were noted in the pulp of the spleen.

Speirs, et al. (115, 116 and 117), also noted an increase in eosinophils following the injection of ascarid extract. They found, though, that upon a second injection, or injection of an already sensitized guinea pig, there was a tremendous increase in eosinophils at the site of injection. The degree of concentration then, depends on the amount of prior sensitization.

If the keratin portion of the ascarid is responsible for the eosinophilia, then after death of the parasite you might expect a greater eosinophilia as the keratin portion of the parasite is more available after death. This was found to be true (Campbell, 22). In hookworm infection, eosinophilia regularly occurs only after death of the parasites (Ashford, et al., 7). If a large portion of the parasites enter the host and die before reaching the intestine, a greater eosinophilia might result or this might be the cause of the eosinophilia, none occurring if all larvae successfully reached their adult home.

Biester and Eveleth (12) reported on the chemical blood changes accompanying ascariasis.

Vaccination Breaks

A vaccination break is a condition where animals sicken following immunization against the hog cholera virus. A hog cholera break occurs when animals sicken with hog cholera: (a) after vaccination (using vaccines) without hyperimmune serum; (b) after the effects of the hyperimmune serum are no longer present; or, (c) when vaccinated animals sicken from a strain of the virus not accounted for in the serum. A serum break occurs usually prior to 14 days after vaccination when the serum fails to hold against the virus. Dick (33) defined a true serum break as one in which susceptible, normal, healthy swine are injected with a composite of viruses and adequate amounts of serum but the serum lacks one or more of the protective counterparts of the composite virus.

Immunization failures were foreseen by Dorset in 1909 when the simultaneous method was put into use. He said that success was not always to be expected (1), (13), (14), (122). Torrey, et al. (122) reported losses as early as 1906. Bennett (10) reported that breaks have occurred since 1908. Smith (114) reported the first large break occurred in 1911 when two per cent of a drove of 219 pigs died following the use of serum and virus. Dick (33) stated that 10 to 12 years following the advent of the simultaneous method, articles describing post-vaccinal sequelae began appearing in professional literature.

Starr (118) in 1916, offered the first report of an attempt to discover the cause of post-vaccinal failures. He thought improper care and feeding following vaccination, and the use of too little serum by economizing owners or administrators were the cause. Cahill (18), Hell (52 and 53), and Kernkamp (66) considered a latent, concomitant or secondary bacterial infection as the cause of vaccination breaks. Birch (15) and Kernkamp (66) also recognized that other conditions affect the well-being of the post-vaccinal pig. Cahill (18 and 19), recognized that sickness following vaccination was due to something other than hog cholera and suggested an abnormal host-antigen relationship. Benner (9), in 1927, was of the opinion that serum breaks were due to impotency of the hyperimmune serum. Birch (15) recommended careful handling of hog cholera biologics to prevent loss of potency.

Variant viruses have been recognized to cause post-vaccinal cholera since 1949 (27), (34), (38), (109), (110), (125). Strains of high pathogenicity, low pathogenicity, and chronic forms have been found and since specificity is the principle behind all immunological processes, variant viruses should be difficult to counteract (33).

Bennett (10) reported his investigations revealed that breaks were not due to immunological products. He also stated the health of the pig at the time of vaccination is no longer accepted as the sole cause of post-vaccination troubles. His observations revealed many breaks associated with atrophic rhinitis-infected herds. Quin (101) supported this and added in addition to atrophic rhinitis, swine influenza-affected pigs are poor

vaccination risks and frequently break with the disease. He also stated that it has been his observation that chick embryos may be totally refractory to certain viruses if the fertile eggs are obtained from flocks receiving antibiotics in their feed, and suggests that perhaps it is possible for pigs fed continuously on antibiotic rations, and from breeding stock fed antibiotics continuously, to become refractory to virus and modified virus.

Some pigs may not develop satisfactory immunity due to inherent inability to respond fully to the antigen (90). Schwarte (109) supported this, stating that individual responses are common and that some pigs are just more susceptible to pathologic strains of the virus than others. Unthrifty, wormy, improperly handled pigs may open the way to infection when the shock of vaccination comes along (27).

Dick and Engelbrecht (34) reported conditions where sows failed to passively immunize their three-week-old offspring though never coming down with the disease themselves and they suggested a higher level of immunity is needed against field virus than is needed against commercial virus.

Cahill (18), in his survey, found that only 19 to 30 per cent of animals affected during hog cholera breaks actually had the virus present. He therefore concluded that a large percentage of breaks are not due to hog cholera.

Breaks in 1949 and 1950 occurred 6 to 10 days after vaccination (1), and other breaks (13), (14), have been reported to occur 30 days, 40 days, and 60 days following vaccination by the simultaneous route.

Dick (33) suggested that no virus is completely composite, i. e., having every strain. Since the original virus used in the simultaneous method was obtained from sick animals in outbreaks and epidemics, it was undoubtedly composite, i. e., polyvalent. Hence it would be reasonable to assume some time a strain not included or one variant might be encountered and the serum would not effectively protect against nor the virus effectively stimulate immunity.

Breaks at three and six weeks following the use of Boyton's tissue vaccine have been reported as well as following the use of crystal violet vaccine (10), (13), (14).

The last major vaccination breaks occurred in the summer of 1950. In Illinois, in a limited field survey, Pratt (98) found mortality to range from 24 to 100 per cent. Necropsy of the afflicted animals revealed septicemia, bronchopneumonia or enteritis or both. Fever and leucopenia had occurred antemortem. After a series of tests, it was concluded that a virus was responsible for at least some of the losses. Variant strains of the virus, although not demonstrated, were not denied as being present. Pratt also considered post-vaccination losses due to a combination of factors which (a) prevent adequate development of immunity after vaccination, or (b) cause deterioration of such active immunity later.

Breaks following the use of modified vaccines were first summarized for 1951 (1), reporting trouble in 361 pigs out of 11,180 vaccinated, a 3.2 per cent average. In 1952, in answer to

a survey of the American Veterinary Medical Association, it was reported that in 719 herds involving 51,996 head in which the lapinized vaccines were used, 1,415 pigs (2.73 per cent) in 63 herds (8.8 per cent) sickened in 3 to 20 days, and 344 pigs died (0.66 per cent) (2). None of these animals received serum at the time of vaccination. With serum, following vaccination of 62,310 pigs in 905 herds, only 359 pigs (0.57 per cent) in 18 herds (1.92 per cent) sickened (in 4 to 14 days). The mortality rate was one-tenth per cent.

Hoffman (55) in a field trial, following losses up to 10 per cent with the use of serum and virus, reported no post-vaccinal difficulties in garbage-fed hogs vaccinated with lapinized vaccines.

Biester and Schwarte (13 and 14), reported post-vaccinal losses occurring 10 days, 14 days, 20 days, 41 days, and 7 to 8 weeks following use of lapinized vaccines.

Quin (101), discussing post-vaccination problems, reported in 1954 that a lethal hog cholera virus was isolated from 23 herds vaccinated with modified virus alone or with serum. In two of the cases, the owners had given rabbit-modified vaccines, and in 10 days a characteristic picture of hog cholera was present. One owner lost 147 out of 153 pigs. Quin concluded that modified vaccines are not safe and harmless as the public has been led to believe.

Schwarte (110) in a survey of vaccination breaks in Iowa in 1955, reported 132 cases against 200 in 1954, and 330 in 1953. These breaks were distributed as follows:

Unvaccinated	50
Serum and virus	20
Serum and modified virus	26
Modified virus only	27
Inactivated virus	3
No history	6
	132

Unfavorable reactions occurred 4 to 275 days following vaccination.

A case report (Fuller, 41) in February, 1957, in which lapinized vaccines were used on garbage-fed hogs, stated a loss of 1 per cent in vaccinating 13,033 pigs since 1952. Prior to this time, using the simultaneous method, losses of 10 to 16 per cent were encountered, the average being around 12 per cent.

