Epidemiol. Infect. (2008), **136**, 1556–1563. © 2008 Cambridge University Press
doi:10.1017/S0950268807000131 Printed in the United Kingdom

Inferior rabies vaccine quality and low immunization coverage in dogs (*Canis familiaris*) in China

R. L. HU[1]*, A. R. FOOKS[2], S. F. ZHANG[1], Y. LIU[1] AND F. ZHANG[1]

[1] *Laboratory of Epidemiology, Veterinary Institute, Academy of Military Medical Sciences, Changchun, China*
[2] *Rabies and Wildlife Zoonoses Group, WHO Collaborating Centre for the Characterisation of Rabies and Rabies-Related Viruses, Veterinary Laboratories Agency, Weybridge, Addlestone, UK*

(*Accepted 14 November 2007; first published online 4 January 2008*)

SUMMARY

Human rabies in China continues to increase exponentially, largely due to an inadequate veterinary infrastructure and poor vaccine coverage of naive dogs. We performed an epidemiological survey of rabies both in humans and animals, examined vaccine quality for animal use, evaluated the vaccination coverage in dogs, and checked the dog samples for the presence of rabies virus. The lack of surveillance in dog rabies, together with the low immunization coverage (up to 2·8% in rural areas) and the high percentage of rabies virus prevalence (up to 6·4%) in dogs, suggests that the dog population is a continual threat for rabies transmission from dogs to humans in China. Results also indicated that the quality of rabies vaccines for animal use did not satisfy all of the requirements for an efficacious vaccine capable of fully eliminating rabies. These data suggest that the factors noted above are highly correlated with the high incidence of human rabies in China.

INTRODUCTION

Rabies is a worldwide zoonosis caused by rabies virus resulting in 55 000 human deaths each year, although this figure is still considered to be a conservative underestimate [1–3]. More than 99% of all human deaths from rabies occur in Asia and Africa [2], with China reporting 2000–3000 human deaths attributed to rabies per annum [4]. Domestic dogs in China pose the greatest threat to humans due to their proximity to humans and their tenacious behaviour once infected. Dog bites are the most important reason for human deaths in Asia and Africa. Among the 2000–3000 human deaths per annum in China, >95% are caused from the bite of a rabid dog [5]. Annually, more than 7 million people are exposed to rabies virus from the bite of a dog throughout the world [1, 6].

The burden of rabies is not evenly distributed across all sectors of society. Children aged <16 years are the major victims of rabies as they are more often in contact with dogs than adults, and more often bitten on the head and neck, which carries a much higher risk than bites to other peripheral regions of the body [7, 8]. Another difference of rabies distribution is also reflected by working patterns, e.g. >60% of reported dog bites in China occur in peasants [9]. The high demand for post-exposure prophylaxis (PEP) in Africa and Asia brings about a substantial economic burden not only because of the high costs of human vaccine, but also because of the considerable indirect costs associated with the patients' travel expenses [10], compared with their low income.

* Author for correspondence: Dr Rongliang Hu, Laboratory of Epidemiology, Veterinary Institute, Academy of Military Medical Sciences, 1068 Qinglong Road, Changchun 130062, China.
(Email: hurongliang@hotmail.com)

The control of human rabies is positively correlated with the status of prevention of rabies in animals, especially in companion animals. This has been demonstrated by many countries and regions in the world [10], most recently by the Pan American Health Organization (PAHO) [11]. Through a concerted vaccine campaign in domestic dogs in Latin America, R_0 was reduced to <1 resulting in the transmission cycle amongst domestic dogs being interrupted and the infection rate being diminished with the subsequent elimination of the disease in the dog population. In Latin America, PAHO demonstrated that a reduction in dog rabies by 81% (and 93%) resulted in an 82% (and 91%) reduction in human rabies cases [12, 13]. In addition, dogs have been vaccinated in several western European countries and North America for many years, resulting in elimination of rabies in the dog population with a concomitant reduction in the burden of human rabies in these continents and countries [14].

