Transboundary and Emerging Diseases

ORIGINAL ARTICLE

Evaluation of Oral Bait Vaccine Efficacy Against Classical Swine Fever in Village Backyard Pig Farms in Bhutan

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Summary

Control and eradication of classical swine fever (CSF) in countries with a high proportion of backyard holdings is a challenge. Conventional attenuated Chinese C-strain vaccines, though safe and effective, are difficult to use in backyard farms due to various practical reasons. The aim of this study was to evaluate the efficacy of the CSF oral bait vaccine in village backyard pig farms and to assess the farmers' knowledge on CSF and motivation on using oral vaccines. The pigs were fed the bait by the farmers themselves; one bait was given on day 0, followed by second bait on the next day. Seventy-three per cent (140 of 193 pigs) of vaccinated pigs had either a slight (2-fold–3-fold; 60 pigs) or significant (at least 4-fold; 80 pigs) increase of the antibody titre against CSFV. A significant increase of the antibody titres was mainly observed in pigs with no pre-vaccination titre (OR = 12, 95% CI = 4–40). The number of pigs with protective antibody titres (≥40) rose from 47 (24%) to 115 (60%) following vaccination. Only 30% of the farmers claimed to be familiar with CSF, although clinical signs they mentioned were rather unspecific and could relate to many other pig diseases. Most of the farmers claimed to be motivated to use oral vaccines if made available. The oral vaccine could be a substitute for the conventional attenuated CSF vaccines in areas where it is logistically difficult for veterinarians to visit. It may therefore be a useful tool to combat endemic CSF disease in regions where the disease continues to have a serious impact on the backyard farmers who depend on pig farming for their sustenance and livelihoods.

Introduction

Classical swine fever (CSF) is one of the most economically important pig diseases worldwide, affecting profitability in large farms and livelihood of small backyard farms (Edwards et al., 2000). In Bhutan, CSF is a notifiable pig disease encountered with sporadic outbreaks. The disease is usually diagnosed by the veterinarian from the clinical signs and case history, but many cases are likely unreported due to remoteness of the outbreak area or failure of farmers to notify a suspicion. There are no scientific data on CSF in Bhutan except for a purposive surveillance study, demonstrating a seroprevalence of 6% (Raika, 1999).

Control and eradication of CSF in countries with high proportion of backyard holdings poses challenges for veterinary services as backyard holdings may act as a reservoir for CSF virus and possible source of infections for commercial farms (Alexander et al., 2011). Lack of proper control strategies coupled with inadequate veterinary services can limit control of CSF especially in the backyard production systems.

Many countries have eradicated the disease through vaccination (Van Oirschot, 2003), and it is still the choice in CSF-endemic countries. This is particularly relevant in developing countries, where control measures such as stamping out and zoning are difficult to apply due to socio-economic reasons. Conventional attenuated Chinese C-strain live vaccines are the most commonly used vaccines against CSF (Suradhat et al., 2007). They are safe and efficiently reduce the CSFV prevalence by increasing herd as well as individual animal immunity (Freitas et al., 2009). Systematic vaccination using conventional vaccines would be a good option for control and eradication of CSF in intensive and industrialized pig production system. However, many developing countries, including Bhutan, have small-scale production systems such as backyard farms and free ranging system, lacking proper registration of pigs and farms (Monger et al., 2014). Furthermore, these farms may be scattered, and in remote areas, they are difficult to reach. Vaccines are also commonly administered via the intramuscular route and therefore require a veterinarian or trained para-veterinarian to administer the same. Therefore, parenteral vaccination in Bhutan is limited only in government breeding farms, and most backyard farms in the village backyard holdings are rarely vaccinated (Raika, 1999). The above-mentioned factors and a lack of adequate veterinary services seriously affect the vaccination coverage in backyard holdings in general.