Hastings (51) stated that "Widespread epizootics (of hog cholera) usually occur during the fall months in years when rainfall and parasites are abundant." Unthrifty, wormy, improperly handled pigs open the way to infection when the shock of vaccination comes along (27). Schwarte (110) stated that most problem cases of hog cholera came from vaccinated herds.

It is apparent by a study of post-vaccinal losses following the use of lapinized vaccines (1), (10), (13), (14), (41), (55), (110), (122), that only a small per cent of hogs vaccinated are involved. They, however, receive the publicity. Also noticeable is the apparent decrease in the number of pigs involved in the outbreaks. However, Hutchings (58) reported that it is difficult to evaluate the new lapinized vaccines as there have been no serious outbreaks since their introduction. He further stated that serious losses have occurred in 1913, 1926, 1949, and 1950; the cycles seeming to occur without respect to the amount and kind of vaccination. Biester and Schwarte (13 and 14), reported that

the new lapinized vaccines produce the same problems as encountered with serum and virus.

MATERIALS AND METHODS

During the course of the experiment, 7 sows of mixed breeding were farrowed in isolation and under as sanitary conditions as possible. A total of 60 pigs were obtained, of which 40 were saved for this project. Each pig was numbered and ear-notched accordingly.

The pigs were divided into 4 groups. Groups I and II were so arranged that experimental and control animals were litter-mates and suckled the same mother. Group I contained 2 controls and 4 experimental animals and Group II, 4 controls and 10 experimentals.

Groups III and IV were divided into control and experimental animals by litters, the experimental animals having no contact with the controls, as was the case in Groups I and II. Group III contained 4 controls and 5 experimental animals, and Group IV, 7 controls and 9 experimentals.

Sows and pigs were housed on concrete floors with similar walls, the pens being completely indoors. The floors were cleaned twice each day and hosed with water once each day. A 16 per cent protein, complete commercial ration was fed prior to farrowing and for the duration of the experiment, to both sows and pigs.

Blood Studies

All blood samples were collected aseptically from the anterior vena cava according to the procedure described by Carle

and Dewhirst (25). The citrated blood was analyzed within the next few hours by methods described by Coffin (28) for total erythrocyte and leucocyte numbers, hematocrit, sedimentation rate, and differential leucocyte counts. Blood smears were made at the time of bleeding and stained with Giemsa's stain within 48 hours. Only cells showing nuclear fragments separated by extremely thin filaments were considered segmented neutrophils.

Pigs were bled 7 to 14 days after birth. This bleeding and a second, 4 days later, taken at the time of ascarid egg inoculation, were used for a base line. Thereafter, blood samples were obtained at intervals of 4 days from both control and experimental animals until their death or termination of the project.

Ascarid Inoculation

Female ascarids were obtained from slaughtered swine at a local slaughter plant. The uteri from mature worms were removed and the eggs expelled into sterile petri dishes where they were mixed with a small quantity of tap water. One drop of merthiolate per dish was added to retard mold growth. These dishes were then incubated at room temperature with a minimum of light. During the course of incubation, examinations were made as to the progress of the embryos. After the eggs had become embryonated, a period of six weeks or more was allowed to elapse before their use.

A human catheter was passed into the stomach of each experimental pig and a culture, previously counted by direct microscopic count of a representative sample, was injected into the stomach of these pigs. Control animals were not inoculated.

Experimental animals from Groups I and II were inoculated with 165,000 live embryonated ova. Groups III and IV were given 125,000 live embryonated ova. These inoculations took place at approximately the same time as the second bleeding period.

Vaccination and Challenge

At approximately eight weeks of age, all remaining experimental and control pigs were vaccinated subcutaneously with two cubic centimeters of modified live hog cholera vaccine. No hyperimmune serum was given. Body temperatures were recorded for five succeeding days. Three weeks from this date, a challenge dose of four cubic centimeters of commercial virulent hog cholera virus was given subcutaneously. The pigs were again temperatured for five succeeding days.

Necropsy and Histopathology

All animals dying during the experiment and those remaining following termination of the project underwent necropsy according to procedures set forth by Roderick (104) and the results were recorded. Representative sections of liver and lung and other organs of interest were taken for histopathological study. Intestinal contents were screened for ascarids.

Tissue sections were fixed in buffered formalin; and mounted and stained with hemotoxylin and eosin, van Gieson's stain and Shorr's stain, according to routine laboratory procedures of the Department of Pathology, Kansas State College, Manhattan, Kansas.

RESULTS AND DISCUSSION

Pigs No. 1 and 2 were control animals for Group I and were first bled at 10 days of age. Their counts were low in total leucocytes (average 7,000 per cubic millimeter), high in lymphocytes (85 per cent), and low in neutrophils (10 per cent) when compared to previously reported results for that age (Table 1). Slight scouring was evident from the 10th to the 12th day but stopped upon the addition of oxytetracycline to the sows' ration. Erythrocyte counts gradually increased after the first bleeding (5,500,000 per cubic millimeter) until termination of the experiment (8,600,000 per cubic millimeter). The hematocrit corresponded to the erythrocyte counts. Total leucocyte counts increased (to 18,000 per cubic millimeter) until two days following vaccination which occurred at 58 days. A leucopenia (11,000 leucocytes per cubic millimeter) occurred at this time, which lasted approximately 10 days before a gradual return to normal. Differential leucocyte counts remained essentially normal except for a slight rise in stab cells and a slight decrease in lymphocytes. Sedimentation rate was increased slightly following vaccination. These pigs were weaned at 72 days of age, and were challenged with virulent virus at 80 days of age. The body temperature remained normal following challenge as well as the blood picture except for a leucopenia less severe than that following vaccination but which required nearly as long to return to normal. This again appeared to be due to a decrease in lymphocytes. Sedimentation rates were increased slightly. At 93 days of age, both

pigs were sacrificed. Pig No. 1, during necropsy, exhibited no gross pathology except for one ascarid in the intestines. Pig No. 2 was similar except for four adult ascarids in the small intestine. These presumably were obtained by ingestion of eggs passing through the experimental pigs unhatched. Histopathology revealed mechanical atelectasis in the lungs of both animals and the liver of pig No. 2 contained more connective tissue than that of pig No. 1, though still within the limits of the "normal" pig. Kidney sections were essentially normal.

Pig No. 3, an experimental animal, was first bled at 10 days of age. The blood picture was essentially the same as pigs No. 1 and 2, but corresponded more closely to counts previously reported by others (Table 1). At 15 days of age, pig No. 3 was inoculated with 165,000 embryonated ascarid ova directly into the stomach by means of a stomach tube. Seven days later the condition referred to as "thumps" was very evident and continued until shortly before death, which occurred at 29 days of age, two weeks from the inoculation date. Necropsy revealed an anemia, increased mucus in the bronchi, lungs emphysematous, and many small areas of pneumonic foci in the apical and cardiac lobes of both lungs. The liver contained multiple white foci, large yellow mottled areas, and areas of congestion. The spleen was slightly enlarged. No parasites were observed in the small intestine. Histopathology of the liver revealed a marked increase in connective tissue, especially around the triads, resulting in a pseudolobule arrangement of the liver cells. These areas exhibited many eosinophils and young fibroblasts within the connective tissue. Fatty changes of the

parenchymal cells occurred around the central veins, many cells were swollen, and pressure atrophy had occurred next to the connective tissue. Some ghost cells were present. The liver in general exhibited degenerative changes. The lung showed evidence of an early pneumonia, with focal edematous areas, leucocytic infiltration, cellular exudate in the bronchi, and many ruptured alveoli. A few eosinophils could also be seen in the supporting tissue of the lung sections. The kidney showed evidence of an early nephrosis. Death was considered to be due to respiratory disturbances.