Since 1996, rabies in China has been increasing, with more ferocity. The officially reported human cases were 2561 cases in 2004, 2548 cases in 2005 and 3215 cases in 2006 [4]. Two types of live rabies vaccines manufactured domestically and four types of inactivated rabies vaccines imported from Europe and the United States have been approved for use to prevent dog rabies in China. However, the imported vaccines are relatively expensive and are not affordable for dog owners in remote rural areas. The live vaccines, although cheap, may not have been utilized correctly in most areas. Moreover, 'owned' dogs are not vaccinated compulsorily against rabies. If dog rabies cannot be properly controlled, the rabies situation will worsen, resulting in an increased public health burden in China. In this report, we provide data related to rabies in humans and dogs in China. The paper illustrates that rabies must be controlled in dogs in China to avert any risk to humans.

METHODS

The occurrence of human rabies in China

The epidemiological data of human rabies are derived from the website from the Chinese Ministry of Health, and recorded monthly and annually [4].

Survey of dog rabies

The animal rabies survey was derived from two resources. One is from the Proceedings of the National Symposium on the Prevention and Control of Zoonoses, held in May 2006 in Beijing [15]; other data or information were obtained by visiting villages in provinces where rabies is endemic or that have been free of rabies for at least 3 years.

Assay of rabies virus neutralizing antibody (VNA) in dogs

Blood samples of dogs from the local communities of a city were collected in a vaccination coverage survey. This was completed by veterinarians who were responsible for the prevention of any outbreak, and the serum samples were taken from dogs by visiting households on a door-to-door basis. Communities were randomly chosen from each city that had undertaken a survey. More than 98% of the dogs in cities including Beijing, Shanghai, Shenzhen, Dongguan, Changchun and Hengshui are pets, however, <2% were watch dogs. Villages where dog serum samples were taken were divided into two types. The first type were villages in counties in Guizhou, Guangxi and Shandong provinces where rabies has occurred severely; the second type were villages in counties in Hebei province where human and animal rabies has not been recorded for at least 3 years, according to the information provided by the Chinese Centre for Disease Prevention and Control (CDC) Information Institute. More than 99% of the dogs from the villages in Shandong, Guizhou, Guangxi and Hebei provinces were watch dogs, and <1% were pets. The serum samples in villages were also collected in the same way as in other cities. Sera, recovered by centrifugation at 15000 ***g*** for 15 min after blood sampling were set for 2 h and cooled for 1 h.

The VNA titre of the serum samples was assayed by fluorescent antibody virus neutralization (FAVN) as described elsewhere [16]. The positive antiserum against rabies virus was provided by AFSSA (Nancy, France); the FITC conjugate was prepared in our laboratory.

Assays of rabies vaccines in dogs and in cell culture

Two kinds of locally produced live attenuated rabies vaccines were used: the first was ERA combined with the canine distemper virus, canine parvovirus, canine adenovirus type-2 and canine parainfluenza virus; the second was Flury LEP. Both vaccines (batch nos. 200602 and 200601004, the brand names of the

https://doi.org/10.1017/S0950268807000131 Published online by Cambridge University Press

vaccines were omitted due to any potential adverse effects on the companies that produce the vaccines in China) were in lyophilized form, and were purchased from an Animal Disease Prevention and Surveillance Centre in the south of China. The positive control vaccines also included two types. One type was prepared in our laboratory according to the live vaccine manufacture guidelines (production no. 2006010205). The second was the Nobivac Rabies inactivated vaccine (batch no. 75118C01) from Intervet, Holland. Ten vials (1 dose/vial for ERA and 10 doses/vial for Flury LEP) of each live rabies vaccine were tested in animals. In total, 50 healthy 4-month-old hybrid dogs weighing 1·8–2·2 kg with no gender selection were purchased from Jilin University Experimental Animal Feeding Centre (Changchun, China). The animals were randomly assigned to five groups, 10 for each type of vaccine and for the negative control group. The dogs were fed in isolated cages throughout the trial. These dogs had not been vaccinated previously against rabies and were confirmed in our laboratory by the FAVN assay [16] to have no rabies VNA before vaccination. The vaccine of each dose was dissolved with 2 ml sterile distilled water and inoculated into the dogs by intramuscular injection in the hind leg. After 4 weeks, blood was collected and the rabies VNA was assayed according to the FAVN protocol [16].