The use of the oral vaccine RIEMSER® Schweinepestoralvakzine, a conventional CSF live vaccine based on the 'C' strain, has been successfully used to control CSF in wild boars (Kaden et al., 2002; von Rüden et al., 2008). The use of oral vaccines against CSF in backyard pigs as an alternative to parenteral vaccination was tested under field conditions and found to be effective (Milicevic et al., 2013). However, along with successful use of oral vaccines, it is important to look at the cultural and socio-economic structure and lack of adequate knowledge and awareness on CSF and its control among backyard farmers, as this will ultimately determine the potential success or failure of oral vaccination. It is therefore important to evaluate the farmer's views on CSF and its control using vaccine and their willingness to use oral vaccine.

This study aims to evaluate the efficacy of the oral bait CSF vaccine used in village backyard pig farms in Bhutan. The study was also conducted to assess the farmers' knowledge of CSF disease and motivation about vaccination at backyard farms.

Materials and Methods

Study area and selection of animals

The study was conducted in Bhutan from November 2013 to February 2014. Bhutan is subdivided into 20 districts, and in each district, there are several villages (Monger et al., 2014). The village backyard farm in Bhutan is characterized by small numbers of pigs (usually 2–4 pigs) reared by the subsistence farmers, either in a small confined pigsty or free-range scavenging system (Timsina and Sherpa, 2005). Pigs are fed well, and the heath conditions in general are good, although the feeds consist of mainly kitchen wastes (leftover foods, vegetable peels) and locally available plants and wild weeds. These backyard farms consist of either local (indigenous) pigs or exotic pigs. Exotic pigs are mainly of European origin, supplied by government breeding farms. These exotic pigs are routinely vaccinated against CSF at weaning age (45–50 days) at the government farms before being sold to the farmers. Local pigs are mainly native pigs produced in the backyard holding system or purchased from elsewhere locally and are in general not vaccinated. They are generally smaller in size compared to exotic pigs.

Twenty-one villages from six districts with different ecological zones were purposively included in the study. These villages are located in the southern regions and selected for the study due to more concentration of pigs in these districts. The villages were selected based on the pig population data provided by the District Livestock Officer from the selected districts. From the selected villages, the backyard farms were selected based on the list of backyard pig farms provided by the livestock extension agent. A total of 224 pigs from 71 village backyard farms from those 21 villages were included in the study. Most of these pigs included were confined, with only a few scavenging pigs. The study was conducted following approval by the Council for Renewable Natural Resources Research of Bhutan (CORRB), an organization within the Ministry of Agriculture and Forests (MOAF), mandated to coordinate research policy and programmes in Bhutan.

The vaccine and vaccination procedure

Oral vaccination was carried out using oral bait vaccine commercially available and licensed RIEMSER® Schweinepestoralvakzine according to the manufacturer's recommendations (Riemser Arzneimittel AG, Greifswald-Insel Riems, Germany). The vaccine is made up by 1.6-ml C-strain 'Riems' vaccine with a minimum titre of 10^4 $TCID_{50}$ ml filled into a blister incorporated in a corn-based bait matrix (Brauer et al., 2006). The study was conducted involving farmers. The farmers were explained about the oral bait vaccine and given specific instruction on how to feed the pigs with the bait. Based on the instruction, the farmers fed their pigs by providing two baits per pigs; one bait given on day 0, followed by the second bait subsequently on the next day. The baits were laid on the floor of the shed before feeding time in the early morning hours letting each pig feed on them. Farmers were asked to observe the bait uptake and any abnormal behaviour or disease after the intake of the bait. The vaccines were stored frozen until use. For the study period, required vaccines were transported in cool boxes with ice pack and handed over to the farmer who fed the bait on the same day. The second bait supposed to be fed on the next day was asked to be stored in the refrigerator.

Sampling procedure

Blood of all vaccinated animals was collected on day 0 and 28 days post-vaccination (dpv) for serological examination to monitor the antibodies against CSF oral vaccine. Whole blood was collected using 10-ml sterile plain Vacutainer tubes and stored overnight at room temperature for serum separation. Each serum was then transferred into a sterile cryo vial and transported in an icebox to the laboratory, sorted and stored at −20°C until analysis. Names of the owner, herd size, age and sex of sampled animal were recorded.