Pig No. 5, an experimental animal, corresponded quite closely with pig No. 3 except that death occurred when 36 days of age, or three weeks from the date of inoculation with embryonated eggs. The blood picture revealed a rapid increase in stab cells. These cells increased from four per cent at time of inoculation to 53 per cent two days prior to death, although the total white cell count remained fairly constant (5-7,000 per cubic millimeter). The sedimentation rate increased three-fold during this time. "Thumps", severe at times, were evidenced from the 22nd day of age until death. Necropsy revealed the blood to be anemic. The entire lung was spotted with localized areas of congestion and the right apical lobe was hemorrhagic. Small areas of pneumonia were seen, especially in the ventral portions of the lung. The liver was mottled and enlarged. Parasites were not found in the small intestines. Histopathology revealed considerable hemosiderin diffusely scattered throughout the liver lobules, and an increase

in connective tissue, especially around the triads. Pseudolobulation, while not extensive, was readily seen. Some of the liver cells were destroyed, lymphocytes were numerous, and young fibroblasts were common. The lungs revealed a pneumonia with cellular infiltration of many lymphocytes, a few polymorphonuclear cells, a few fibroblasts, and a few eosinophils. A considerable amount of cellular debris was present in the bronchi and alveoli. Many bronchioles were enlarged and the lung was edematous in these areas. Death of the animal probably was due to respiratory embarrassment resulting in anoxia.

Pigs No. 4 and 6 were both experimental animals. They differed from pigs No. 3 and 5 in that both survived the course of the experiment. Both were bled at 10 days of age and they exhibited some scouring between the 10th and 12th days. At 15 days of age, they were inoculated with 165,000 embryonated ascarid eggs and both exhibited "thumps" about 6 days later, though not as severe as pigs No. 3 and 5. Four days following inoculation, total circulating eosinophils in pig No. 6 had risen from less than 1 per cent to 5 per cent. By the 8th day, the count was over 6 per cent; by 12 days, 5 per cent; and by 20 days had decreased to less than 1 per cent. Pig No. 4 showed only a 3 per cent increase in circulating eosinophils 4 days after inoculation. Other blood studies revealed counts similar to those previously reported by other authors (Table 1) until 2 to 3 days following vaccination (at 58 days of age) when a leucopenia developed dropping the total leucocyte count an average of 4,000 cells per cubic millimeter.

The lymphocyte count increased temporarily at the expense of the neutrophils. Sedimentation rate was doubled for pig No. 6 and remained within normal ranges for pig No. 4. Both animals were weaned at 72 days of age. A leucopenia of less severity also followed the challenge with virulent virus (at 80 days of age), the blood picture being similar to that following vaccination. There was no increase in body temperature after either vaccination or challenge. Both pigs were sacrificed and underwent necropsy at 93 days of age. The livers showed evidence of minor fibrosis and the lungs exhibited a repaired pneumonia. Four adult ascarids were found in pig No. 6 but none in pig No. 4. Histopathologically, the liver contained a slight increase in connective tissue as compared to control pigs, No. 1 and 2. The lungs exhibited a chronic condition with thickened septal walls containing many macrophages. The kidneys appeared essentially normal in both animals.

Control animals for Group II were pigs No. 7, 8, 9, 10, and 23. Pig No. 10 was removed 20 days from the beginning of the project and placed on another experiment. The first bleeding of this group occurred at 15 days of age, the blood picture closely resembling those listed in Table 1. Significant blood changes did not occur until vaccination (at 55 days of age) when a leucopenia followed in four days, the leucocyte count dropping from an average of 16,600 per cubic millimeter to 12,400 per cubic millimeter. A slight rise in sedimentation rate and a slight decrease in per cent of lymphocytes was noted at this time. Twelve days later, following vaccination, the blood picture had returned to normal. Body temperature elevation was not noted during this period. The

pigs were weaned at 62 days of age. Following challenge at 76 days of age, a leucopenia resulted very similar to that which occurred following vaccination. Again no temperature rise was evidenced following challenge. All four pigs remained healthy throughout the experiment and were sacrificed at 89 days of age. Necropsy revealed no gross pathology and no parasites were found in the intestines. Microscopic sections of the liver, lung, and kidney did not disclose any outstanding pathological changes.

Group II experimental animals were numbers 11 to 18 inclusive and numbers 26 and 28. Pigs No. 14 and 26 died during the experiment and are discussed separately.

Pig No. 14 was inoculated with 165,000 embryonated ascarid ova at 21 days of age. Within 7 days, a severe coughing was evident ("thumps") accompanied by listlessness and depression. This condition persisted for 10 days, during which time the hair coat became dull and roughened, and the pig became emaciated. The animal seemed to recover partially until approximately 30 days after ascarid inoculation when the original symptoms recurred. He appeared weak and anemic and in 14 days was dead. The blood picture of this animal showed a peak erythrocyte count (6,000,000 per cubic millimeter) at 31 days of age (12 days post-inoculation) and decreased to 4,000,000 per cubic millimeter 2 days prior to death. The hematocrit was correspondingly deficient. Total leucocyte counts had remained similar to the control animals except during the relapse period when it was greatly elevated and reached 31,000 per cubic millimeter just prior to death. Following vaccination, the total leucocyte count had dropped 5,000 per

cubic millimeter. Sedimentation rate was increased throughout the experiment. Immature forms of neutrophils predominated (47 per cent) in the differential counts, especially near the terminal end. Eosinophil counts rose from less than 1 per cent to 27 per cent 12 days following inoculation and as late as the 36th day post-inoculation remained at 18 per cent. Necropsy revealed pneumonic lungs, a fibrotic liver and the usual findings of a septicemia, including hemorrhagic lymph nodes, congested spleen, liver and kidneys; and petechia on serosal and mucosal surfaces. No parasites were isolated by screening the intestinal contents. Histologically, the liver revealed an increased amount of connective tissue with diffuse pseudolobulation of the liver cells. Eosinophils were numerous in both the connective tissue and the liver sinusoids. Many of the liver cells were swollen and some had undergone pressure atrophy. The connective tissue around the triads was especially abundant. The lungs were pneumonic, with cellular infiltration of polymorphonuclears, macrophages, and unidentified lymphoid cells. The bronchi were filled with exudate and solidified areas were numerous. The kidney exhibited only congestion.

Pig No. 26 differed only slightly from pig No. 14, just described. The blood picture was essentially normal except for an increase (to five per cent) in the number of circulating eosinophils eight days after inoculation. Symptoms of respiratory distress were severe at this time. This pig died 20 days after inoculation. Necropsy revealed gross pathology similar to pig No. 4. Histopathology consisted chiefly of an increase in

connective tissue throughout the liver and resulting in pseudo-lobulation of the liver cord cells. Many of these cells were atrophic. Eosinophils had invaded the connective tissue, especially in the mass of connective tissue surrounding the hepatic triads. A few ghost cells were also visible. Sections of the lung revealed consolidation with lymphocytic infiltration. Several foci of necrosis were evident in scattered locations but in no particular pattern.

Experimental pigs No. 11, 12, 13, 15, 16, 17, 18, and 28 all survived the course of the experiment. They were not particularly good-doing animals though supplemental feed was available to them as soon as they would eat. Five to 7 days following inoculation, all developed "thumps" which gradually disappeared in 2 weeks time. Vaccination was at 55 days of age and challenge at 76 days. No body temperature changes were noted at either time. Blood studies indicated the characteristic leucopenia following vaccination and challenge previously described for other pigs. Recovery occurred in an average time of 12 days. A decrease in numbers of circulating lymphocytes seemed to be one of the chief factors resulting in the leucopenia. Eosinophil counts were elevated by the 8th day post-inoculation and reached an average of 13 per cent 12 days after inoculation. Pig No. 16 had a 7 per cent count at 8 days, 16 per cent at 16 days, and 23 per cent at 20 days for the maximum percentage of circulating eosinophils found. Only 1 animal (pig No. 15) failed to reach a 10 per cent count at 8 to 12 days post-inoculation. At 89 days of age, these pigs were sacrificed and a necropsy performed. Pig No. 18 had 1 intestinal parasite

present; the remaining pigs were negative for parasites. This was assumed to be due to the large quantities of milk consumed by this group of pigs necessitated by decreased production of the dams due to mammitis and constipation. Many of the lungs revealed areas suggestive of a previous pneumonia with repair having taken place. The livers contained scattered white areas which were visible only on close inspection. Other gross lesions were not apparent other than one pig (No. 16) which had a nephrosis of both kidneys due to an inguinal hernia which allowed a section of intestine to become looped over and around the ureters compressing the ureters and resulting in retention of urine and pressure atrophy of the kidneys. Microscopically the liver contained increased connective tissue with suggestions of pseudolobulation. The connective tissue was mainly interlobular with many old and a few young fibroblasts. The liver cells appeared normal. Eosinophils were not observed in these sections. The lung contained many areas of thickened septa due chiefly to cellular infiltration of macrophages. These changes were thought to be evidence of a repaired or a chronic condition. Kidney sections were essentially normal.