At the end of the trial, i.e. 6 months after the vaccination, the five groups of dogs were challenged with 10^6 mouse LD_{50} of a street virus (D961, passaged and maintained in mouse brain), intramuscularly injected in the masseter muscle. The dogs were observed for clinical signs indicative of rabies such as behavioural changes, swallowing difficulties, salivation, loss of appetite, aggressive behaviour, paresis, paralysis and death, and recorded for the progress of the disease. After clinical signs of rabies appeared, the dogs were euthanized with a barbiturate solution administrated intravenously. For those animals that survived the challenge, the VNA in serum was measured by FAVN [16].

All the animal experiments involving the vaccination and challenge of dogs were approved by the Veterinary Institute, Academy of Military Medical Sciences Research Ethics Committee [SYXK (M) 2002-001]. All analyses was performed in accordance with regulations of Experimental Animal Management and Welfare of China (2002 version).

Another two vials of each of the two commercial live vaccines were restored with MEM to 2 ml/dose,

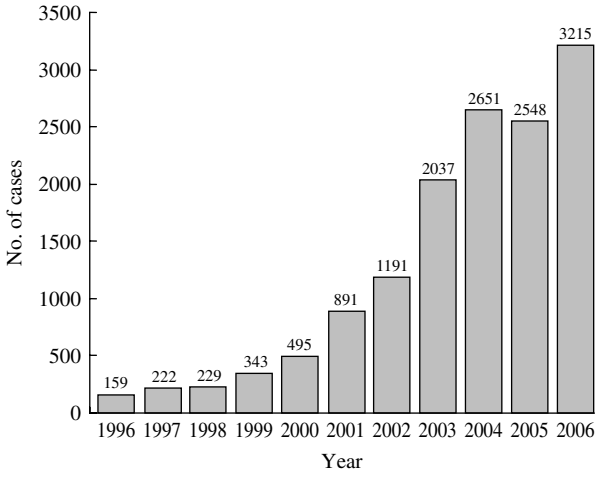

Fig. 1. Annual cases of human rabies deaths in China (1996–2006).

160 μl were removed and inoculated into the first row of Vero cells and BHK-21 cells in 96-well cell culture plates, serial tenfold dilution with octoplets for each dilution were performed for each kind of vaccine. After 48 h incubation at 37 °C, the cells were stained with FITC conjugate and $TCID_{50}$ was calculated according to methods described elsewhere [17].

Assay of rabies virus antigen in dog brain specimens

Dog brains were collected from dog slaughterhouses located in villages and counties in Guangxi, Guizhou and Hebei provinces, where dog meat is commonly eaten as a custom. Dogs were derived from the same areas where blood samples were collected for the VNA assay. The dogs are sold to slaughterhouses by dog dealers who purchase the dogs from different villages. During the collection of brain specimens, the dogs were euthanized and the blood collected. The specimens were removed either by opening the skull or by a method previously described [18]. Brain tissues were transferred to a sterile tube and frozen at −20 °C, they were then stained on a smear slide with FITC conjugate according to a method previously described [19].

RESULTS

Epidemiology of human rabies in China

Since 1996, human cases of rabies have continued to increase in China (Fig. 1). Most of the deceased patients were peasants from rural areas. For example, among the 2548 people who died of rabies in 2005,

https://doi.org/10.1017/S0950268807000131 Published online by Cambridge University Press

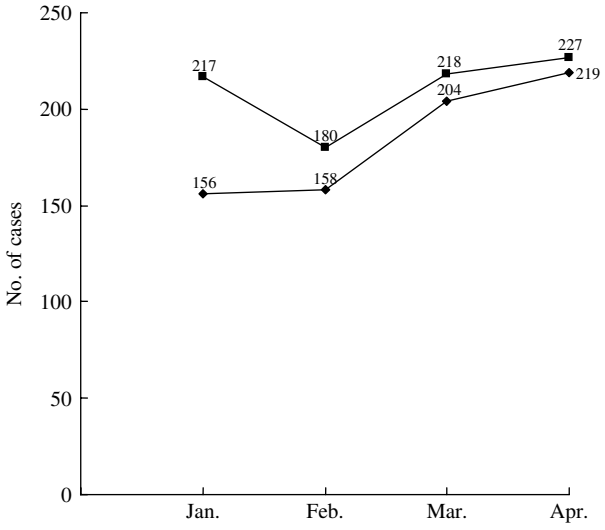

Fig. 2. Human rabies cases in China (January to April). –◆–, 2006; –■–, 2007.