Thirty-one animals (14%) of the 224 animals were not available for sampling at 28 dpv because they had either been sold or slaughtered. Therefore, they were excluded from the study.

Questionnaire

Face-to-face interviews were applied to the pig farmers by the first author using a structured questionnaire during both the first and second visits to the farm. The questionnaire contained only 'close-ended' questions in three parts. The first part covered general aspects such as herd size, knowledge of farmer on CSF, vaccine and vaccination programme, and experiences in pig rearing. The second part of the questionnaire contained information on individual pigs such as age, breed, source of pigs, vaccination status and number of baits eaten. The third part covered information collected at the end of the field trial about farmers' experience on feeding the bait, their view on the use of oral bait vaccine, willingness to pay if charged and pig's response to the bait.

Detection of antibodies

To determine the effectiveness of oral vaccination, all sera collected before and after vaccination were tested for antibody titres by means of the virus neutralization test (VNT) based on CSFV strain, Alfort/187 (Dahle and Liess, 1995), as described by Terpstra et al. (1984) and Kaden et al. (2004). The tests were conducted at the Central Veterinary Laboratory, Lelystad, Netherlands.

Briefly, 100 $TCID_{50}$ of CSFV strain 'Alfort/187' were incubated at 37°C for 1 h with 2-fold serial dilutions of the serum to be investigated. Then, to each serum-virus mixture, PK-15 cells were added, and after an incubation at 37°C (5% CO_2) for 4 days, medium was removed and cells were fixed by adding 4% formaldehyde. A mixture of two horseradish peroxidase-conjugated monoclonal antibodies against CSFV was used to label virus-infected cells. Antibody titres were expressed as the reciprocal value of the neutralization titre (ND_{50}).

Vaccine response investigation

Response to CSF oral bait vaccine was interpreted by comparing pre- and post-vaccination titres of individual pigs. 'No change' was defined as a post-vaccination titre that was less than twice the pre-vaccination titre. A 'slight increase' was defined as a post-vaccination titre that was 2 to 3 times the pre-vaccination titre. A 4-fold (or more) rise in titre was considered a 'significant increase'. Furthermore, pigs were considered to have a protective titre whenever the neutralizing antibody titres were above 40 ND_{50} (Terpstra and Wensvoort, 1988). This cut-off was also used by Milicevic et al. (2013) to determine whether pigs were protected or not against disease post-oral vaccination.

Data Management and Analysis

Data management and analysis was carried out using SPSS software, IBM SPSS version 20 (SPSS., Chicago, IL, USA). For prevalences, a 95% confidence interval was calculated using the exact binomial confidence intervals (Clopper and Pearson, 1934). A logistic regression was performed to assess the effect of age (>90 or <90 days), breed (exotic or local) and pre-vaccination titre (≥10), as independent variables) on seroconversion rate of vaccinated animals using either titre (≥40 = 1; else 0) as dependent variable.

Results

Antibody titres at the time of vaccination (pre-vaccination)

On the day of the vaccination (prior to vaccination), 51 of the 193 pigs (26%; 95% CI: 20–33) already had an antibody titre of at least 10 ND_{50} (Tables 1 and 2, with further details on type and age of pigs). Exotic pigs were significantly more likely of having pre-vaccination titres ≥40 than local pigs (OR = 28, 95% CI: 10–76). Fifty-six per cent of exotic pigs (42/75) had pre-vaccination antibody titres greater than 40 ND_{50} compared to 4% (5/118) local pigs.

Oral vaccine response

In 140 of the 193 pigs (73%, 95% CI: 66–78), either a slight (60 pigs) or significant (80 pigs) increase of the antibody titre against CSFV was observed (Tables 1 and 2). Figure 1 demonstrates detailed plots showing the relation between pre-vaccination and post-vaccination titre for individual pigs.