Experiments with Group III were conducted under similar conditions as Groups I and II except that inclement weather prevailed throughout most of the period. A severe infestation of lice, in spite of treatment, weakened both control and experimental animals and as a result, mortality was high. The etiology of these losses could not be established by necropsy findings in many cases.

Experimental pigs of Group III were bled at nine days of age and were inoculated with 123,000 embryonated ova four days later.

Blood studies at this time revealed an erythrocyte count of 4,000,000 per cubic millimeter and a leucocyte count of 7 to 11 thousand per cubic millimeter. Differential counts revealed 60 to 70 per cent lymphocytes and 30 to 40 per cent neutrophils. Circulating eosinophils were negligible. Four days following inoculation, all experimental pigs (Nos. 1A through 5A) developed a respiratory distress, lasting 5 to 7 days, and severe in all but pig No. 2A. Pigs No. 5A, 4A, 3A, and 1A died at 23, 25, 26, and 32 days post-inoculation, respectively. They had been in poor condition and failed to make normal gains. Blood counts preceding death indicated little change from the base counts given above, with the following exceptions: pigs No. 3A and 5A showed an increase in neutrophils with a shift to the left at the expense of lymphocytes, with pig No. 3A having a total leucocyte count of 48,000 per cubic millimeter two days prior to death. Pig No. 1A survived the longest, and the eosinophil count reached 21 per cent, 12 days post-inoculation. The lungs of all four pigs, upon necropsy, had areas of pneumonia with solidification of lower lobes and emphysema of upper portions. Pig No. 1A, surviving the longest, exhibited more pronounced signs of pneumonia. Pig No. 3A had a large abscess on the costal surface of the right lung, probably accounting for the high leucocyte counts prior to death. The livers of these pigs were mottled with indications of fibrosis. Histologically, the lung sections contained areas of consolidation with polymorphonuclear and lymphocytic infiltration. The liver sections revealed an increase in connective tissue with excessive pseudolobulation, especially in

pig No. 4A. Many of the liver cells were undergoing degenerative changes. Eosinophils were numerous, and especially abundant around the triads and interlobular connective tissue. These were the first sections to show eosinophils of significant numbers in the sinusoids and central veins. Histologically, the kidney sections were essentially normal.

Since only one pig remained in experimental Group III, the experiment was not completed; pig No. 2A being sacrificed at 57 days post-inoculation. The blood picture varies from those summarized in Table 1 in that the eosinophil count had greatly increased following inoculation. Six per cent circulating eosinophils were found 8 days after inoculation. Twelve days later, this had increased to 13 per cent, and by 16 days had dropped to 8 per cent. Twenty-four days post-inoculation, this had decreased to 3 per cent circulating eosinophils. Necropsy findings revealed a mottled liver with many fibrotic foci, and extensive adhesions of the pericardium and pleura with evidence of a recovered pneumonia in the lungs. No parasites were screened from the intestinal contents. Histological examination revealed areas of the lung containing many macrophages and a liver with only a moderate connective tissue increase, but with many eosinophils observed in the sinusoids and interlobular connective tissue.

Controls for Group III were pigs No. 6A, 7A, 8A, and 9A. All four died during the course of the experiment with symptoms resembling acute Salmonellosis, both grossly and histologically.

Blood counts corresponded with those reported by other workers for normal healthy pigs (Table 1). Just prior to death, their blood pictures became very erratic. Inclement weather, a poor milking mother, and a serious louse problem were considered to play an important role in these losses.

Experiments with Group IV were conducted under similar conditions as those of previous groups. Inclement weather and a louse infestation were present and complicated this experiment as they did for Group III.

Pigs No. 17A, 18A, 20A, 21A, 22A, 23A, 24A, and 25A were designated as experimental pigs and inoculated with 123,000 embryonated ascarid ova 16 days after birth. Respiratory distress ("thumps") was apparent within 5 days, accompanied by loose stools, rough hair coat, and general unthriftiness. Twelve days post-inoculation, pig No. 22A succumbed. The leucocyte count increased to 19,250 per cubic millimeter from 9,800 per cubic millimeter with a marked shift to the left. Circulating eosinophils were minimal (one to two per cent). Necropsy findings revealed considerable gray hepatization and compensatory emphysema of the lungs. The liver was mottled with many white foci. Histological sections of the lung revealed many macrophages within the lung tissue and alveoli, and pneumonic areas were numerous. Liver sections again revealed pseudolobulation with considerable connective tissue present. Eosinophils were concentrated around the triads, while a few were seen in the parenchyma proper. The liver cells around the central veins of some lobules were undergoing fatty degeneration.

The remainder of Group IV experimental pigs lived to conclude the experiment. Blood studies revealed a picture similar to that previously related for Groups I and II experimental. Erythrocyte counts were low throughout the experiment due probably to a louse infestation that would not respond to treatment. Leucocyte counts decreased from an average of 13,500 per cubic millimeter to 11,000 per cubic millimeter following vaccination and from 13,200 per cubic millimeter to 11,100 per cubic millimeter following challenge. Sedimentation rates increased slightly following both vaccination and challenge. Hematocrit determinations corresponded with the lowered erythrocyte counts. Except for a rise (to three per cent) in eosinophils 8 days after inoculation, differential blood counts resembled those previously reported for normal healthy pigs (Table 1). The high eosinophil count persisted for 16 days at which time it returned to less than 1 per cent. Body temperature increases were not detected throughout the experiment. These pigs were sacrificed at 74 days of age and a detailed necropsy performed. The condition of the pigs at this time was, in general, one of unthriftiness. Grossly, the lungs revealed many areas suggestive of a repaired pneumonia. The livers contained many fibrotic foci and showed the mottled appearance of previously described livers of experimental pigs. Pigs No. 21A and 24A contained several small abscesses on the surface of the livers. From 3 to 12 ascarids were found in the intestinal screenings of each pig. Histological sections of the liver of pig 17A were essentially normal. The livers of pigs No. 18A, 20A, and 23A had a slight increase in interlobular connective tissue and many mature

fibroblasts. The liver of pig No. 25A exhibited a moderate amount of pseudolobulation.

In the livers of pigs No. 21A and 24A, several areas were observed not previously seen in any other sections. The liver of pig No. 21A had one large foci of eosinophils (larger than a high power field) surrounded by lymphocytes. These areas were surrounded by a layer of connective tissue with many young fibroblasts. The liver cells at the margin of the connective tissue had been destroyed. Sections of liver from the other pig (No. 24A) contained several of these foci. These differed only in that eosinophils and lymphoid cells were mixed and surrounded by a connective tissue layer. Lung sections from these pigs revealed only mechanical atelectasis.

Pigs No. 10A, 11A, 13A, 14A, and 15A were used for control animals for Group IV. Blood studies were almost identical to those described for Group II control animals. No temperature rises were evidenced following vaccination and challenge. Parasites were not found in the intestinal screenings. Gross lesions were negative as were histopathological findings.

Pig No. 12A was also a control animal for Group IV. This pig, at 26 days of age, was found dead, and necropsy revealed signs of acute Salmonellosis. Death of this pig occurred at the same time as the death of pig No. 9A and was also attributed to Salmonellosis.

Table 2 summarizes the gross pathology observed in the experimental pigs. Table 3 summarizes the histopathology found in the livers of the experimental pigs.

Table 2. Gross pathological findings in experimental pigs.

