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EFFECT OF THE ROUTE OF FOOT-AND-MOUTH DISEASE VIRUS INFECTION OF PIGLETS ON THE COURSE OF DISEASE

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Summary. – Three different routes of Foot-and-mouth disease virus (FMDV) infection of piglets, namely intranasal (i.n.) through drops, intradermal (i.d.) into the foot, and intramuscular (i.m.) were compared regarding the onset and severity of the disease. The results showed that the i.d. injection of the virus resulted in the fastest onset of the disease. The i.m. injection led to a delayed onset, but the final effect was identical with i.d. injection. Moreover, the i.m. injection was simpler to perform and easier to evaluate. Therefore, the i.m. injection of piglets is recommended as the optimal infection route for evaluation of the FMDV vaccine potency.

Keywords: Foot-and-mouth disease virus; challenge routes; intranasal drops; intradermal injection; intramuscular injection; pig

Introduction

Foot-and mouth disease is an acute, febrile, and contagious vesicular disease affecting cloven-hoofed animals. In recent years, pigs infected with FMDV have been reported around the Chinese mainland such as Taipei and Hong Kong (Feng *et al*., 2003; Chen *et al*., 2006; Dunn *et al*., 1997; Yang *et al*., 1999). Vaccination is the most important control and eradication strategy for FMDV, especially the vaccination with oil-adjuvanted vaccine used in the developing countries (Balamurugan *et al*., 2004; Lubroth *et al*., 2007; Mason *et al*., 2003; Grubman *et al*., 2004). Vaccine potency is very important parameter that is estimated in the vaccinated animals by evaluating their resistance to the live virus challenge. OIE (Office International des Epizooties) states two challenge methods for the examination of FMDV vaccine potency in pigs: (i) i.d. injection into the heel bulbs and (ii) i.m. injection into the ear-root-neck area (OIE terrestrial manual, 2009). Unfortunately, it is not indicated which one is more suitable.

In this report, we compared three different routes of FMDV infection of piglets in order to find the optimal one for the challenging animals for examination of the FMDV vaccine potency.

Materials and Methods

Animals and virus strains. For experimental infection we used 45-day-old long white pigs seronegative for FMDV NSP 3ABC type O and Asia 1 antibodies and two different virus strains. OH/99 strain was isolated from pigs (Liu *et al*., 2004) and Asia 1/JS/05 strain (GenBank Acc. No. EF149009) was isolated from cattle and adapted to the pigs.

Experimental infection. Thirty-six non-vaccinated piglets were randomly divided into 6 groups with 6 animals in each group. Each group of animals was inoculated in a different challenge route and kept in separate rooms. OH/99 strain was used to infect the groups 1, 2, and 3. The i.n. drops and i.m. injection dose applied to the 1st and 3rd group, respectively, was 10,000 ID_{50}/pig in the volume of 2 ml. I.m. injection was applied in the ear-root-neck area. The i.d. dose applied to the 2nd group was 10,000 ID_{50}/pig in the volume

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Abbreviations: FMDV = Foot-and-mouth disease virus; i.n. = intranasal; i.d. = intradermal; i.m. = intramuscular; OIE = Office International des Epizooties; p.i. = post infection

Table 1. The course of infection of piglets with FMDV OH/99 strain applied by different routes

Infection of a group	Pig (No.)	Disease symptoms on day p.i.									
		1	2	3	4	5	6	7	8	9	10
Group 1 (i.n.)	1	_	fever	fever	_	_	_	_	_	_	_
	2	_	fever	fever	fever	_	_	_	_	_	_
	3	_	_	_	_	_	_	_	_	_	_
	4	_	_	_	_	+	+	+	+	+	+
	5	_	fever	fever	fever	_	_	_	_	_	_
	6	_	_	fever	fever	_	_	_	_	_	_
Group 2 (i.d.)	7	_	+	+	+	+	+	+	+	+	+
	8	_	_	+	+	+	+	+	+	+	+
	9	_	_	+	+	+	+	+	+	+	+
	10	_	+	+	+	+	+	+	+	+	+
	11	_	+	+	+	+	+	+	+	+	+
	12	_	+	+	+	+	+	+	+	+	+
Group 3 (i.m.)	13	_	_	_	+	+	+	+	+	+	+
	14	_	_	+	+	+	+	+	+	+	+
	15	_	_	+	+	+	+	+	+	+	+
	16	_	_	_	+	+	+	+	+	+	+
	17	_	_	_	+	+	+	+	+	+	+
	18	_	_	_	+	+	+	+	+	+	+