Table 1. Response to oral vaccination with classical swine fever bait vaccines in exotic and local pigs, depending on pre-vaccination titre. No change is a post-vaccination response <2× the pre-vaccination titre, a slight increase 2–3× the pre-vaccination titre, and a significant increase ≥4× the pre-vaccination titre; *n* denotes the number of pigs for each category of pre-vaccination titres

	Post-vaccination titre											
	Exotic pigs (*n* = 75)				Local pigs (*n* = 118)				Total (*N* = 193)			
Pre-vaccination titre	*n*	No change (%)	Slight increase (%)	Sign. increase (%)	*n*	No change (%)	Slight increase (%)	Sign. increase (%)	*n*	No change (%)	Slight increase (%)	Sign. increase (%)
<10	32	6 (19)	9 (28)	17 (53)	110	18 (16)	41 (37)	51 (46)	142	24 (16)	50 (35)	68 (48)
10–30	1	0	1 (100)	0	3	0	1 (33)	2 (67)	4	0	2 (50)	2 (50)
≥40	42	30 (71)	6 (14)	6 (14)	5	4 (80)	1 (20)	0	47	34 (72)	7 (15)	6 (13)
Total	75	36 (48)	16 (21)	23 (31)	118	22 (19)	43 (36)	53 (45)	193	58 (30)	59 (31)	76 (39)

Table 2. Response to oral vaccination with classical swine fever bait vaccines in young and older pigs, depending on pre-vaccination titre. No change is a post-vaccination response <2× the pre-vaccination titre, a slight increase 2–3× the pre-vaccination titre, and a significant increase ≥4× the pre-vaccination titre; *n* denotes the number of pigs for each category of pre-vaccination titres

	Post-vaccination titre											
	Young pigs (<90 days, *n* = 39)				Older pigs (≥90 days, *n* = 154)				Total (*N* = 193)			
Pre-vaccination titre	*n*	No change (%)	Slight increase (%)	Sign. increase (%)	*n*	No change (%)	Slight increase (%)	Sign. increase (%)	*n*	No change (%)	Slight increase (%)	Sign. increase (%)
<10	35	7 (20)	12 (34)	16 (46)	107	17 (16)	38 (36)	52 (49)	142	24 (17)	50 (35)	68 (48)
10–30	2	0	1 (50)	1 (50)	2	0	1 (50)	1 (50)	4	0	2 (50)	2 (50)
≥40	2	2 (100)	0	0	45	32 (71)	7 (16)	6 (13)	47	34 (72)	7 (15)	6 (13)
Total	39	9 (23)	13 (3 3)	17 (44)	154	49 (32)	46 (30)	59 (38)	193	58 (30)	59 (31)	76 (39)

From the logistic regression analysis, a significant increase of the antibody titres was mainly observed in pigs with no pre-vaccination titre (OR = 12, 95% CI = 4–40). Conversely, age (OR = 1, 95% CI = 0.9–1) and breed (OR = 0.9, 95% CI = 0.4–2) did not have an influence on the significant increase in the antibody titres 28 dpv.

Protective antibody titres post-vaccination

Titres that were considered protective (≥40) were observed in 47 of the 51 pigs with pre-vaccination titres (Tables 1–3). Following vaccination, the number of pigs with protective antibody levels (≥40) rose from 47 (24%) to 115 (60%) (Table 3). Pigs that had pre-vaccination antibody titres ≥40 are most likely to remain protected with protective titres (≥40) 28 dpv (OR = 7, 95% CI: 2.3–19; $P = 0.001$).

Farmer's knowledge and perception on CSF and vaccination through a questionnaire

The feedback of the pig farmers based on the questionnaire is shown in Table 4. The median herd size was 3 (with 2 and 8 at the 10 and 90 percentile). Of the farmers interviewed, 41% were familiar with CSF and clinical signs related to CSF. The main three clinical signs regarding the CSF that the farmers answered were off-feed, fever and recumbency. Vaccination against CSF at the backyard level is not a regular practice, and none of the farmers interviewed said that they vaccinate at their backyard farms. The main reasons being that the pigs were already vaccinated, the vaccine was not available, or they did not know how and where to obtain the vaccine.

