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PATHOLOGICAL PICTURES OF RABBITS AND PIGS INFECTED WITH TWO LAPINIZED HOG CHOLERA VIRUSES

ROBERT C. T. LEE[1] and S. S. LAI[2]

Clinically and pathologically, two lapinized HC vaccine viruses, the LPC-Chinese (LPC) and Chinese (Chinese) strains, caused no abnormal reactions in pigs, but induced similar fever responses, the enlargement of lymph nodes (LN), particularly mesentery LN, and histopathological changes in the brain, such as neuronal satellitosis, neuronophagia and perivascular cuffings in rabbits.

Both lapinized HC viruses had a similar viral distribution pattern in rabbits and pigs. Lymphatic tissues were mainly infected with the viruses. The result indicated that these two lapinized HC vaccine viruses had identical pathogenicity and viral distribution in rabbits and pigs. Therefore, it may suggest that these two viruses have the same origin.

Vaccination with attenuated live viruses has been considered as an effective method of controlling hog cholera (HC), particularly using a lapinized attenuated HC vaccine[8]. However, the use of live vaccine was prohibited in some countries as eradication programs developed. Knowledge on attenuated HC viral vaccine initiated by Koprowski *et al.*[4] and Baker[1] with lapinized HCV has been expanded by a variety of techniques used for attenuation.

The LPC strain widely used for HC control in Taiwan was originally introduced from the Philippines in 1952[6]. The imported LPC virus being passaged in rabbits for 150 times still caused severe reactions and death in pigs. However, no response was noted in rabbits inoculated intravenously with the virus. More than 800 passages of the LPC virus in rabbits and many experiments related to the safety, efficacy, virus shedding and virulent reverse test of the vaccine were carried out[9,10,11]. The experimental data of the latter passages indicated that the LPC virus was highly safe and potent in piglets, even in the newborns[11]. Although the LPC virus has been widely used in the field for HC control, so far these has been no report concerning the pathology of the vaccine virus infected rabbits and pigs, which is an important key to determining the degree of attenuation. In addition, a so-called Chinese strain of lapinized HC vaccine has been widely used for HC control in many Asian and European countries. The vaccine was also very safe and efficacious. However, as cited in many scientific papers, its origin was unknown[2,3]. Therefore, this report describes the pathological pictures of rabbits and pigs infected with the LPC and so-called Chinese strains of HC vaccine, which may prove the identity of these two viruses.

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1. Council for Agricultural Planning and Development, Taiwan, ROC.
2. Taiwan Provincial Research Institute for Animal Health, ROC.

MATERIALS AND METHODS

Animals

Sixteen specific pathogen-free (SPF) pigs aged 4 to 5 weeks and 16 commercial rabbits weighing 1 to 1.5 kgs were intramuscularly and intravenously inoculated respectively with the LPC and Chinese strains of lapinized HC vaccine viruses, both contain-

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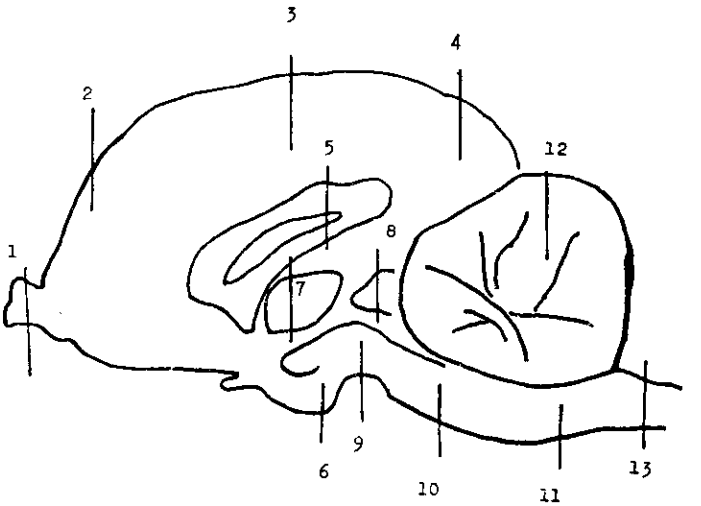