67% were peasants, 17% were students, 8% were 'under-age' children not attending school, and 2% were 'office/factory' workers, and 2% were babies. Of these, 91% of the students and the children were also from peasant families. Moreover, almost all the workers were also peasants from rural areas who had worked in the cities. All these data suggests that rabies occurs much more often in rural areas than in cities in China.

It apeared that the number of human rabies cases reduced from 2561 in 2004 to 2548 in 2005. However, in 2006, a total of 3215 human rabies cases were officially reported, reaching a new peak. Meanwhile, in the first 4 months of 2007, the number of cases continued to increase rapidly (Fig. 2) compared to the same period in previous years. The situation remained the same, i.e. most of the new cases occurred in rural areas.

Number of dogs in China

According to the preliminary statistical data, it was estimated that around 130 million dogs are present throughout the country in China. The accurate number, however, is not fully clear. Of these, at least more than half of the dogs are present in rural areas.

We lack accurate data on animal rabies in China as there is not an accurate nationwide surveillance system similar to that for human rabies surveillance. These data are basically derived from the occurrence of human rabies, mainly from the local branches of the China CDC in each county and province. In 2005, the number of confirmed rabid dogs in China was only 145.

We investigated several villages including Tancheng and Cangxian (Shandong province), Xingren and Puding (Guizhou province), and Guiping (Guangxi province), where rabies is a serious problem and where dogs were killed almost every day because of dog-biting incidents. We also investigated Raoyang (Hebei province), which was free of rabies for at least 3 years and is sporadically epidemic.

In these villages and provinces, 'suspect' dogs are usually killed after biting an individual. The dogs are killed and the carcass is usually abandoned in a river, pool or more often buried. Seldom is a dog that is killed submitted to a laboratory for rabies diagnosis.

Immunization coverage in dogs assayed by FAVN

In total, 1653 dog sera were collected from the metropolitan area of Beijing, Changchun, Shenzhen, Shanghai, Dongguan and Hengshui. All serum samples from Beijing (311) and Shanghai (230) were tested by the FAVN test and shown to have a VNA of $\geqslant 0{\cdot}5$ IU, with the average level measured at $2{\cdot}27 \pm 0{\cdot}43$ (mean $\pm$ S.D.). Dog serum samples from some communities of Shenzhen, Dongguan and Changchun had a VNA titre of $\geqslant 0{\cdot}5$ IU/ml; while in other communities of Shenzhen (110), Dongguan (98) and Changchun (78), the VNA-positive rate was 80%, but none of the dogs tested reached a VNA titre of 0·5 IU/ml ($0{\cdot}09 \pm 0{\cdot}32$ IU/ml). In Hengshui, a medium-sized city in the north of China, the VNA-positive rate and immunization coverage in dogs were substantially lower, at 41·6% ($0{\cdot}06 \pm 0{\cdot}43$ IU/ml) and 25·2% ($0{\cdot}87 \pm 0{\cdot}26$ IU/ml) on average, respectively.

In rural areas, 1320 samples in total from Tancheng and Cangxian (Shandong province), Xingren and Puding (Guizhou province), and Guiping (Guangxi province), where rabies prevails severely and Raoyang (Hebei province), where rabies is rare and had not been recorded for 3 years, were collected and assayed for VNA titre by FAVN, the positive rate of VNA present in all samples was very low, ranging from 1·2% to 2·8% (data partly shown in Table 1). The average VNA titre among the antibody-positive dogs was ($0{\cdot}69 \pm 0{\cdot}12$ IU/ml).

Quality of rabies vaccines for dogs

We immunized 10 dogs with each locally produced live vaccine of the two kinds commercially available

https://doi.org/10.1017/S0950268807000131 Published online by Cambridge University Press

Table 1. *Immunization coverage and rabies virus prevalence in dogs from different regions of China*

County	Number of dog specimens (sera or brains)	Immunization coverage	Virus-positive rate in brain
Guiping	97	2 (2·1%)	—
	235	—	5 (2·2%)
Xingren	83	1 (1·2%)	—
	187	—	12 (6·4%)
Raoyang	252	7 (2·8%)	—
	332	—	0 (0)

Table 2. *The challenge result of dogs vaccinated with different rabies vaccines*