With regards on the use of oral bait vaccine, all farmers successfully fed the vaccine during the trial period, and, except for two farmers, they said the administration of vaccine as a bait was easy. These two farmers had fed the bait after feeding time. All farmers were willing to vaccinate their pigs if the oral vaccine is made available, but ten (14%) farmers still preferred a veterinarian to come and vaccinate their pigs.

Discussion

The objective of this study was to evaluate the efficacy of oral bait CSF vaccine in village backyard pig farms in a field experiment. Here, we report on the oral vaccine trial in

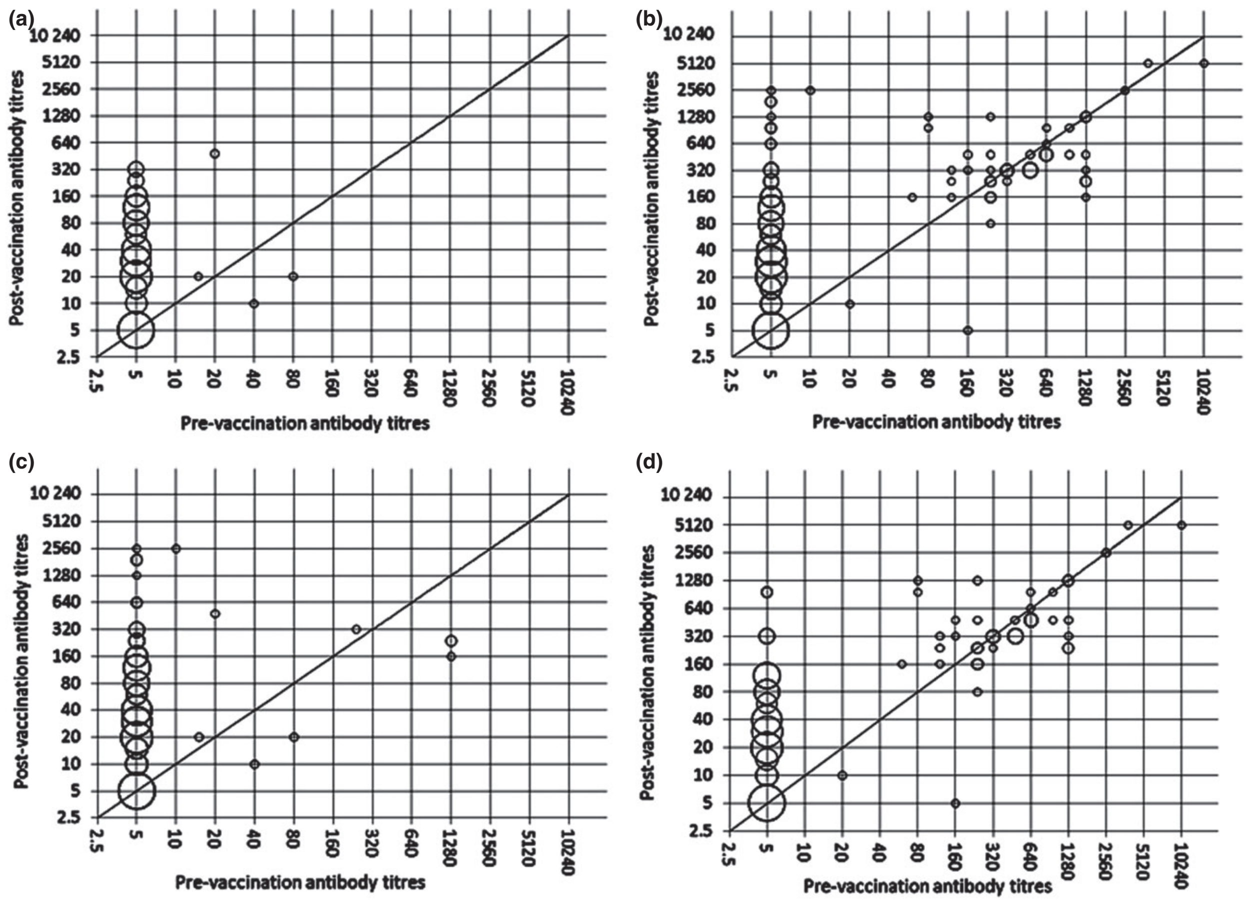

Fig. 1. Relation between pre-vaccination and post-vaccination titres for young pigs (a), older pigs (b), local pigs (c) and exotic pigs (d).

