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Berl Münch Tierärztl Wochenschr 125,  
191–196 (2012)  
DOI 10.2376/0005-9366-125-191

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Verlagsgesellschaft mbH & Co. KG  
ISSN 0005-9366

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Eingegangen: 19.09.2011  
Angenommen: 31.12.2011

Online first: 07.05.2012

[http://vetline.de/zeitschriften/bmtw/  
open\\_access.htm](http://vetline.de/zeitschriften/bmtw/open_access.htm)

### Summary

### Zusammenfassung

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Code Statement:  
0005-9366/2012/12505-191 \$ 15.00/0

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## Short-term interval baiting to combat the re-emergence of fox rabies in Rhineland Palatinate (Germany) in 2005

### *Kurzzeitintervallbeköderung zur Bekämpfung der Tollwut bei Wiedereinschleppung am Beispiel von Rheinland-Pfalz im Jahr 2005*

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In 2005, the final phase of terrestrial rabies eradication in Germany was put at risk by a severe setback due to re-introduction of the disease in Rhineland-Palatinate from neighbouring Hesse after seven years of absence. The rapid westward spread of the disease prompted veterinary authorities to react swiftly and apply a new yet unproven vaccination strategy to rapidly increase herd immunity in an almost unprotected fox population to stop the epidemic. The cornerstones of this emergency oral rabies vaccination strategy, i. e. vaccination intervals, identification of high risk spots, real time epidemiological assessment, capable to eliminate rabies within 13 months after incursion are described here. This strategy may be used as a template to tackle similar emergency situations in Europe in the future.

**Keywords:** emergency vaccination strategy, fox, Germany, oral rabies vaccination, rabies, re-infection

2005 wurde die Endphase der Tollwuteradikation in Deutschland durch einen schweren Rückschlag infolge der Wiedereinschleppung der Tollwut aus dem benachbarten Hessen nach Rheinland-Pfalz, sieben Jahre nach erfolgreicher Tilgung, gefährdet. Die schnelle westliche Ausbreitung der Tollwut veranlasste die Veterinärbehörden unverzüglich zu reagieren und eine bis dahin unerprobte Impfstrategie anzuwenden, um die Immunisierungsrate in einer fast ungeschützten Fuchspopulation innerhalb kürzester Zeit aufzubauen und somit die Epidemie zu stoppen. Die Eckpfeiler dieser Notfallimpfstrategie wie Auslageintervalle, Identifikation von Risikogebieten sowie die zeitnahe epidemiologische Auswertung werden beschrieben, die innerhalb von 13 Monaten in der Lage war, die Tollwut erneut zu tilgen. Diese Strategie könnte als Vorlage bei zukünftigen ähnlichen Notfallsituationen bei der Tollwutbekämpfung in Europa dienen.

**Schlüsselwörter:** Notfallimpfstrategie, Deutschland, orale Immunisierung, Fuchs, Tollwut, Reinfektion

## Introduction

Oral rabies vaccination of foxes (ORV) is without doubt the most (cost-) effective method of rabies control in wildlife. During the past 25 years, the implementation of ORV considerably improved the rabies situation in Europe (Müller, 2000). Since 1978 a continuous decrease in rabies incidence has been reported Europe-wide and eventually the rabies front was repelled eastwards and elimination of terrestrial rabies was achieved for the most part of Western and Central Europe. To date, as a result of ORV, several European countries have been officially recognised as being free of terrestrial rabies, i. e. Finland (achieved rabies free status in 1991), the Netherlands (1991), Italy (1997), Switzerland (1998), France (2000, regained status in 2010), Belgium and Luxembourg (2001), the Czech Republic (2004), Germany (2008), and Austria (2008) (Anonym, 2008; Cliquet and Aubert, 2004; European Commission, 2002; Matouch and Vitasek, 2005). Other Middle and East European countries have made considerable progress in rabies control in wildlife too, resulting in a significant decrease in rabies incidence in recent years. However, there are still years to go in order to reach the goal of complete elimination of terrestrial rabies in Europe.