Pig No.	Age at death (days)	Anemia	Pneumonic foci on lungs	Liver cirrhosis	No. A.l. found in int.[1]	Other findings
3	29	Yes	Numerous	Severe	0	Spleen enlarged
4	93	No	Repaired areas	Minor	0	None
5	36	Yes	Numerous	Severe	0	Apical lobe of lung hemorrhagic
6	93	No	Repaired areas	Minor	4	None
11	89	No	Repaired areas	Minor	0	None
12	89	No	Repaired areas	Minor	0	None
13	89	No	Repaired areas	Minor	0	None
14	68	Yes	Numerous	Severe	0	Generalized septicemia
15	89	No	Repaired areas	Minor	0	None
16	89	No	Repaired areas	Minor	0	Nephrosis
17	89	No	Repaired areas	Minor	0	None
18	89	No	Repaired areas	Minor	1	None
26	50	No	Numerous	Severe	0	Generalized septicemia
28	89	No	Repaired areas	Minor	0	None
1A	32	Yes	Numerous	Moderate	0	Compensatory emphysema of lung
2A	57	No	Repaired areas	Moderate	0	Adhesions of pericardium and pleura
3A	26	Yes	Numerous	Moderate	0	Abscess on costal surface of lung

Table 2 (concl.).

Pig No.	Age at death (days)	Anemia	Pneumonic foci on lungs	Liver cirrhosis	No. A.l. found in int.[1]	Other findings
4A	25	Yes	Numerous	Moderate	0	Compensatory emphysema of lung
5A	23	Yes	Numerous	Severe	0	Compensatory emphysema of lung
17A	74	Yes	Repaired areas	Moderate	3	None
18A	74	Yes	Repaired areas	Moderate	6	None
20A	74	Yes	Repaired areas	Moderate	5	None
21A	74	Yes	Repaired areas	Moderate	11	Numerous small abscesses on liver
22A	28	Yes	Few areas	Severe	0	Compensatory emphysema of lung
23A	74	Yes	Repaired areas	Moderate	7	None
24A	74	Yes	Repaired areas	Moderate	12	Numerous small abscesses on liver
25A	74	Yes	Repaired areas	Moderate	9	None

[1] Number of Ascaris lumbricoides found in the small intestine at autopsy.

During the interval of the preceding experiments, fecal samples of all sows were negative for parasite eggs. Except for those instances mentioned, the sows remained in good health throughout the experiment.

Table 3. Histopathology of the liver of experimental pigs.

Pig No.	Age at death (days)	Increased connective tissue: Capsule	Triads	Pseudo-lobulation	Eosinophils: In sinusoids	In C. T.[1]	Liver cells
3	29	Marked	Marked	Marked	Few	Numerous	Degenerative changes
4	93	Normal	Slight	Slight	None	None	Normal
5	36	Moderate	Marked	Moderate	None	Few	Many destroyed
6	93	Normal	Slight	Slight	None	None	Normal
11	89	Normal	Normal	Slight	None	None	Normal
12	89	Normal	Normal	Slight	None	None	Normal
13	89	Normal	Normal	Slight	None	None	Normal
14	68	Moderate	Marked	Marked	Numerous	Numeroua	Swollen, pressure atrophy
15	89	Normal	Normal	Slight	None	None	Normal
16	89	Normal	Normal	Slight	None	None	Normal
17	89	Normal	Normal	Slight	None	None	Normal
18	89	Normal	Normal	Slight	None	None	Normal
26	50	Moderate	Marked	Marked	Few	Numerous	Degenerative changes
28	89	Normal	Normal	Slight	None	None	Normal
1A	32	Slight	Moderate	Moderate	Few	Numerous	Degenerative changes
2A	57	Slight	Moderate	Moderate	Few	Numerous	Degenerative changes
3A	26	Slight	Moderate	Moderate	Numerous	Numerous	Degenerative changes

Table 3 (concl.).

Pig No.	Age at death (days)	Increased connective tissue: Capsule	Triads	Pseudolobulation	Eosinophils: In sinusoids	In C. T.[1]	Liver cells
4A	25	Moderate	Marked	Marked	Numerous	Numerous	Degenerative changes
5A	23	Slight	Moderate	Moderate	Numerous	Numerous	Degenerative changes
17A	74	Normal	Normal	Normal	None	None	Normal
18A	74	Normal	Normal	Slight	Few	Few	Normal
20A	74	Normal	Normal	Slight	Few	Few	Normal
21A	74	Normal	Moderate	Moderate	Few	Numerous	Eosinophilic foci in liver
22A	28	Moderate	Severe	Severe	Few	Numerous	Degenerative changes
23A	74	Slight	Slight	Slight	Few	Few	Normal
24A	74	Normal	Moderate	Moderate	Few	Numerous	Eosinophilic foci in liver
25A	74	Normal	Slight	Moderate	Few	Few	Normal

[1] Connective tissue.

This experiment supports the view that pigs exposed to large numbers of ascarids become unthrifty during the first weeks of life. With few exceptions, experimental pigs could easily be recognized by their rough hair coat and slow growth rate.

Blood studies indicated that following vaccination with modified vaccines and challenge with virus, a transitory leucopenia results. This was not too severe and is supported by the work of commercial companies (44) although detailed information on blood studies following vaccination with lapinized vaccines could not be found or was not made available from reluctant commercial firms.

Cole (29) reported that leucopenia is a regular phenomenon following vaccination with serum and virus, and in many cases is then followed by a leucocytosis resulting in a higher leucocyte count than was present before vaccination. His experiment (on 55- to 90-pound pigs, using 35 cubic centimeters of serum and 2 cubic centimeters of virus) indicated a drop of from 8 to 20 thousand in numbers of circulating leucocytes in 3 to 5 days following vaccination. These changes returned to normal by the 6th to the 7th day and exceeded the original counts in some cases by the 8th day. According to Cole (29) the leucopenia following the simultaneous vaccination method became less severe as increased amounts of serum were given, and the smaller the dosage of serum, the greater the leucopenia. Serum given alone does not cause a reduction in leucocytes.

A leucopenia was observed in these experiments, not only following vaccination but also following challenge (Plate I, Fig. 2). The leucopenia was less severe than that reported following the use of serum and virus (29) and is supported by work of others (44). The leucopenia developed within four days followed by a mild leucocytosis. The leucopenia observed following challenge was less severe but slower developing and was also followed by

EXPLANATION OF PLATE I

Fig. 1. Averages of erythrocyte counts during the course of the experiment.

Fig. 2. Averages of leucocyte counts during the course of the experiment.

A. Time of inoculation of experimental animals.
B. Time of vaccination of all animals.
C. Time of challenge of all animals.

PLATE I

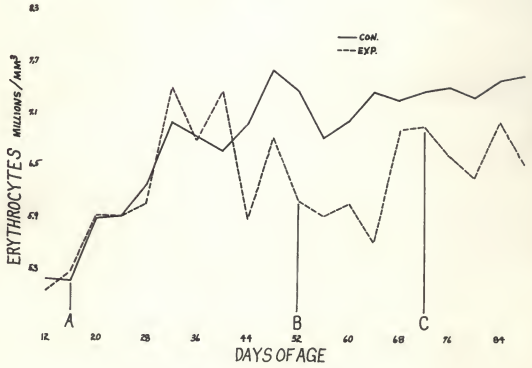

Fig. 1

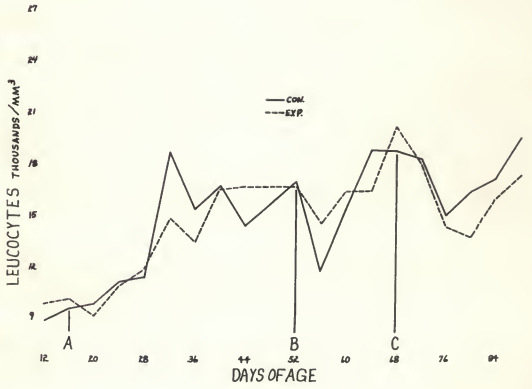

Fig. 2

a leucocytosis.