Table 3. Protective antibody titres against classical swine fever at 0 and 28 days post-vaccination

		Titre ≥40	
Pigs	*n*	Pre-vaccination (%)	Post-vaccination (%)
Total	193	47 (24)	115 (60)
Type			
Exotic pig	75	42 (56)	59 (79)
Local pig	118	5 (4)	56 (47)
Age			
Young pig (<90 days)	39	2 (5)	18 (46)
Older pig (>90 days)	154	45 (29)	97 (63)

village backyard farms involving farmers to give the vaccine themselves. Furthermore, a questionnaire was used during and after the trial to know farmer's perception about CSF and its control and their motivation to use the oral vaccine.

In our study, it was found that 73% (140/193) of pigs responded to the vaccine with an increase of the antibody titre 28 dpv. Out of these, 43% responded by developing a 'slight increase' in antibody titres (2-fold or 3-fold) and 57% by a 'significant increase' (4-fold or higher) in antibody titres, showing a response to the vaccine in vaccinated pigs in a field situation.

Because this study was carried out in a field situation, 26% of pigs were found to have antibody titres against CSFV before vaccination (pre-vaccination antibodies). These pigs were mainly exotic pigs, supplied from the government breeding farms with vaccination history. The exotic pigs having pre-vaccination antibody titres greater than 40 ND_{50} remained protected (titres ≥40) after 28 dpv. Pre-vaccination antibody titres against CSFV in local pigs may be attributed to past exposure to natural infection or vaccination. Vaccination in local pigs is rare, and recovery from previous infection is therefore the most likely cause for antibody titres. This could, however, not be verified because there was no information on a history of any illness associated with CSF in these pigs.

The seroconversion rate after vaccination was influenced by the presence or absence of antibodies against

Table 4. Characteristic of farmer's response to the questionnaire on experience in pig rearing, knowledge and perception of classical swine fever (CSF), vaccination and willingness to use oral vaccine against CSF

Variables	Median (Percentile 10, 90)	Total	Yes (%)	No (%)
Herd size	3 [2, 8]			
How many years of experience in rearing pigs?	30 [10, 43]			
Are you familiar with classical swine fever?			29 (41)	42 (59)
If yes, number of clinical signs that could be mentioned (maximum three per interview)		69		
Off-feed		25		
Fever		21		
Recumbency		14		
Diarrhoea		4		
Cough		3		
Skin reddening		2		
Do you vaccinate pigs after purchasing?			0 (0)	71 (100)
If not, reasons for not vaccinating				
Pig already vaccinated		27		
Vaccine not available		16		
Don't know how/where to get the vaccine		14		
No disease around		9		
Don't know about vaccination		5		
How did you experience the administration of the bait?				
Difficult		0		
Intermediate		2		
Easy		69		
Would you consider using oral vaccine if made available?			71 (100)	0 (0)
If yes, preference for carrying out the vaccination				
Application by a veterinarian		10		
Application by farmer himself		32		
No preference (either a veterinarian or himself)		29		

CSFV at the time of vaccination (pre-vaccination titres). Pigs that were seronegative (<10 ND_{50}) at the time of vaccination responded better to the vaccine by developing a 'significant rise' in antibody titres (4-fold or higher) than pigs that already had pre-vaccination antibody titres. In this study, age did not have any influence on the seroconversion. In previous studies, carried out in wild pigs and domestic pigs, the oral vaccines based on the 'Chinese strain' (C-strain) were found to provide a better immune response in older animals (>3 months) because of a better uptake of vaccine baits than in younger pigs (Brauer et al., 2006; von Rüden et al., 2008; Milicevic et al., 2013). We found that bait uptake was not a problem, irrespective of the age groups. Most young pigs were between the age of 2 and 3 months and were able to feed on the bait. This may also be because all the pigs were fed individually by farmers and therefore had an equal chance of taking up the baits, while otherwise older pigs may dominate the younger pigs. This makes the use of oral baits ideal in backyard farms with small numbers of pigs where individual pig can easily be fed.

