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Experimental Polychlorinated Biphenyl Toxicosis in Germfree Pigs

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ABSTRACT

The effects of polychlorinated biphenyls were studied in eight germfree pigs. Beginning at fourteen days of age, two pigs each were fed daily 12.5, 25, 50 and 100 mg/kg body weight of polychlorinated biphenyls as Aroclor 1254. Three germfree pigs were negative controls. Clinically the treated pigs had inappetance, a hemorrhagic diarrhea, erythema of the nose and the anus, retarded growth, distended abdomen and at the higher dose levels, incoordination and coma followed by death. Deaths occurred in 11 to 35 days after exposure.

At necropsy, the piglets exhibited grossly enlarged mottled liver, erosions of the gastric mucosa, hemorrhages through the mesentery and the intestinal wall, a fibrinous pericarditis, a hypoplastic thymus and congested swollen thyroid glands. The histopathological lesions included hepatic centrolobular necrosis, interstitial myocarditis, endocarditis, myopathy of the muscles, gastric erosions and colitis. All of the organs examined for polychlorinated biphenyls had elevated residue levels which were particularly high in the fat, liver, psoas muscle, brain and kidney and were higher than has been reported in conventional pigs fed approximately equal concentrations of polychlorinated biphenyls.

The severity of clinical signs, pathological changes and tissue concentrations were directly related to the dose administered and were more pronounced in the germfree pigs than has been described in conventional pigs. These differences appear to be related to the absence of a microbial flora in the germfree intestine and/or to lesser developed detoxifying enzyme systems in the liver of the younger pig.

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Aroclor is a registered trademark of the Monsanto Co. The aroclors are complex mixtures of polychlorinated biphenyls and are described by a four digit number. The first two digits indicate that the materials are polychlorinated biphenyls and the last two digits indicate the percent chlorine content in the mixture, e.g. Aroclor 1254 is a polychlorinated biphenyls 54% chlorine content.

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RÉSUMÉ

Les auteurs ont étudié l'effet des diphényles polychlorés, chez huit porcelets axéniques. Dès l'âge de 14 jours, quatre groupes de deux porcelets reçurent respectivement 12.5, 25, 50 et 100 mg de diphényles polychlorés par kilogramme de poids vif, sous la forme d'Aroclor 1254. Trois autres porcelets axéniques servirent de témoins. Les porcelets expérimentaux manifestèrent de l'inappétence, une diarrhée hémorragique, de l'érythème nasal et anal, un retard de croissance et une distension abdominale; ceux qui avaient reçu la plus forte dose d'Aroclor 1254 manifestèrent en plus de l'incoordination, du coma et finirent par mourir. Les mortalités survinrent entre le 11e et le 35e jour de l'expérience.

La nécropsie de ces porcelets révéla les lésions macroscopiques suivantes: hypertrophie et aspect nodulaire du foie, érosions de la muqueuse stomacale, hémorragies du mésentère et de la paroi intestinale, péricardite fibrineuse, hypoplasie du thymus, hyperémie et hypertrophie des thyroïdes. Les lésions histologiques incluaient de la nécrose hépatique centro-lobulaire, de la myocardite interstitielle, de l'endocardite, de la myopathie, des érosions gastriques et de la collte. La recherche de l'Aroclor 1254 dans divers organes en révéla une quantité élevée, particulièrement dans le tissu adipeux, le foie, les psoas, le cerveau et les reins. Cette quantité s'avéra supérieure à celle qu'on avait déjà retrouvée dans les organes des porcs conventionnels auxquels on avait donné des concentrations équivalentes d'Aroclor 1254.

La gravité des signes cliniques, des lésions macroscopiques et histologiques, ainsi que la quantité de résidu tissulaire, se révélèrent directement proportionnelles à la dose d'Aroclor 1254 et plus marquées chez les porcelets

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for WEE virus, eastern equine encephalitis virus was transmitted experimentally via the oral route in a herbivorous species, the snowshoe hare (31). In nature, cannibalism among squirrels is observed frequently along prairie highways due to roadkills during the summer. Therefore, it is possible that oral exposure of squirrels to high titres of WEE virus would occur upon the ingestion of infected squirrel carcasses. Recovery of WEE virus from renal tissue and the occasional recovery from urine indicated WEE virus could be excreted via the urinary tract. Arbovirus viruria had been reported previously in clinical human cases (26). In view of the viruria and the susceptibility to intranasal exposure it is possible that WEE virus could be transmitted via an excretory-inhalation pathway from animal to animal. However, the survival of the virus in voided squirrel urine would be questionable because of the sensitivity of WEE virus to fluctuations in temperature and pH (18).