Batch number or vaccine types	Dogs vacci-nated	Specific death	Survived	Protection
200602 (local)	10	9	1	10%
200601004 (local)	10	8	2	20%
Intervet	10	0	10	100%
Laboratory manufactured	10	0	10	100%
Negative control	10	10	0	0

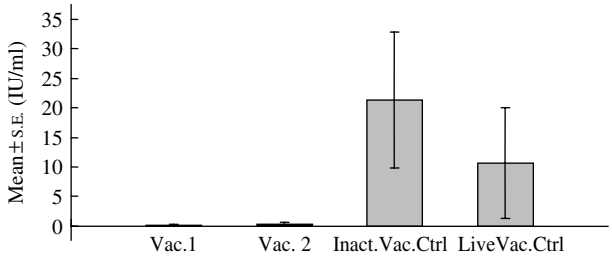

Fig. 3. Comparison of the neutralizing antibody levels between the two commercial live vaccines and the two (one inactivated, one live) positive controls. Vac.1, A live rabies vaccine combined with other vaccines; Vac.2, a live vaccine containing rabies virus alone as indicated by the label; Inact.Vac.Ctrl, an imported rabies vaccine; LiveVac.Ctrl, a live vaccine manufactured in our own laboratory. The neutralizing antibody was assayed 2 weeks after the vaccination, which is similar to that in vaccine efficacy assay in vaccine manufacture.

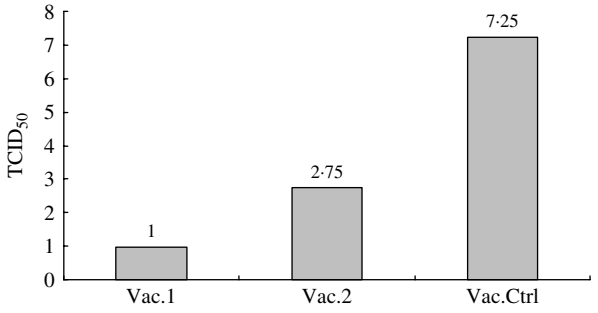

Fig. 4. The assay of efficacy ($TCID_{50}$) of two commercial vaccines and a laboratory-produced live vaccine on cells. Vac.1, The $TCID_{50}$ of a live rabies vaccine combined with other canine vaccines; Vac.2, the $TCID_{50}$ of a live vaccine containing rabies virus alone; Vac.Ctrl, the $TCID_{50}$ of a live vaccine manufactured in our own laboratory.

in China. After 4 weeks, the VNA titre of 1/10 (0·08 ± 0·18 IU/ml) in the first group and 3/10 (0·29 ± 0·45 IU/ml) in the second group of the immunized dogs reached 0·5 IU/ml. The two positive vaccines elicited 100% antibody response, which reached the minimum VNA threshold in dogs (10/10) 4 weeks after vaccination. The attenuated live vaccine control (produced in our laboratory) elicited a VNA titre between 2·34 and 27·7 IU/ml (13·63 ± 9·12 IU/ml) and the inactivated rabies vaccine elicited a VNA titre between 4·67 and 43·16 IU/ml (18·75 ± 12·10 IU/ml) (Fig. 3). Both the seroconversion and the mean VNA titre were significantly higher ($P \leqslant 0{\cdot}01$) than that induced by the two types of commercial live vaccines.

In the challenge trial with the street rabies virus, dogs had been observed for 3 months (Table 2). Dogs that survived the challenge developed a high level of VNA to rabies virus ranging from 17·77 to 65·22 IU/ml, which indicated a strong anamnestic immune response.

We restored four vials of the commercial live vaccines with distilled water to 2 ml and removed 160 μl from each. The vaccines were inoculated onto eight wells of Vero cells with one batch and BHK-21 cells with another. After culture at 37 °C for 48 h, the cells was stained using an anti-rabies FITC conjugate, the average virus titre of the two vaccines was shown to be $10^{1{\cdot}0}$/ml in Vero cells and $10^{2{\cdot}75}$/ml in BHK-21 cells. In comparison, the virus titre of the live vaccine control that elicited a VNA between 2·34 and 27·7 IU/ml in dogs was $10^{7{\cdot}25}$/ml in BHK-21 cells (Fig. 4).