1. Olfactory bulb
2. Frontal lobe
3. Motor lobe
4. Occipital lobe
5. Corpus callosum
6. Hypophsis
7. Thalamus
8. Corpora quadrigemina
9. Peduncle
10. Pons
11. Medullar Oblongata
12. Cerebellum
13. Spinal cord

Fig. 1. Schematic drawing of brain, showing areas where samples were collected.

ing about 10^6 rabbit infective dose (RID) for pathological studies. These SPF pigs and three rabbits were also inoculated with a virulent HC virus, an ALD strain containing 10^4 MLD. One SPF pig and one rabbit served as non-inoculated controls. Two pigs and two rabbits were killed 3, 5, 7 and 10 days after inoculation. One pig and one rabbit were killed 3, 5 and 7 days after inoculation. Rectal temperature and clinical signs were daily recorded. All visceral organs, lymph nodes and the central nervous system (CNS) were collected for histopathological studies and virus isolation. The brain was sectioned as indicted in Fig. 1.

Viruses

The LPC strain virus, passaged in rabbits 816 times, obtained from the infected spleen was used. The so-called Chinese strain virus of unknown origin was obtained from the National Institute of Animal Health (NIAH) of Japan. The virus was passaged in rabbits 3 times before use. Both virus strain-infected spleens contained about 10^7 RID per gram. The Western equine encephalomyeolitis (WEE) virus grown in swine testicle (ST) cells was used in the interference test. The GPE^- virus, an attenuated HC vaccine virus obtained from the NIAH of Japan, was also grown in ST cells and used in the interference test.

Virus isolation and the interference test

Tissues were aseptically collected and ground with sterile sand in a mortar, and 10% suspension (w/v) was made in MEM with 10x antibiotics. The suspension was centrifuged at 1,800 G for 30 minutes and supernatants were used for virus isolation. The interference test was previously described by Shimizu *et al.*[13]. Two days old ST cells grown in microplates were inoculated with 0.05 ml of tissue emulsion. Three wells of ST cells were used for each tissue emulsion. The ST cells were rinsed with PBS saline twice after one hour's incubation at 37°C in a 5% CO_2 chamber. A 0.05 ml maintenance medium (with 2% fetal calf serum) was added to each well. The plates sealed with tape were incubated for 5 days at 37°C. After 5 days of incubation, the

cultural fluid was sucked out and a 0.05 ml maintenance medium containing 1,000TCID$_{50}$ GPE$^-$ virus was added to each well and incubated for another 5 days at 37°C. After incubation, the cultural fluid was sucked out again and a 0.05 ml maintenance medium containing 1,000 TCID$_{50}$ WEE virus was added to each well. The plates were incubated and the interpretation was made 3 days after incubation. The LPC virus was present in the tissue emulsion if ST cells showed no cytopathic effect (CPE). The LPC virus instead of the test tissue emulsion were used as a positive control for the test. Control tests were also made to prove that the GPE$^-$ and WEE viruses caused no CPE and CPE in ST cells respectively.

Pathological procedures

All visceral organs and CNS tissues were placed in 10% formaldehyde in buffered saline (pH 7.0). Following fixation, tissue blocks were embedded in paraffin-wax and sections, cut at 6 μm, were stained with Ehrlich's haematoxylin and eosin (H & E).

RESULTS

Clinically, all tested pigs were normal; but 3 pigs inoculated with virulent viruses died on the 3rd day. As indicated in Fig. 2, the LPC and Chinese strains caused similar fever responses in rabbits. All inoculated rabbits had fever responses on the second and third days, while rabbits killed in later periods had the 2nd peak of fever reaction on the 5th-6th days or 6th-7th days.

No significant lesions were found in the pigs inoculated with the two lapinized HC viruses, while remarkable hemorrhagic LNs were noted in the three pigs inoculated with the ALD strain died on the third day. In all tested rabbits, no gross change was noted in the tissue other than the LN, particularly in the mesenteric LN.