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axéniques de cette expérience, comparativement aux porcelets conventionnels d'expériences antérieures. Ces différences seraient attribuables à l'absence de flore bactérienne intestinale, chez les porcelets axéniques, et/ou à un développement moindre des systèmes enzymatiques de détoxication du foie, chez les jeunes porcs.

INTRODUCTION

Polychlorinated biphenyls (PCB) have been widely used in the organic chemical industry as plasticisers in paints, resins and plastics and in industry in heat transfer units and insulators. They have become among the most widely distributed and persistent pollutants of the ecosystem and have been detected in various forms of animal life (7, 18, 19, 21, 22, 23). Polychlorinated biphenyls have been found to be present in agricultural products (13, 15, 16, 32) and in human tissues and blood samples (6, 21, 22). In animals they tend to accumulate in fatty tissues and in tissues and organs rich in fat. Pathological lesions due to PCB have been found in various organs of mammals but most severely affected is the liver (10, 17, 20, 26, 35). In birds the cardiovascular system is damaged most severely, hydropericardium and edema being the most prominent lesions (11, 12, 25, 30, 35). Lethal effects have been reported in chicken (11, 12, 25, 30) and in mink (5, 26, 31). Reproductive disorders due to PCB have been recorded in various species of animals (5, 10, 20, 24, 26, 27, 31).

Previous work with conventionally reared pigs indicates that they tolerate relatively high doses of PCB without showing clinical signs of toxicosis or grossly detectable pathological lesions, even though they may contain high levels of the chemical in their tissues (28, 29). Concern has been expressed that such animals may pass packing plant inspection as fit for human consumption (29). It would therefore be of value to find a model similar but more sensitive to PCB than the conventional pig. Due to the above consideration and because there are indications that the presence of microbes may modify the reaction of animals to PCB (9, 14), it was considered important to determine the nature and site of PCB action in germfree pigs. In this study the clinical response, pathological lesions and tissue levels of germfree pigs fed PCB was investigated and compared to similar previous observations in conventional pigs.

MATERIALS AND METHODS

Eleven colostrum deprived germfree Yorkshire piglets were used for the experiment. They were derived by hysterectomy, reared in sterile isolators and monitored for microbial contaminants according to methods described previously (2). Beginning at fourteen days of age, eight of the pigs, two animals per group, were fed daily 12.5, 25, 50 and 100 mg/kg body weight respectively of PCB as Aroclor 1254 in olive oil added to the morning meal. It was dispersed in sterile condensed cow's milk by means of Tween 60. Three control pigs were fed a similar diet containing no Aroclor. All animals were observed for clinical signs of intoxication and after death their organs and tissues were examined for gross and histopathological lesions and for PCB content. Necropsies were performed on principals when they died or when they were killed while moribund.

During necropsy the animals and selected organs were weighed and the values obtained were used for calculation of the weight of each organ expressed as a percentage of total body weight. Small pieces of the organs and tissues were fixed in neutral buffered formalin and routinely processed to paraffin in a histokinette. Sections were cut at five microns and stained with H&E and PAS stains. Samples of various tissues were removed for PCB analysis and stored at -20°C until analysed. Polychlorinated biphenyls were extracted from tissues using the FDA multipesticide residue method (8). Compounds were detected by means of a Micro-Teck, Model 220[1], gas-liquid chromatograph, equipped with a ^{63}Ni high temperature electron capture detector. The quanti-

[1]Tracor Inc., Augusta, Texas.

[2]Infotronics Ltd., Shannon, Ireland.

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tation of PCB was performed by measuring total peak area as the detector response, using Infotronics Model 208[3] automatic digital integrator equipped with a baseline tracking and drift corrector.

Clinical Signs

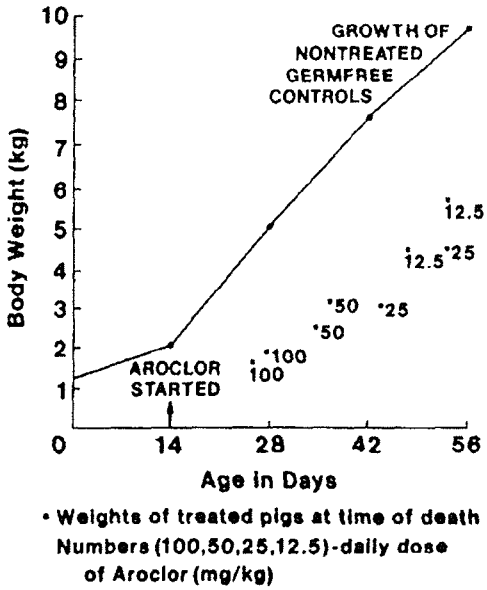
The principal clinical signs of PCB toxocosis were inappetance, erythema of the nose and the anus, diarrhea, distended abdomen and retarded growth and at dose levels higher than 25 mg/kg incoordination and coma followed by death within three weeks after exposure.