Detection of rabies virus in dog brain specimens

The positive rate of rabies virus antigen detection using the fluorescent antibody test in dog brain specimens was 2·2% (5/235) and 6·4% (12/187) in dogs from the counties of Xingren (Guizhou province), and Guiping (Guangxi province), respectively.

https://doi.org/10.1017/S0950268807000131 Published online by Cambridge University Press

There was no antigen-positive staining in the brain specimens of 332 dogs collected from two villages in Raoyang (Hebei province), where the VNA positive rate (above 0·5 IU/ml) in dogs was 2·8% and where no human rabies cases had been reported (Table 1).

DISCUSSION

People bitten by dogs in China, especially those in remote rural areas did not usually take any prophylactic measures against rabies. For those patients who did seek prophylaxis, only one or two of the recommended measures were taken, with little attention to the seriousness of the wound. This is one of the principal reasons that human deaths have occurred, especially in these rural areas. It also indicated that the dogs in these rural areas had not been well vaccinated. These observations were substantiated in our subsequent testing of dog sera, brain tissues and the currently used vaccines. The observed increased cases of human rabies could not be ultimately resolved by the improved surveillance and the use of PEP for treatment of the human rabies alone. Closer attention is now required to investigate the infectious source and reservoir of rabies, namely the domestic dogs, in China.

In contrast, the surveillance on animal rabies suggested that the incidence of rabies in animals is low, e.g. animal rabies in 2005 was reported as only 145 cases. This number is likely to be an underestimate, as dog bites often happen to people and the majority of the people bitten by dogs are usually seen by doctors in a local CDC who universally accept the use of PEP. The dogs that bit people were killed without exception and, unfortunately, seldom were the 'suspect' dogs reported to a specific veterinary/administrative department for rabies testing. Moreover, people living in rural areas are not educated to report dog-bite exposures. Currently, an animal CDC system is now under construction in China and a guidance of animal rabies control is being prepared. Therefore, the surveillance of animal rabies in China is expected to improve.

In assaying rabies virus antigen in dog brain specimens, the dogs were primarily purchased by dog dealers from villages and sold to the dog slaughterhouses. Most of the dogs are watch dogs. But when there are two or more dogs in one family due to uncontrolled breeding, as dogs in villages are not restricted by their owners, and when the owners could not rear them, one of the dogs would be selected for sale. Therefore all the dogs bought by dog dealers were adults and are randomly collected.

According to our survey, the local veterinary departments have rarely undertaken a mass vaccination unless human rabies cases are increasing in a short period or a governmental-run mass vaccination campaign is implemented, mainly because these villages are remote and the houses are scattered. To vaccinate a dog in these areas is relatively expensive and difficult for both the veterinarian and the dog owners. Moreover, human rabies deaths are relatively rare compared to other deaths in these areas and therefore no special attention has been focused on rabies.

The rabies-positive rates of dog brain specimens of between 2·2% and 6·4% in Xingren and Guiping counties are extremely high. The high percentage of rabies virus existing in dogs in endemic areas also indicates the severity in animal rabies. In fact, before we collected the brain specimens, we consulted the Chinese CDC Information Institute. The occurrence of human rabies in these two counties was among the highest in China at that period. Rabies virus in dogs was shown to be consistent with the high incidence of human rabies cases from the Chinese CDC. This implied that many of the dogs had been infected with rabies virus and some of them were at the incubation stage of rabies during that specific period. Without prompt and suitable measures, the spread of rabies in animals will become more serious, especially in unvaccinated and naive populations. We are now tracking the occurrence of rabies in both humans and animals in these areas.

In spring and autumn of each year, the Ministry of Agriculture, acting on behalf of the Chinese government, undertakes nationwide mass rabies vaccination campaigns for dogs. Previously, this approach has played an important role in rabies control in specific cities and towns. For reasons that are unclear, however, vaccination is not effective in certain regions, especially rural areas. For example, vaccination is not usually implemented by design but by the status of a rabies epidemic, i.e. if there is a rabies occurrence in a region that year, vaccination will be subsequently undertaken. If, however, there is no rabies the following year, the yearly or half-yearly vaccination programme will be cancelled, possibly and partly because of a lack of finance. Therefore the year afterwards rabies will inevitably re-emerge. We suggest that the rabies VNA in dogs after compulsory vaccination should, therefore, be regularly and randomly checked

to determine the immunization coverage or to check for seroconversion. The government-funded administrative department should check the implementation of vaccination by monitoring the VNA level of the dog population and checking for the presence of virus in 'suspect' animals.