The mesenteric LNs in rabbits killed on the third day enlarged 3-4 times in size as compared with those in the control rabbits (Fig. 3). The enlargment of LNs decreased gradually thereafter. Those rabbits

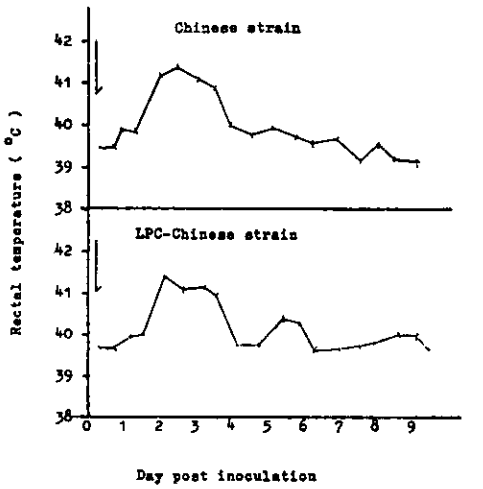

Fig. 2. Rectal temperature response of rabbits intravenously inoculated with two attenuated hog cholera viruses, LPC-Chinese and So-called Chinese strains.

Fig. 3. Enlarged (3-4×) Mesentery lymph node of rabbit infected with LPC-Chinese HC virus, 3 DPI.

killed on the tenth day had slightly swollen mesenteric LNs. Macroscopical changes of LNs in the rabbits induced by the two lapinized and virulent HC viruses did not show any significant differences.

No significant histopathological change was seen in the CNS and visceral organs of all but 1 pig inoculated with the two lapinized HC viruses (Table 1). The exceptional pig inoculated with the LPC virus when killed on the third day showed mild perivascular cuffings in the olfactory bulb (Fig. 4). Three pigs which died on the third day did not show any microscopical

Table 1. Distribution of Brain Lesions in Pigs Infected with Different Strains of Hog Cholera Virus

Site	LPC				Chinese			
	3	5	7	10	3	5	7	10
Olfactory bulb	—c	——	——	——	—	——	——	—
Cerebrum	——	——	——	——	—	——	——	—
Frontal	——	——	——	——	—	——	——	—
Motor	——	——	——	——	—	——	——	—
Occipital	——	——	——	——	—	——	——	—
Corpus callosum	——	——	——	——	—	——	——	—
Hypophysis	——	——	——	——	—	——	——	—
Thalamus	——	——	——	——	—	——	——	—
Corpora quadrigemina	——	——	——	——	—	——	——	—
Brain stem								
Peduncles	——	——	——	——	—	——	——	—
Pons	——	——	——	——	—	——	——	—
Medulla oblongata	——	——	——	——	—	——	——	—
Cerebellum	——	——	——	——	—	——	——	—
Spinal cord	——	——	——	——	—	——	——	—

c : Perivascular lymphocytic cuffing.
—: One "—" indicate one pig examined.

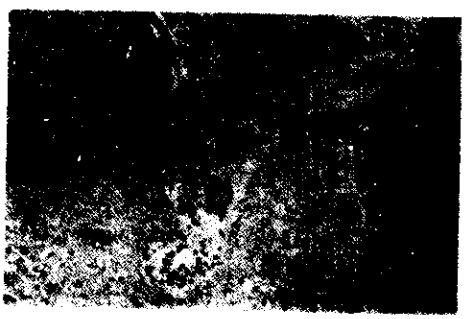

Fig. 4. Lymphocytic perivascular cuffings of olfactory bulb from pig infected with LPC-Chinese HC virus, 3 DPI, H & E. ×40.

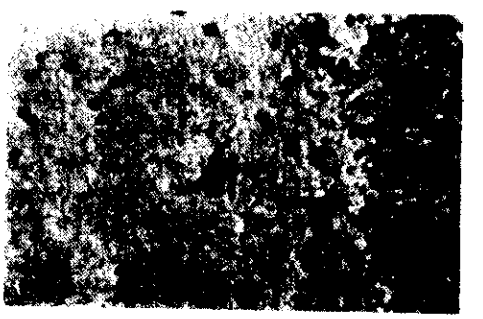

Fig. 5. Neuronal satellitosis of cerebellum from rabbit infected with LPC-Chinese HC virus, 3 DPI, H & E, ×80.