Regardless of the dosage level, the treated pigs had reduced appetites beginning from five to seven days after exposure to the chemical. The pigs fed Aroclor 1254 at the 12.5 mg/kg level regained their appetites about three weeks after exposure while the appetites of the pigs fed the higher levels became progressively poorer until prior to death they had a complete anorexia.

The onset of diarrhea in the pigs fed 100 and 50 mg of Aroclor per kg body weight was first observed nine to eleven days after exposure and in those fed 25 and 12.5 mg/kg on day 19. Feces of the pigs fed the lower levels remained liquid and light brown to dark brown in color until termination of the experiment, while in those fed the higher levels the feces changed to black and tarry and in extreme cases contained visible blood. The ani became inflamed and one pig fed 12.5 mg/kg had a prolapse of the rectum.

The abdomena of all the treated animals became distended. Those pigs fed Aroclor 1254 at 100, 50 and 25 mg/kg levels were incoordinated, progressed to semicomatose or comatose state and either died or were killed *in extremis*. The deaths of pigs belonging to the respective groups occurred on day 11 (100 mg/kg), days 20 and 21 (50 mg/kg) and days 32 and 40 (25 mg/kg) after exposure to the chemical. Of the animals fed 12 mg/kg, the pig that had *prolapsus recti* was in a semicomatose state on day 35, when it was killed and necropsied. The other pig of this group remained in a relatively good state of health until it was killed on day 40 after exposure or at 53 days of age.

Microbiological examination of samples collected at weekly intervals and at necropsy indicated that the animals had been bacteriologically sterile throughout the experiment. The growth rates of the pigs exposed to Aroclor 1254 were reduced as compared to the normal growth curve of germfree pigs fed similar diets without the chemical (Fig. 1). The three control pigs remained healthy. One of them was killed at 34 days of age and the other two were killed at 53 days of age.



• Weights of treated pigs at time of death
Numbers (100,50,25,12.5)-daily dose of Aroclor (mg/kg)

Fig. 1. Weight gains of germfree pigs fed Aroclor 1254, as compared to the weights of negative controls.

Necropsy and Histopathological Observations

At necropsy the principals were observed to be small in size for their age with long hair, emaciated and dehydrated to varying degrees. Lesions were seen in the liver and in the cardiovascular, digestive, immunological and certain elements of the endocrine systems.

In all of the animals the most obvious lesion was a hypertrophied mottled liver. The degree of hypertrophy of both the livers and kidneys increased in relation to the levels of Aroclor in the diet. All pigs, regardless of the dosage level had microscopic lesions of hepatic centrolobular necrosis of varying severity (Fig. 2).

One pig in each of the treated groups had a serosanginous fluid in the peritoneal cavity and hemorrhages through the serosa of the mesentery and intestinal wall. A pig

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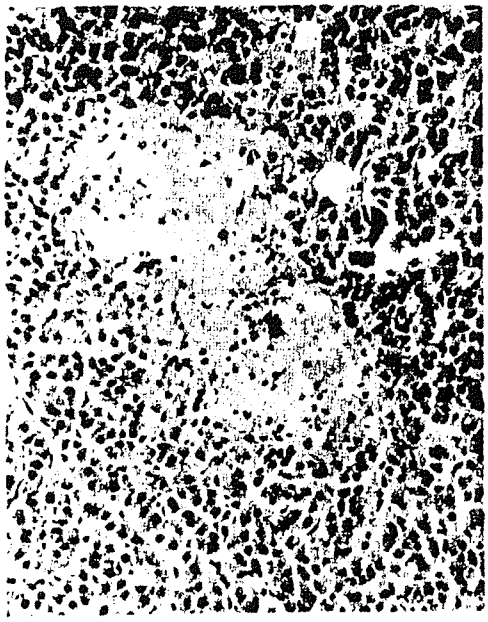

Fig. 2. Centrolobular necrosis in liver of germfree pig fed 100 mg/kg of PCB. Note the position of two necrotic foci slightly off center in the liver lobule. H & E. x200.

which had received PCB at a dose level of 50 mg/kg had fibrinous pericarditis (Fig. 3) and a slight interstitial myocarditis. Interstitial myocarditis (Fig. 4) along with endocardial thickening was seen in another pig fed PCB at the level of 100 mg/kg. Five of the PCB fed pigs had slight microscopic lesions of myopathy in the psoas and gastrocnemius muscles (Fig. 5).