There was no rabies virus-positive result from the dog specimens of the two villages at Raoyang (Hebei province). At the same time, the rabies VNA in dogs from the same area was not detected or the antibody titre was very low in a small percentage of dogs. According to the information from the Chinese CDC, no incidence of human rabies in this area had been recorded for 3 years. This might be related to the following: first, this area is far from the endemic regions and consequently the dogs were not able to be infected; second, Raoyang (Hebei province) is a large geographical rural area, although dogs were not restricted to cages, they had a home and were less likely to stray and had less chance to become infected; third, the number of dogs in this county is overall less than that in the counties in Guizhou and Guangxi provinces and the dogs are not evenly fed between villages making dog-to-dog transmission difficult. As there had been no rabies in this area in the past years, dog owners (most are peasants) were unwilling to have their dogs vaccinated and consequently no or a low level of rabies VNA could be detected. Since the middle of 1990s, human rabies has mainly occurred in the south part of China. However, the occurrence of rabies is now spreading from the south to the north and is gradually penetrating into the provinces located in the north part of China. Shandong, a province in the middle of China with 10–20 human rabies cases each year, is in the south and a neighbour of Hebei. The rabies threat to Hebei has been becoming obvious. Without an appropriate vaccine and a strict vaccination policy for animals, the possibility of the spread of animal and human rabies from the south to the north exists, especially for dogs without any rabies immunity.

Currently, four types of inactivated vaccines and two types of live vaccines for dog use have been approved by the Chinese government. The inactivated vaccines are all imported from foreign companies, e.g. Intervet, Fort Dodge, Merial and RabVac, the price of which, ranging from US$1·5 to US$8 per dose, is relatively high for local residents. The locally produced live vaccines are cheap, US$0·01–0·14 per dose, but only 20–40 million doses are produced annually nationwide. In the developed large cities, especially Beijing, Shanghai, Shenzhen and Dongguan, live vaccine is not allowed to be used for dogs according to local regulations. However, as rabies vaccination is not mandatory for dog owners, there are still some who avoid having their dogs vaccinated because of the high price of registration and vaccination. In those semi-urbanized cities, different types of vaccines including both the inactivated and live-attenuated viral vaccines are allowed for use in dogs. But the government suggests that dogs subjected to close contact with their owners should be vaccinated with an inactivated vaccine. Similarly, a high number of dogs could not be vaccinated because of a number of reasons such as high registration and vaccination fees, poor administration, and difficulties in implementation. In rural areas, no regulations have been issued yet to restrict the use of vaccine type. For those who can afford vaccination, or when the local government pays the cost of rabies vaccination, their dogs will be vaccinated. Nevertheless, the live rabies vaccines may not be appropriately distributed to the rabies endemic areas. At least at this stage, most of the dogs in rural areas have not been vaccinated.

In addition, we have concerns about the potency of some vaccines for veterinary use in China, especially the locally produced vaccines. The WHO recommends that all rabies vaccines for animal use must have a minimum antigen content of 1 IU/dose and be tested using the NIH protocol [20, 21]. Commercial vaccines purchased from Western countries are too expensive for vaccinating dogs throughout China. While the level of virus titre contained in the locally produced live vaccine may be affected by many other factors, e.g. cold-chain, storage, additives, or the difference of cell lines used for the titration. Nevertheless, the lyophilized rabies vaccine should keep its infectivity stable under suitable conditions such as refrigeration at $-20\ ^{\circ}C$. Therefore, the quality of the approved vaccines sold or supplied in China should be randomly inspected by an independent institution of the vaccine manufacturers, in spite of the vaccine quality control used in manufacturing.

As both human and animal rabies occurs mainly in rural areas, the costs to a family following the death of a family member from rabies are exorbitant. For this reason, it is recommended that attention to the vaccination of dogs in rural areas be a priority. Not only the vaccine production but also the vaccination regimen for dogs should be supervised by a veterinary administration and should be strictly controlled

under the surveillance of the administrative department(s).