(–) = normal; (+) = blister (except for i.d. infected foot).

of 0.2 ml and was injected into the heel bulb. Asia 1/JS/05 strain was used to infect the groups 4, 5, and 6. The i.n. drops and i.m. injection dose applied to the 4th and 6th group, respectively, was 10,000 ID_{50}/pig in the volume of 2 ml. I.m. injection was applied in the ear-root-neck area. The i.d. dose applied to the 2nd group was 10,000 ID_{50}/pig in the volume of 0.2 ml and was injected into the heel bulb. All infected pigs were observed daily for 10 days post infection (p.i.). The first day was recorded at 24 hrs p.i.

Table 2. The course of infection of piglets with FMDV Asia 1/JS/05 strain applied by different routes

Infection of a group	Pig No.	Disease symptoms on day p.i.									
		1	2	3	4	5	6	7	8	9	10
Group 4 (i.n.)	19	_	_	_	_	_	_	_	_	_	_
	20	_	_	_	_	_	_	_	_	_	_
	21	_	_	_	_	_	_	_	_	_	_
	22	_	_	_	_	+	+	+	+	+	+
	23	_	_	fever	fever	_	_	_	_	_	_
	24	_	_	_	_	_	_	_	_	_	_
Group 5 (i.d.)	25	_	_	+	+	+	+	+	+	+	+
	26	_	+	+	+	+	+	+	+	+	+
	27	_	_	+	+	+	+	+	+	+	+
	28	_	+	+	+	+	+	+	+	+	+
	29	_	_	+	+	+	+	+	+	+	+
	30	_	+	+	+	+	+	+	+	+	+
Group 6 (i.m.)	31	_	_	_	+	+	+	+	+	+	+
	32	_	_	_	+	+	+	+	+	+	+
	33	_	_	_	+	+	+	+	+	+	+
	34	_	_	+	+	+	+	+	+	+	+
	35	_	_	_	+	+	+	+	+	+	+
	36	_	_	_	+	+	+	+	+	+	+

(–) = normal; (+) = blister (except for i.d. infected foot).

Results and Discussion

In the first group of animals infected with i.n. drops, four pigs had a fever after two or three days, but they stayed without the blisters. One pig developed a blister in the upper margin of coronet on the day 4 p.i. In the second group of animals infected i.d., four pigs showed the blisters on day 2 p.i. and two pigs showed the blisters on day 3 p.i. In the third i.m. infected group, two pigs developed the blisters on day 3 p.i. and remaining four pigs developed the blisters on day 4 p.i. (Table 1). Thus, all twelve pigs in the second and third group showed similar clinical symptoms, but the onset of the disease was delayed in pigs of the group 3. We tested also the infectivity of both FMDV strains Asia 1/JS/05 and OH/99 using three infection routes. We found that both strains behaved similarly in the experimental infection of pigs. This result demonstrated that the three infection routes for both virus strains had similar effect on the course of disease (Table 1, 2).

It followed that the i.d. challenge was the most sensitive infection route due to the blisters appearing in two days p.i. Next to the i.d. challenge was i.m. challenge due to the blisters appearing on day 4 p.i. Infection with the i.n. drops was the slowest way of challenge, since only one pig showed the blisters after 5 days of infection. However, three to four workers were needed to catch and fix pigs in the procedure of i.d. challenge. The pigs kicked and hurt the workers, so this kind of infection was laborious and dangerous. Furthermore, the i.d. challenged pigs should develop a blister in the other foot than the injected one to ensure that the pig was really infected. On the other hand, the i.m. infection needed one or two workers to fix the head of a pig and to inject the animal. The infection was ensured when the blister appeared just on one foot. Thus, the i.m. challenge required simple manipulation and the observation of blisters was uncomplicated. The experimental results showed that the i.m. and i.d. challenges were closely pathogenic. Hence, i.m. challenge is the best route for challenging pigs and it is highly recommended for the evaluation of FMDV vaccine potency.

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