The stomachs of the pigs fed 100 and 50 mg/kg of Aroclor contained excessive mucus and one pig that was given the higher dosage level had large areas of erosion of the mucosa and hemorrhages along the greater curvature. All exposed pigs had colitis regardless of the dose of PCB administered. The walls of the caecum and of the colon were thickened and the mucosa was congested. The contents of the large intestines of the pigs receiving 100 and 50 mg/kg of Aroclor were black in colour and positive for occult blood. These animals had microscopic lesions in the stomach and in the colon, consisting of moderate infiltration of the gastric mucosa with inflammatory cells and excessive secretion of mucus and lesions of late colitis (Fig. 6).

All of the treated animals had grossly observable swollen and congested thyroids and one of the pigs fed Aroclor at the 12.5 mg/kg level appeared to have a swell-



Fig. 3. Pericarditis in germfree pig treated with 50 mg/kg of PCB. Note two foci of inflammatory cells (arrows) in the pericardium. H. & E. x200.



Fig. 4. Myocarditis in germfree pig fed 100 mg/kg of PCB. Note discrete foci of inflammatory cells (arrows). H & E. x400.

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TABLE I. Relative Weights of Internal Organs of Germfree Pigs Fed Aroclor 1254[a]

Organ	Daily Dose of Aroclor (mg/kg body weight)				
	Neg. Conctr. Organ Weight	12.5	25.0	50	100
		—	Expressed as % of body weight		
Liver	2.9	7.51	8.81	10.43	12.60
Kidneys	0.52	0.62	0.98	1.17	1.18
Testicles	0.14	0.09	0.11	0.08	0.08
Spleen	0.16	0.16	0.11	0.06	0.11
Thymus	Normal	Reduced	Much red.	Absent	Absent

[a]The recorded values represent arithmetical means from two pigs of each of the treated groups

ing of the adrenals. The thymus was hypoplastic and in those pigs fed Aroclor for the longest period of time at the lower levels both the thymus and body fat appeared to be absent. The spleen and the testicles of the treated pigs were reduced in size and weight as compared to those of the control pigs. The weights of selected organs of the nontreated controls and the treated pigs expressed as a percentage of the total body weight are presented in Table I. No lesions were observed in the organs and tissues of the control pigs and no significant histological lesions were seen in the lungs, kidneys, spleens, adrenals, thyroid and thymus glands of the treated animals.

Tissue Distribution of PCB

The concentrations of PCB in tissues of the treated animals are shown in Table II. The highest concentrations of PCB occurred in the adipose tissues of pigs given 50 mg/kg and 100 mg/kg. Piglets fed 12.5 mg/kg and 25 mg/kg of PCB were emaciated to the point that adipose tissues were not available for analysis. The hepatic levels were next to adipose tissues in concentrations but were substantially lower than the former. Brain concentrations were consistently lower than those in liver and muscle. The levels were also high in testicles. The ratios of brain versus blood concentrations were nearly identical in all animal analysis and varying from 4.1 to 4.5.

DISCUSSION

In conventional pigs, clinical signs of toxicosis and gross pathological lesions have been observed only following administration of high doses of PCB or after prolonged exposure. Feeding PCB to mature sows has resulted in reduced fertility and chronic septicemia with liver hypertrophy and gastric erosions (10). There is also evidence that PCB interfere with the reproductive function of the male pig (27, 29). In growing conventional pigs, low doses of PCB stimulate growth, while high doses suppress the growth rates (28, 29).

Multiple, but generally mild histological lesions due to PCB have been observed by the cited authors in conventional pigs of all ages. These include hepatitis, focal hepatic necrosis and cirrhosis, acute interstitial nephritis and nephrosis, erosions of the gastric mucosa, degeneration of the skeletal muscle and myocardium and



Fig. 5. Myopathy in germfree pig treated with 25 mg/kg of PCB. Note unevenness in the diameter of the fibres of each muscle bundle. The larger fibres are necrotic, and a few of them have centrally located nuclei (arrows). H & E. x400.