Four weeks after vaccination, 60% of the vaccinated pigs was considered to be protected (titre ≥40) in our study, which was less than the 68% found in a previous study by Milicevic et al. (2013). One of the reasons for this difference may be the number of baits fed to the pigs: 2 per pig in our study and 4 in the Milicevic study. Theoretically, the vaccine dose in one bait is sufficient to induce a full protective immunity. However, both the uptake of the bait itself and the uptake of the vaccine from the blister inside the bait are uncertain factors in the oral vaccination. Multiple baits may therefore be needed to increase the success rate of the oral vaccination. Vaccine baits are relatively expensive compared to vaccine for parenteral application. The need to use multiple baits may therefore increase the costs of oral vaccination in backyards to an unacceptable level. What is still acceptable will depend on the overall losses due to CSF and the perceived chance for an individual farmer to be confronted with CSF in his pigs. This will differ for different regions, and no general rule on the maximum number of baits for a cost-effective vaccination can therefore be given.

An alternative approach may be to feed each pig 2 baits, but with 2 weeks in between. Thus, the immune response may benefit from a booster response, which may result in a higher rate of protection compared to giving the two baits with only 1 day in between. This needs to be evaluated in a further study though.

In this study, we used questionnaires to understand the farmer's background knowledge of CSF and their motivation to use an oral vaccine. They said that feeding the pigs with oral vaccine by themselves was much easier and convenient, compared to a parenteral vaccine. For one, they did not have to catch the pigs individually. They were willing to vaccinate themselves if oral vaccines were made available to them. This will overcome the difficulty faced in many developing countries having predominantly backyard farms and a free ranging system. Furthermore, this will also save time and resources without having to involve a veterinarian or paraveterinarian. There will be a better vaccination coverage by covering scattered and remote areas, which otherwise would be difficult to reach by a vaccinating team.

For successful control of CSF in endemic areas, alongside successful vaccination programme, it is also important to have adequate knowledge and awareness on the disease. In this study, only 30% of the farmers interviewed claimed to be familiar with CSF and its clinical signs. When asked about the clinical signs, those most often mentioned were very general and could apply to many pig diseases. Except for two farmers that mentioned reddening of the skin, no other clinical signs that are more typical for CSF were mentioned. It is therefore important that the farmers are sensitized through effective awareness on CSF and its clinical symptoms. It is also important to have the views of farmers in other regions of the country on the CSF and clinical signs.

Many countries rely on vaccination for the control of CSF. However, vaccination using conventional parenteral vaccine is difficult in backyard farms due to various practical difficulties, one reason being to be applied via the intramuscular route requiring the participation of a veterinarian or trained para-veterinarian.

Based on previous studies and results of this study, we can conclude that the oral bait vaccine using the 'Chinese strain' of CSFV can successfully induce a protective immunity in the vaccinated pigs reared under backyard village conditions. The oral vaccine may be a substitute for parenteral vaccines in areas where it is logistically difficult for veterinarians to visit, owing to remote and scattered location of the pig farms. Such easy-to-administer oral bait vaccines could be cost-effective, as there is no need to involve a veterinarian, but this also depends on the number of baits needed for a sufficient success rate of the vaccination. It has the potential to be a useful tool to combat endemic CSF disease in regions where it continues to have a serious impact on the backyard farmers who depend on pig farming for their sustenance and livelihoods, although an optimal oral vaccination scheme with the right balance between efficacy and cost-effectiveness will require some more research.

Conflict of Interests

Authors do not have conflict of interest in regard to the findings from this study.

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