Runnells (105) stated that the leucopenia of hog cholera was really a lymphocytopenia. The lymphocyte picture in this experiment indicated a shift to the right following vaccination (Plate II, Fig. 3). The sedimentation rate increased following vaccination and challenge (Plate II, Fig. 1). A mild anemia was noted in the experimental pigs during the experiment (Plate I, Fig. 1).

Blood studies on control animals (Table 4) did not always follow those previously reported and summarized in Table 1. Lymphocytes decreased rather than increased and neutrophils increased instead of decreasing. The erythrocyte counts increased in numbers from birth to seven weeks of age. Leucocytes increased until approximately 50 days of age and then remained rather constant.

Eosinophils appeared in the circulating blood of experimental animals in 4 to 8 days following inoculation with embryonated ascarid eggs (Plate II, Fig. 2). Histological sections of animals succumbing a short time following inoculation indicated that circulating eosinophils appeared sometime later than the eosinophilic infiltration of tissues (liver and lung). The results of unpublished work at this station substantiate these findings. Eosinophils were seen in the tissues (liver, especially) in 24 hours or less post-inoculation (Plate III, Fig. 1; Plate IV, Fig. 1).

The significance of the eosinophilic foci found in the liver sections from pigs No. 21A and 24A can only be speculative (Plate V). It has been reported (116) that eosinophils concentrate around invading parasites. The areas of eosinophils observed were

EXPLANATION OF PLATE II

Fig. 1. Averages of sedimentation rates during the course of the experiment.

Fig. 2. Averages of eosinophil counts during the course of the experiment.

Fig. 3. Averages of lymphocyte counts during the course of the experiment.

A. Time of inoculation of experimental animals.
B. Time of vaccination of all animals.
C. Time of challenge of all animals.

PLATE II

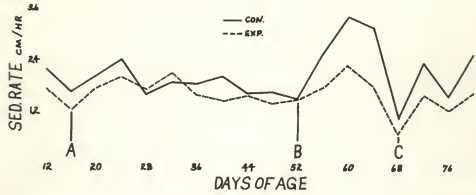

Fig. 1

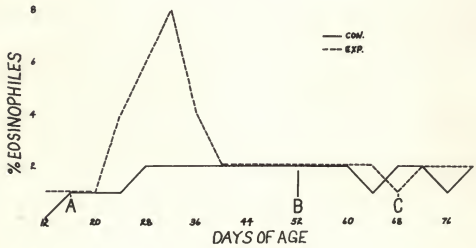

Fig. 2

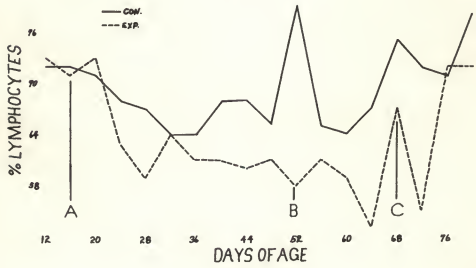

Fig. 3

Table 4. Summary of blood studies of control pigs.

Days of age	RBC 10^6/mm^3	WBC 10^3/mm^3	Sed mm/hr	Hem %	Poly %	Stab %	Lym %	Mono %	Eos %	Bas %
10	4.71	7.53	2.64	20.4	17	4	78	1	-	-
14	4.78	11.19	1.27	21.8	19	8	73	1	1	-
18	5.27	10.32	1.83	23.4	18	6	72	2	1	-
22	5.39	10.87	1.76	24.5	17	18	65	-	-	-
26	5.48	12.65	2.00	25.2	13	16	69	2	-	-
30	5.93	12.28	1.77	25.5	17	14	66	1	2	-
34	5.66	10.88	1.54	27.1	13	14	71	1	1	-
38	6.24	14.90	1.19	27.4	12	20	65	1	2	-
42	5.67	13.00	1.32	26.7	11	18	69	1	1	-
46	6.38	14.17	0.28	26.9	9	22	67	1	1	-
50	6.32	15.30	1.27	27.8	8	24	66	1	1	-
54	6.60	14.50	1.33	29.7	10	21	65	2	3	-
58[1]	6.16	14.64	1.96	29.0	9	23	65	2	1	-
62	6.65	13.82	2.09	30.1	13	19	64	1	3	-
66	6.42	15.67	2.30	30.9	12	22	62	1	1	-
70	6.25	15.77	1.76	31.5	10	22	63	3	2	-
74[2]	6.51	16.46	2.03	29.9	8	17	72	2	1	-
78	6.37	14.96	1.45	29.8	10	21	67	1	1	-
82	6.51	14.30	1.82	30.0	11	20	69	-	-	-
86	7.29	16.08	2.19	32.0	11	20	66	1	2	-
90	7.15	17.26	2.28	33.0	2	18	77	2	1	-
94	7.63	16.82	1.90	32.5	9	34	55	1	1	-

[1] Vaccination with modified hog cholera vaccine occurred at this time.

[2] Challenge with live hog cholera virus occurred at this time.

EXPLANATION OF PLATE III

Fig. 1. Connective tissue mass containing numerous eosinophils. (Hematoxylin and Eosin, x450)

A. Liver cells.
B. Connective tissue.
C. Eosinophils.
D. Vein with erythrocytes.

Fig. 2. Liver of experimental pig. (Hematoxylin and Eosin, x95)

A. Thickened capsule.
B. Pseudolobule.

PLATE III

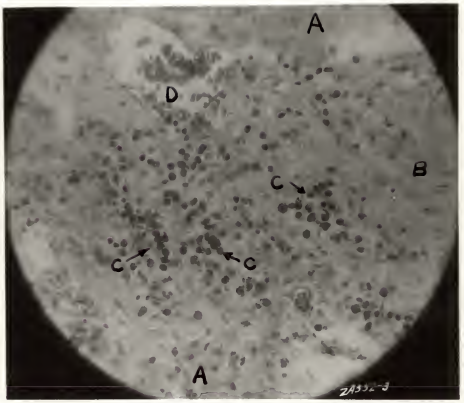

Fig. 1

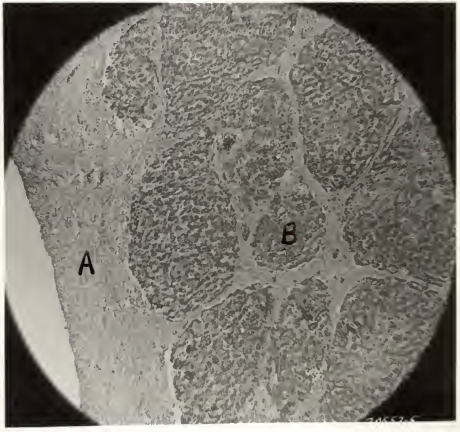

Fig. 2

EXPLANATION OF PLATE IV

Fig. 1. Hepatic triad of normal pig. (Hemotoxylin and Eosin, x450)

Fig. 2. Hepatic triad of experimental pig. (Hemotoxylin and Eosin, x450)

A. Liver cells.
B. Connective tissue.
C. Eosinophils.

PLATE IV

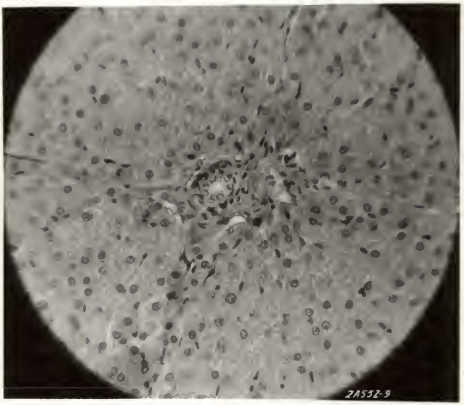

Fig. 1

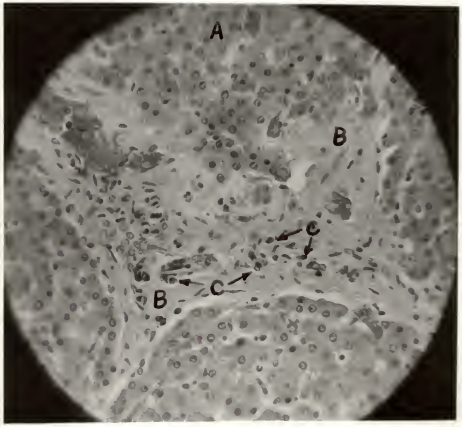

Fig. 2

EXPLANATION OF PLATE V

Fig. 1. Eosinophilic foci with surrounding lymphocytic cells. (Hemotoxylin and Eosin, x95)

A. Foci of eosinophils.
B. Connective tissue surrounding A.
C. Area of lymphoid cells.
D. Connective tissue surrounding C.
E. Liver cells.