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TABLE II. Concentrations (in μg/g wet tissue) of Polychlorinated Biphenyls In Various Tissues of Germfree Piglets Given Daily Graded Doses of Aroclor 1254*

Tissue	Dose Level			
	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Brain	8.0	11.7	34.0	55.1
Heart	3.2	17.2	24.8	77.1
Kidney	6.9	6.8	20.8	101.7
Liver	9.6	40.0	65.9	202.5
Lung	5.3	7.1	26.5	—
Psoas muscle	15.3	16.3	64.4	122.4
Testicle	5.3	8.6	27.3	54.3
Bile	5.3	65.7	75.1	101.6
Fat	—	—	1864.0	2315.5
Blood	1.77	—	8.2	12.5

*The recorded values represent arithmetic means from two pigs

lesions in the brain. The described lesions have not been consistent but one or more have been observed in some of the exposed animals, the liver lesions being present most consistently.

In germfree pigs, clinical manifestations of toxicosis appeared earlier, the growth was suppressed more severely and the incidence of fatalities was higher and they occurred after a shorter period of exposure than has been observed in conventional pigs. Abdominal distention and signs of intestinal irritation as seen in the gnotobiotic pig have not been reported in the conventional animal.

The degree of liver hypertrophy of the germfree pig greatly exceeded that of conventional pigs (Table I). Unlike the conventional pig but similar to poultry (11, 12) some of the germfree pigs had a hydropericardium and ascites and like mink (5, 26, 31) they had black tarry feces and hemorrhages in the gastrointestinal tract and in the abdominal cavity. Hyperplasia and dysplasia of the gastric mucosa, even though observed in a mild form in the conventional pig, were more pronounced in the germfree pig. Lesions resembling these have been observed in primates fed PCB (3, 4). The hypoplastic thymus observed in most of the PCB fed germfree pigs, similar to that reported in the rabbit (33, 34), indicates an immunosuppressive effect of Aroclor 1254 in the pig.

There are reports that PCBs have an unfavourable effect upon the reproductive function in several species of animals including swine (10, 27, 29), mink (5, 26, 31) and poultry (24). While no direct assessment of the reproductive capacity of the germfree pigs could be made in this study because these animals did not reach a sufficient age, the reduction in the size and weight of the testicles of the male germfree pigs exposed to PCB as compared to those of the negative controls would suggest a deleterious effect of PCB upon the reproductive organs.

As compared to conventional pigs, germfree pigs appear to accumulate higher levels of PCB residues in their tissues. For example, the following residue levels were observed in conventional pigs fed 15 mg/kg body weight daily for six weeks (29) and in germfree pigs fed 12.5 mg/kg daily for five and seven weeks (Table I).



Fig. 6. Late stage of inflammation in the colon of germfree pig treated with 100 mg/kg of PCB. Note fibroblasts (F) and capillaries (arrows) in the colonic mucosa, indicating early repair. x300.

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	Conventional pigs	Germfree pigs (mean)
brain	6.6 ppm	8.2 ppm
fat	122.9 ppm	insufficient sample
liver	7.8 ppm	9.6 ppm
psoas muscle	4.9 ppm	15.3 ppm

In the same two experiments the conventional pigs consumed over a 12 week period a total of 860 mg/kg of Aroclor 1254 and germfree pigs fed 50 mg/kg of body weight during 20 days consumed 1000 mg/kg body weight of the PCB. Here the respective tissue levels were as follows:

	Conventional pigs	Germfree pigs
brain	8.8 ppm	34 pmm
fat	226.2 ppm	1864 ppm
liver	5.9 ppm	65.9 ppm
psoas muscle	5.9 ppm	64.6 ppm

These differences, even though arrived at in experiments that are not directly comparable, would suggest a more efficient absorption or retention of PCB by the germfree pig.

The results of the present study thus indicate that the germfree piglet is fatally affected by doses of PCB which are either of no or only minor clinical and gross pathological consequence in somewhat older pigs maintained under conventional conditions. The response of the germfree pig is in many respects similar to that of its conventional counterpart but it is more severe. In addition germfree piglets display lesions that have not been observed in conventional pigs but have been seen in a variety of other species exposed to PCB, including primates. When graded doses of PCB were administered to germfree pigs, correlation could be made between clinical signs, gross and histopathological lesions and residue levels of PCB in the tissues. As concentration of PCB in blood increased with the dose administered, and as the brain levels increased correspondingly, the ratios of brain versus blood concentrations may be indicative of PCB transfer across the blood-brain barrier in the germfree pig.