CNS lesions except that peripheral hemorrhage was observed in several LNs.

Rabbits inoculated with the LPC strain and killed on the third day showed neuronal satellitosis in the frontal and motor lobes, pons, and copora quadrigemina of cerebrum and cerebellum (Table 2), (Fig. 5). There were no particular microscopical changes in the CNS of rabbits inoculated with the LPC strain virus killed on the fifth and seventh days, but mild neuronal changes were found in the rabbits inoculated with the Chinese strain virus. The perivascular cuffings in the medulla oblongata were detected in rabbits killed on the tenth day. Besides the CNS, LNs were the only tissues showing microscopical changes. A hyperplastic cortical area accompanied with some hetero-

Table 2. Distribution of Brain Lesions in Rabbits Infected with Different Strains of Hog Cholera Virus

Site	LPC				Chinese				ALD		
	3	5	7	10	3	5	7	10	3	5	7
Olfactory bulb	−−	−−	−−	−	−−	−−	−−	−−	−	−	−
Cerebrum											
Frontal	−+	−−	−−	−	−+	−−	−−	−−	c	−	−
Motor	−+	−−	−−	−	−−	−+	−−	−−	−	−	−
Occipital	−−	−−	−−	−	−−	−−	−−	−−	−	−	−
Corpus callosum	−−	−−	−−	−	−−	−−	−−	−−	c	−	−
Hypophysis	−−	−−	−−	−	−−	−−	−−	−−	−	−	−
Thalamus	−−	−−	−−	−	−−	−−	−−	−−	−	−	−
Corpora quadrigemina	+−	−−	−−	−	−−	−−	−−	−−	−	−	−
Brain stem	−−	−−	−−	−	−−	−−	−−	−−	−	−	−
Peduncles		−−	−−	−	−−	−−	−+	−−	c	+	−
Pons	−+										
Medulla oblongata	−−	−−	−−	c	+−	−−	−−	−−	−	−	−
Cerebellum	++	−−	−−	−	−−	−−	−+	−−	−	−	−
Spinal cord	−−	−−	−−	−	−−	−−	−−	−−	−	−	−

+: satellitosis and neuronophagia.
c: perivascular lymphocytic cuffing.
One "−" or "+" indicate one pig examined.

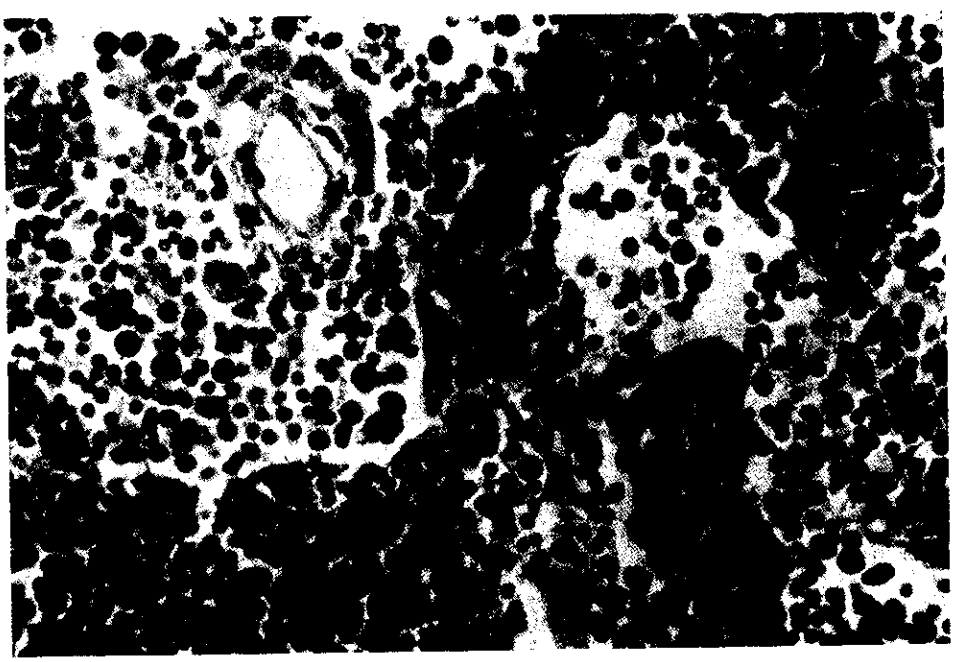

Fig. 6. Lymph node of rabbit infected with LPC-Chinese HC virus. 3 DPI, H & E, ×128. Numerous lymphoblast, macrophages and heterophils filled in sinus as in medullary lymphatic cords were moted.