Fig. 2. Foci of eosinophils and lymphoid cells. (Hemotoxylin and Eosin, x95)

A. Triad with increased connective tissue.
B. Area of eosinophils and lymphocytes.
C. Liver cells.

PLATE V

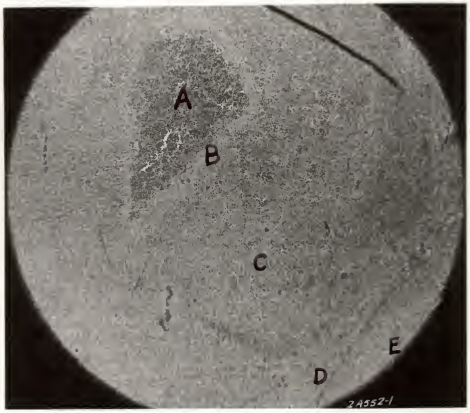

Fig. 1

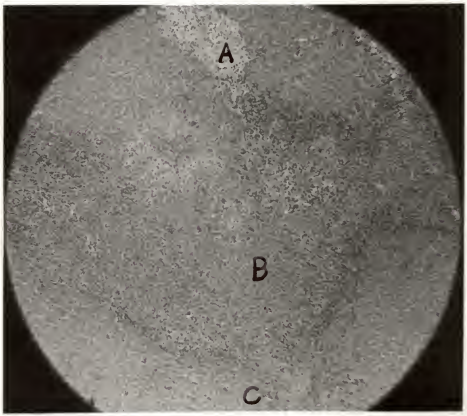

Fig. 2

perhaps former locations of larvae as they passed through during migration. Perhaps the larvae became stranded for a period of time in these areas. Had serial sections of these livers been made, perhaps more of these areas would have been observed.

Attempts to isolate larvae from infected animals by maceration of liver and lung tissue was unsuccessful. These attempts were made on animals dying 10 or more days post-inoculation. Similar results have been reported (64) for animals 11 or more days post-inoculation, and it is considered that the larvae have already migrated through the liver and lung by this time. Other work at this station indicates that perhaps some or many larvae may by-pass the liver and go directly to the lungs.

Death of the experimental pigs, other than those suspected of having contacted Salmonellosis, was considered due to pneumonia with resulting respiratory embarrassment and anoxia.

The abscess found on the lung of pig No. 3A was in all probability caused by migrating larvae carrying pathogenic bacteria during their migration. Plate VI shows ascarid larvae in the lung during their migration.

Three control animals were noted to have ascarids in their intestines at necropsy. These were observed only in Groups I and II where both control and experimental animals suckled the same dam and remained in the same pen. It is logical to assume that following inoculation of experimental animals with embryonated ascarid eggs, some of the larvae were passed in the feces unhatched and were picked up by control pigs in spite of sanitary procedures. Intrauterine infection cannot be dismissed, although

EXPLANATION OF PLATE VI

Fig. 1. Ascarid larvae in lung (arrows). (Van Gieson's Stain, x95)

Fig. 2. Ascarid larva in lung. (Van Gieson's Stain, x450)

PLATE VI

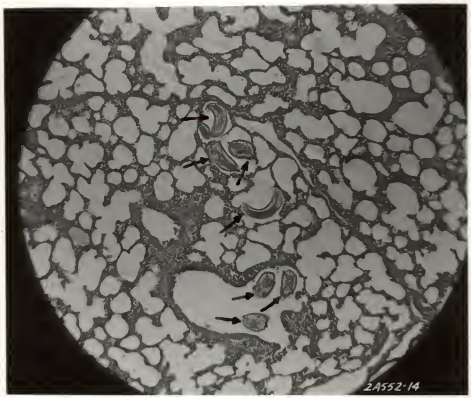

Fig. 1

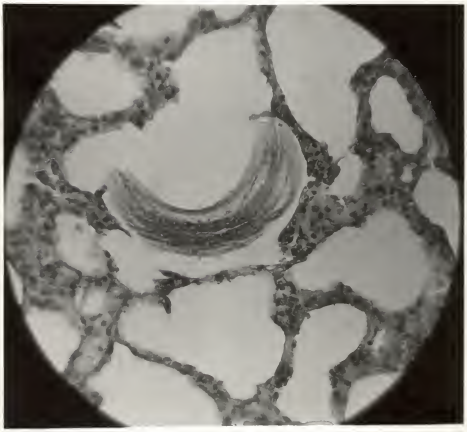

Fig. 2

the erratic larvae would have had to have been migrating for a considerable length of time in the sow's body.

Considerable amounts of milk were fed supplementally to Group II pigs and this could possibly account for the relatively few parasites present at necropsy. Spindler, quoted by Morgan and Hawkins (88), indicated that milk is detrimental to adult ascarids in the intestines. Kelley (64) stated that diet has an influence as to whether or not the worms become established in the intestine and that pigs receiving a milk diet seldom have ascarids in the small intestine.

It is interesting to note that Mercer, et al. (80) reported on a case of human ascariasis where the patient, with apparently a considerable intake of embryonated eggs, failed to reveal any ova or adult worms in the intestine. He implied that the larvae may be trapped or destroyed in the liver and lungs by hyperallergic inflammatory reactions.

The livers of experimentally infected pigs revealed many round, whitish foci on the surface; these findings are also frequently observed at abattoirs. Histological examination of these livers revealed a pronounced connective tissue infiltration (Plates III, IV, VII, and VIII).

Biester and Eveleth (12) stated that swine raised under controlled sanitary conditions fail to show well-developed interlobular connective tissue and that excess connective tissue in most swine livers is due, in most part, to parasitic infection. Elias, et al. (40), on discussing the "normal" pig liver, stated that it is not a normal mammalian liver and that compared to human

EXPLANATION OF PLATE VII

Fig. 1. Normal liver of pig. (Hematoxylin and Eosin, x95)

A. Hepatic triad.
B. Central vein.

Fig. 2. Liver of experimental pig. (Hematoxylin and Eosin, x95)

A. Hepatic triad with increased connective tissue.
B. Liver cells (pseudolobule).

PLATE VII

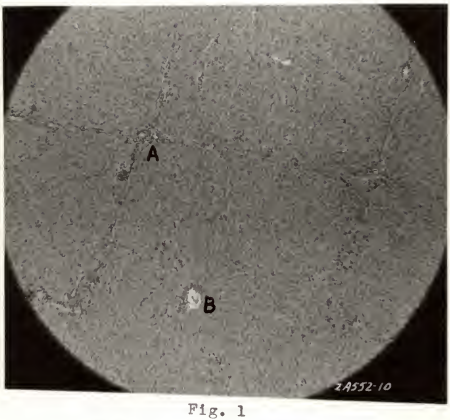

Fig. 1

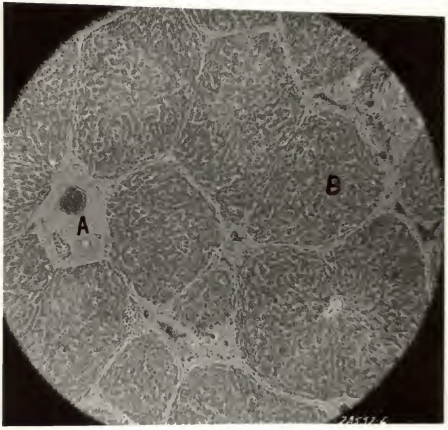

Fig. 2

EXPLANATION OF PLATE VIII

Fig. 1. Liver of normal pig. (Van Gieson's Stain, x95)