The greater susceptibility to PCB of the germfree pigs used in this study as compared to conventional pigs was probably related to the absence of bacteria in the digestive tract of the germfree pigs and to their younger age.

The observation that hydroxy metabolites of PCB have been identified in bacteria in *in vitro* studies suggests that also *in vivo* part of the ingested PCB may be metabolized by the intestinal bacterial flora. Germfree pigs, having no such flora, would therefore have available for absorption a greater proportion of the ingested chemical than would the conventional animal. Our findings that germfree pigs actually had higher PCB residues in their tissues than those detected in conventional pigs fed approximately equal quantities of PCB per unit body weight would support this assumption. Being younger than the conventional pigs that have been investigated, the germfree pigs likely possessed less developed detoxifying enzyme systems in their livers than did the conventional pigs and therefore probably would be affected more severely by the toxic effects of PCB even if the quantities absorbed were equal.

On the basis of this work it appears that germfree pigs where the effects of PCB are not masked by the presence of environmental and intestinal microbes could provide a sensitive and useful tool for the study of PCB toxicosis even in relatively low concentrations. Studies of PCB in gnotobiotic animals associated with a defined flora may further elucidate the role of specific microbial organisms in the absorption and retention of PCB by higher animals.

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San Miguel Sea Lion Virus Fed to Mink and Pigs

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ABSTRACT

Mink became infected with San Miguel sea lion virus when fed ground meat from seal carcasses showing vesicular-like lesions in the skin. The mink also contracted the infection when they were fed San Miguel sea lion virus infected pig meat or cell culture propagated virus. San Miguel sea lion virus infection in mink was inapparent but the virus was isolated from blood and rectal swabs. Pigs treated similarly with the same virus preparations given to mink developed a severe vesicular disease syndrome similar to that produced by vesicular exanthema of swine. In a separate trial, pigs fed a large sample of commercial ground seal meat did not develop disease signs or antibodies. Further work is needed to assess the hazard of introducing San Miguel sea lion virus into swine on the same premises when potentially San Miguel sea lion virus infective seal meat is fed to mink.

RÉSUMÉ

Le fait d'alimenter des visons avec de la viande hachée provenant de carcasses d'otaries à fourrure qui présentaient des lésions cutanées ressemblant à des vésicules, fit réaliser qu'on les infectait avec le virus de l'otarie de San Miguel. Les visons contractèrent aussi l'infection après avoir absorbé de la viande de porc ou des cultures tissulaires qui contenaient ce virus. L'infection passa inaperçue, mais on isola le virus à partir du sang et d'écouvillons du rectum de ces visons. L'utilisation de porcs au lieu de visons, dans une expérience similaire, se traduisit par l'apparition d'un syndrome vésiculaire grave et semblable à celui que provoque l'exanthème vésiculaire du porc. Au cours d'une autre expérience, les porcs qui avaient mangé un échantillon commercial considérable de viande hachée d'otarie à fourrure ne développèrent pas de signes de maladie, ni d'anticorps. Il faudra effectuer d'autres expériences afin de préciser le danger d'introduire le virus de l'otarie de San Miguel chez des porcs qui vivent sur la même ferme que des visons auxquels on donne de la viande d'otarie à fourrure qui pourrait receler ce virus.

INTRODUCTION

San Miguel sea lion virus type 5 (SMSV-5) has been isolated from a vesicle on a skin sample obtained from the northern fur seal (*Callorhinus ursinus)* found on the Pribilof Islands in the Bering Sea (3). Observers of the annual culling of three and four year old bachelor males from the rookeries on the Pribilof Islands noticed that certain seals had ruptured vesicle-like lesions on the skin of their flippers. San Miguel sea lion virus was isolated from meat ground from seals with skin lesions after the seals had been skinned and evisserated (John C. Sawyer, personal communication). Large amounts of seal meat are a by-product of the annual culling. Ground seal meat is then shipped to the U.S. mainland as mink feed. Some mink ranchers keep pigs to feed on the ground seal meat that drops under the mink cages. Thus, feeding seal meat to both mink and pigs may represent a potential danger to the swine industry because SMSV is indistinguishable physiochemically and morphologically from vesicular exanthema of swine virus (VESV) (2). Furthermore, SMSV and VESV produce similar vesicular disease features in pigs. The purpose of this study was to determine whether mink or pigs would become infected by feeding on seal meat infected with SMSV.

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