Table 3. Viral Distribution of Rabbits Infected with Two Lapinized HC Viruses, LPC-Chinese and So-Called Chinese Strains

DPI	3		5		7		10	
Organ	L	C	L	C	L	C	L	C
Lymph nodes	+	+	+	+	+	+	+	+
Mandibular	+	+	+	+	+	+	+	−
Hepatic	+	+	+	+	+	+	+	+
Mesentery	+	+	+	+	+	+	+	+
Renal	+	+	+	+	+	+	+	+
Inguinal	+	+	+	+	+	+	+	+
Lung	−	−	−	−	−	−	−	−
Liver	−	−	−	−	−	−	−	−
Pancreas	−	−	−	−	−	−	−	−
Kidney	−	−	−	−	−	−	−	−
Spleen	+	+	+	+	+	+	−	−
Adrenal gland	−	−	−	−	−	−	−	−
Brain	−	−	−	−	−	−	−	−
Tonsil	+	+	+	+	+	+	−	−
Duedinum	−	−	−	−	−	−	−	−
Jejunum	−	−	−	−	−	−	−	−
Ilium	−	−	−	−	−	−	−	−
Heart	−	−	−	−	−	−	−	−
Testes	−	−	−	−	−	−	−	−

L: LPC-Chinese strain C: So-called Chinese strain
+: Virus was recovered −: Virus was not recovered

Table 4. Viral Distribution of Pigs Infected with Two Lapinized HC Viruses, LPC-Chinese and So-Called Chinese Strains

DPI	3		5		7		10	
Organ	L	C	L	C	L	C	L	C
Lymph nodes	−	−	+	+	+	+	+	+
Mandibular	−	−	+	+	+	+	+	+
Hepatic	−	−	+	+	+	+	+	+
Mesentery	−	−	+	+	+	+	+	+
Renal	−	−	+	+	+	+	+	+
Inguinal	−	−	+	+	+	+	+	+
Lung	−	−	−	−	−	−	−	−
Liver	−	−	−	−	−	−	−	−
Pancreas	−	−	−	−	−	−	−	−
Kidney	−	−	−	−	−	−	−	−
Spleen	−	−	+	+	+	+	−	−
Adrenal gland	−	−	−	−	−	−	−	−
Brain	−	−	−	−	−	−	−	−
Tonsil	−	−	+	+	+	+	+	+
Duedinum	−	−	−	−	−	−	−	−
Jejunum	−	−	−	−	−	−	−	−
Ilium	−	−	−	−	−	−	−	−
Heart	−	−	−	−	−	−	−	−
Testes	−	−	−	−	−	−	−	−

L: LPC-Chinese strain C: So-called Chinese strain
+: Virus was recovered −: Virus was not recovered

phils, macrophages and lymphoblasts infiltration in sinuses was noted in the LNs of the rabbits killed on the third day (Fig. 6). However, the intensity of lymphadenopathy in rabbits killed in the later periods diminished gradually. Only very mild cellular reactions of LNs were observed on the tenth day. The degree and types of tissue changes in the CNS and LNs of the pigs and rabbits inoculated with the Chinese strain virus were mainly similar to those induced by the LPC strain. Perivascular cuffings were also detected in the CNS of rabbits inoculated with the ALD strain when killed on the fifth and seventh days.

The virus distribution in the tissues of infected rabbits was tabulated in Table 3. These two lapinized HC viruses induced similar virus distributions in the rabbits. The viruses were recovered from all test lymphatic tissues, lymph nodes, tonsile and spleens on the third up to the tenth day, with the exception of tonsils and spleens from which the virus was not recovered 10 days after inoculation with either the LPC or Chinese strain.