A. Connective tissue.
B. Liver cells.

Fig. 2. Liver of experimental pig. (Van Gieson's Stain, x95)

A. Connective tissue.
B. Liver cells.

PLATE VIII

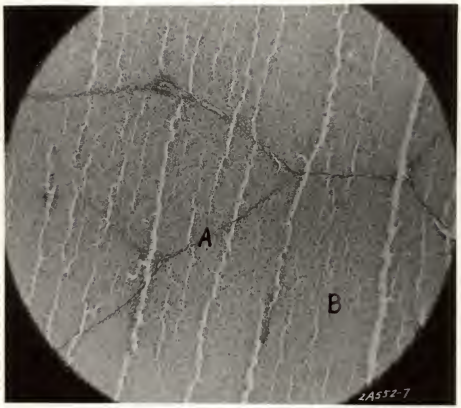

Fig. 1

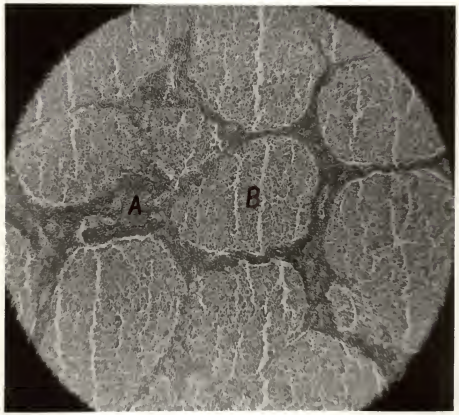

Fig. 2

livers, the pig's liver is cirrhotic. Johnson (61) reported on the anatomical development of the pig, indicating that the completion of the formation of connective tissue septa is not accomplished until several months after birth. With due consideration to these reports, the results of this experiment indicated that the amount of connective tissue present in so-called "normal" livers of pigs, has been over-emphasized. Control livers from this experiment exhibited only a thin line of connective tissue separating lobules, even in those animals living three months or more (Plate IV, Fig, 1; Plate VII, Fig. 1; and Plate VIII, Fig. 1).

SUMMARY AND CONCLUSIONS

Experimental animals infected with ascarid larvae failed to develop post-vaccinational problems when immunized with lapinized vaccines.

Larvae of Ascaris lumbricoides had apparently no effect on the immune responses of infected pigs as the degree of immunity was adequate to protect against a challenge dose of live virus. The experimental and control pigs did not develop a temperature response following immunization or challenge.

The major gross clinical symptom observed following inoculation with ascarid larvae was a respiratory distress ("thumps") which occurred 4 to 7 days later and existed 7 to 14 days.

Total leucocyte counts indicated that a leucopenia followed vaccination against hog cholera with lapinized vaccines. This leucopenia existed for a longer period in ascarid-infected animals than in control animals. When a challenge dose of live virus was

administered to these animals, a similar syndrome was observed except that the leucopenia was of longer duration and less severe. Ascarid-infected pigs recovered more slowly from the leucopenia following immunization and challenge than did the control animals.

Lymphocyte counts decreased slightly at vaccination and challenge whereas the neutrophil, monocyte, and basophil counts remained essentially normal.

Routine blood studies in these experiments indicated that a milk anemia accompanied the ascarid infection, and hematocrit determinations corresponded with these findings.

Eosinophil counts increased in ascarid-infected animals by the fourth day following inoculation and continued to increase until the 12th to 16th day, when these counts decreased to pre-infective levels in 20 to 28 days following inoculation.

Sedimentation rates increased only slightly in the experimental pigs following ascarid infection. Vaccination and challenge of experimental and control animals increased the sedimentation rates. The increase following immunization was greater than that following challenge.

Pigs dying 5 to 10 days following inoculation with infected ascarid larvae manifested an early cirrhosis of the liver and pneumonic areas throughout the lungs on necropsy. Microscopic examination of liver sections indicated an increased thickness of the capsule, increased connective tissue around the triads, and pseudolobulation of the liver parenchyma, presumably due to increased intralobular connective tissue. Eosinophils were numerous within the connective tissue described, and a few were observed in

the sinusoids of the lobules in some sections.

Cirrhosis of the liver, though less pronounced, was observed in experimental pigs necropsied at the termination of the experiment. Lung sections contained many areas indicative of repaired pneumonia.

Major damage due to migrating ascarid larvae was in the liver but lung damage resulting in pneumonia was no doubt a greater stress factor in decreasing the resistance of the animals to infection. Liver damage by invading ascarid larvae is apparently not sufficient to alter immune responses to hog cholera vaccines. Apparently many larvae are capable of by-passing the liver and going directly to the lungs.

The fact that only a minimal amount of connective tissue was observed in the liver sections of control animals indicates that the previously reported "normal cirrhotic" pig liver has been grossly over-emphasized.

ACKNOWLEDGMENTS

Grateful acknowledgment is given to Dr. M. J. Twiehaus, Major Instructor and Head of the Department of Pathology, who suggested and directed this experimental study; to Dr. Dean S. Folse, Associate Professor of Pathology, for his helpful criticism and advice; and to Dr. Lee M. Roderick, Professor of Pathology, for his help in the microscopic studies. Their patience, understanding, and direction were clear, concise, and greatly appreciated.

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THE EFFECT OF ASCARIS LUMBRICOIDES INFECTION ON IMMUNITY PRODUCTION BY LAPINIZED HOG CHOLERA VACCINES

by

KENNETH DUANE WEIDE

B. S., Kansas State College
of Agriculture and Applied Science, 1956
D. V. M., Kansas State College
of Agriculture and Applied Science, 1958

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This study was undertaken in an attempt to determine the difficulty veterinarians sometimes encountered in immunizing swine against hog cholera, using attenuated vaccines. Field observations indicated that frequently herds of swine in which losses occurred following immunization had severe ascarid infection and considerable damage had been done to the liver by migrating larvae. An attempt has been made here to investigate the effects of the lapinized hog cholera vaccines on the young pig, and to study the effects of ascarids and their migrating larvae on the growing pig, and their resulting influence, if any, on the immunizing potential of such infected pigs.

Forty baby pigs, farrowed in isolation and in a sanitary environment, were divided into experimental and control animals. The experimental animals were inoculated with embryonated ascarid ova orally, soon after birth. Both control and experimental animals were then kept under similar conditions and at approximately eight weeks of age were vaccinated with rabbit modified hog cholera vaccine and in two to three weeks challenged with a large dose of lethal hog cholera virus.

Blood samples from both control and experimental animals were taken at four-day intervals and routine laboratory examinations were made. All animals dying during the experiment and those remaining following termination of the project were carefully autopsied. Histological examinations were made of the livers and lungs of all animals.

Blood studies indicated that a leucopenia followed immunization against hog cholera using lapinized vaccines. This leucopenia

lasted longer and was slower in returning to normal in ascarid-infected pigs. Lymphocyte counts increased in all pigs following vaccination and challenge whereas neutrophils, monocytes, and basophils remained essentially normal. Erythrocyte counts indicated a mild anemia in ascarid-infected pigs. Eosinophil counts increased in experimental animals following inoculation. Immunization and challenge with virus increased sedimentation rates in all animals.

Microscopic examination of liver sections of experimental pigs indicated an increased thickness of the capsule, increased connective tissue around the triads, and pseudolobulation of the liver parenchyma, presumably due to increased intralobular connective tissue. Eosinophils were numerous within the connective tissue described, and a few were observed in the sinusoids of the lobules in some sections.

Major pathological changes due to migrating ascarid larvae were in the liver, but lung damage resulting in pneumonia was no doubt a greater stress factor in decreasing the resistance of the animal to infection. Liver damage by invading ascarid larvae is apparently not sufficient to alter immune responses to hog cholera vaccines. Apparently many larvae are capable of by-passing the liver and going directly to the lungs.

The fact that only a minimal amount of connective tissue was observed in the liver sections of control animals indicates that the previously reported "normal cirrhotic" liver of the pig has been grossly over-emphasized.