The data presented here might not represent the whole status of rabies in every province in China. However, it at least reflects the current situation of rabies in epidemic areas of China. If vaccination coverage in dogs decreases from the required level of 70%, or is not maintained, rabies will re-establish rapidly as the R_0 will be >1 resulting in the continual spread of rabies virus amongst domestic dogs. For these reasons, we suggest that the Chinese government should put rabies vaccination for dogs as a high priority.

ACKNOWLEDGEMENTS

Financial support for this study was provided by National Natural Science Foundation key project (Approval no. 30630049) and China National '973' Programme (Approval no. 2005CB52300). A.R.F. was funded by the UK Department for Environment, Food and Rural Affairs (Defra) grant SEV3500.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Knobel D, *et al.*** Re-evaluating the burden of rabies in Asia and Africa. *Bulletin of World Health Organization* 2005; **83**: 360–368.
2. **Fooks AR.** Rabies in Europe – editorial. *Eurosurveillance* 2005; **10**: 1–4.
3. **Mallawa M, *et al.*** Rabies encephalitis in a malaria-endemic area of Malawi, Africa. *Emerging Infectious Disease* 2007; **13**: 136–139.
4. **Ministry of Health of the People's Republic of China.** (http://www.moh.gov.cn).
5. **World Health Organization.** WHO Expert Consultation on Rabies. First Report WHO Technical Report Series, No. 931 2005.
6. **Coleman P, Fevre E, Cleaveland S.** Estimating the public health burden of rabies. *Emerging Infectious Disease* 2004; **10**: 140–142.
7. **Pancharoen C, *et al.*** Rabies exposures in Thai children. Wilder. *Environmental Medicine* 2001; **12**: 239–243.
8. **Fevre E, *et al.*** The epidemiology of animal bite injuries in Uganda and projections of the burden of rabies. *Tropical Medicine of International Health* 2005; **10**: 790–798.
9. **Zhang YZ, *et al.*** Human rabies in China. *Emerging Infectious Diseases* 2005; **11**: 1983–1984.
10. **Cleaveland S, *et al.*** Canine vaccination – providing broader benefits for disease control. *Veteriary Microbiology* 2006; **117**: 43–50.
11. **Belotto AJ.** The Pan American Health Organization (PAHO) role in the control of rabies in Latin America. *Development of Biologicals* (*Basel*) 2004; **119**: 213–216.
12. **Schneider MC, *et al.*** Epidemiological situation of human rabies in Latin America in 2004. Report from Organización Panamericana de la Salud, 2005.
13. **Belotto A, *et al.*** Overview of rabies in the Americas. *Virus Research* 2005; **111**: 5–12.
14. **Finnegan CJ, *et al.*** Rabies in North America and Europe. *Journal of the Royal Society of Medicine* 2002; **95**: 9–13.
15. **Association of Science and Technology and Ministry of Health of China.** *Proceedings of the National Symposium on the Prevention and Control of Zoonoses*, Bejing, 2006, pp. 1–68.
16. **Cliquet F, Aubert M, Sagne L.** Development of a fluorescent antibody virus neutralisation test (FAVN test) for the quantitation of rabies-neutralising antibody. *Journal of Immunological Methods* 1998; **212**: 79–87.
17. **Reed LJ, Muench HA.** A simple method of estimating fifty per cent end-points. *American Journal of Tropical Medicine and Hygiene* 1938; **27**: 493–497.
18. **Aubert MFA, Cliquet F, Barrat J.** Rabies. *OIE Manual of Standards for Diagnostic Tests and Vaccines*, 3rd edn. Paris: Office International des Epizooties, 1996, pp. 188–217.
19. **Meslin F-X, Kaplan MM, Koprowski H.** *Laboratory Techniques in Rabies*, 4th edn. Geneva: World Health Organization, 1996, pp. 55–65.
20. **WHO Expert Committee on Biological Standardization.** Requirements for rabies vaccine for veterinary use (amendment 1992). Forty-third report. Geneva: World Health Organization, 1994 (WHO Technical Report Series, No. 840), Annex 6.
21. **Wunderli PS, *et al.*** The protective role of humoral neutralizing antibody in the NIH potency test for rabies vaccines. *Vaccine* 1991; **9**: 638–642.