The virus distribution in the tissues of the infected pigs was shown in Table 4. Both the LPC and Chinese strain viruses also had similar virus distributions in pigs. Viruses were only isolated from lymphatic tissues, lymph nodes, tonsils and spleens five to ten days after inoculation. No virus could be recovered from the other parenchymal organs of infected rabbits and pigs.

DISCUSSION

The original seed virus of the LPC strain of HC vaccine was introduced from the Philippines in 1952 by Lee[6]. The virus being serially passed in rabbits for 150 passages was still virulent to pigs. Further rabbit passages of the virus were carried out by our workers[6,9]. A typical fever response in rabbits inoculated with a 100 or higher rabbit passage virus was constantly noted. Lin *et al.* used the 111 rabbit passage virus for the virulence reverse test[11]. They found that the virus regained its virulence causing piglets to die at the 6th back passage in piglets. However the virus became stable and completely lost its virulence, but retained good immunogenicity when rabbit passages reached 500.

The seed virus of the LPC strain currently used for vaccine production is of the 816th rabbit passage. The high rabbit passage virus is highly safe and immunogenic to pigs. Twenty reverse passages of the virus in SPF pigs gained no increased virulence[11]. Unlike the low passages of the lapinized HC virus, the Rovac strain induced histopathological changes either in the brain or in the lymphatic tissues of pigs[14], but the 816 rabbit passages of the LPC strain virus did not induce any microscopic changes in piglets. Histopathologically, mild encephalitits and lymphadenopathy were noted in rabbits inoculated with the LPC virus. Clinically, the virus proved more virulent to the rabbits in terms of fever response, as compared with the early rabbit passage virus. However, unfortunately there were no pathological data on rabbits inoculated with the lower rabbit passage virus. Therefore, the virulence of the virus for rabbits could not be concluded as the rabbit passages increased.

The virus distributions of infected rabbits and pigs were similar to previous reports[12,13]. The lapinized virus mainly multiplied in lymphatic tissues such as lymph nodes, spleens and tonsils. The virus persisted longer in the lymph nodes of rabbits and pigs. However, unlike the virulent HC virus, the tonsil did not prove more susceptible to the lapinized HC virus as indicated by Lin[12] and Liu[13]. Tissues other than those of lymphatic origin did not show any viral multiplication. In addition, low titers of viremia were found in infected rabbits, but not in infected pigs which may result in no or few viruses shedding out of the infected pigs as indicated by the failure to recover the virus from all secretions and excretions, also by the failure to establish contact infection[12]

The experimental results indicated that

these two lapinized HC vaccine viruses were identical in pathogenicity and pathogenesis for rabbits and pigs. The origin of the Chinese strain virus was unknown as cited in many scientific papers by European researchers[2,3]. However, LPC strain viruses of 469 and 760 rabbit passages had been officially exported to the Ryukyu islands in 1959 and to South Vietnam in 1966. Therefore, the Chinese strain was possibly introduced from those areas. Recently, Lai *et al.* also proved that these two lapinized viruses were identical in many virological aspects[5]. The experimental data suggest that the Chinese strain virus may have originated from the LPC strain virus originally developed in the Republic of China on Taiwan.

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猪及兎感染二種兎化猪瘟病毒之病理學變化

李崇道[1]　賴秀穗[2]

猪隻經本省開發之中華民國 LPC 株兎化猪瘟疫苗與歐洲使用之所謂中國株兎化猪瘟疫苗注射後，在臨床及病理學上，均無不正常之反應，惟這些疫苗注射兎子後在臨床上則引起典型之熱反應，病理學上引起淋巴之腫大（特別是腸間淋巴）及輕度之非化膿性腦炎。

二種兎化猪瘟疫苗注射於猪及兎子後病毒在體內之分佈情形兩者頗爲類似，均以淋巴系統爲主要感染組織。本試驗結果顯示上述二種疫苗，病毒之來源可能相同。

1. 行政院農業發展委員會。
2. 臺灣省家畜衛生試驗所。