Once fox rabies is successfully eradicated in a given area, re-emergence of the disease is considered a permanent threat. Re-emergence of rabies in free(d) areas can be caused by two different scenarios, e. g. local disease spread or a sudden emergence without apparent connection to an epidemic. A local disease spread is always associated with the persistence of the disease in neighbouring regions resulting in a frequent invasion of infected foxes due to the permanent infection pressure into already freed areas. On condition of a proper surveillance system, a local disease spread can be well anticipated and prevented by an on time establishment of an effective vaccination cordon (European Commission, 2002). In contrast, the introduction of single rabies infected animals into a rabies free area most likely due to the importation/translocation of a rabid animal and subsequent spillovers into wildlife or a long distance migration of an infected reservoir animal, are unexpected events. Such a scenario can occur at any place at any time independent of the rabies situation in neighbouring regions. Existing WHO and EU recommendations for ORV mainly address situations in which endemic rabies is to control (European Commission, 2002; World Health Organisation, 2005). Unfortunately, emergency situations have never been contemplated in detail and it remains open whether current recommendations can be considered adequate bearing in mind the fact that whatever the reasons for a re-emergence of rabies is, in such a worst case scenario the disease would meet a totally unprotected, i. e. susceptible fox population likely characterized by a high density. On these conditions rabies is expected to spread rapidly at high incidence and all measure would need to focus on rapid response planning to eliminate the disease as quickly as possible.

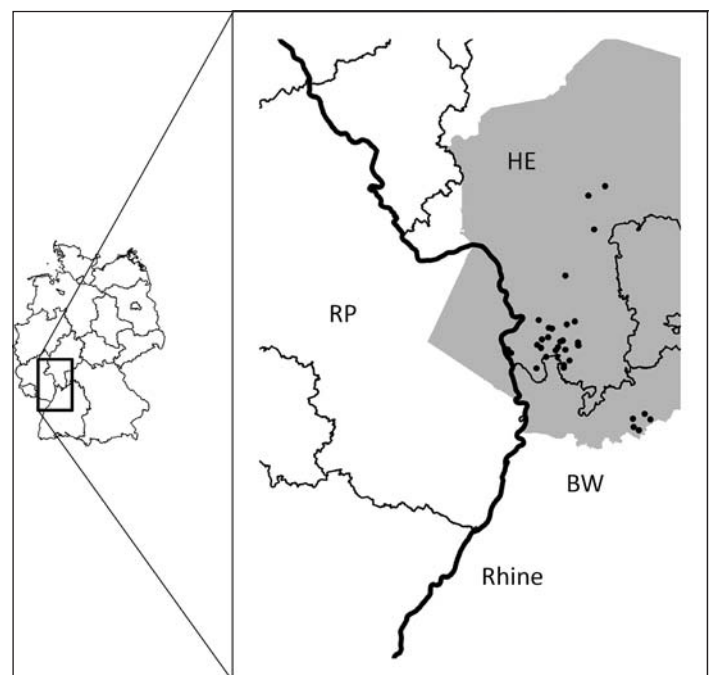
During the past three decades Germany's efforts to achieve a rabies free status had often been challenged and was put at risk by a severe setback in the final phase of rabies elimination. Although rabies had already been eliminated from much of its territory, fox rabies re-emerged in Rhineland-Palatinate (RP) in 2005 after a long period of absence (Müller et al., 2005; Müller et al.,

2012a). The rapid westward spread prompted veterinary authorities to react swiftly and apply a new yet unproven vaccination strategy that was developed from a vaccination approach used after re-infection of Carinthia, Austria in 2002 (Vogl, 2002). This paper aims at presenting the ORV strategy developed and successfully used that time. This strategy may be used as a template to tackle similar emergency situations in Europe in the future.

## Case scenario – chain of events

After more than 20 years of vaccination, the last rabies cases occurred in 1998 and eventually rabies could be eliminated in Rhineland-Palatinate (RP). Nevertheless, ORV campaigns continued in border areas in the southeast for more than three years, because rabies had not been successfully controlled in neighbouring Hesse (HE). Despite large-scale and long-term vaccination rabies had persisted in urban and periurban areas of southern HE (Müller et al., 2005; Müller et al., 2012a).

Rabies paved its way through urban and periurban settlements in HE reaching metropolitan Frankfurt/Main in 2001 and the border triangle between RP, HE, Bavaria (BY) and Baden-Wuerttemberg (BW) another two years later. In 2004 the rabies situation deteriorated in the southernmost districts of HE (Müller et al., 2005). Although BY and BW had maintained a preventive vaccination belt along the common border with HE for over three years, an adjacent area in BW finally became re-infected in December 2004. Also, an additional vaccination campaign in winter time in HE could not prevent rabies closing in on the Rhine River. As a result, veterinary authorities in RP established a 25 km deep preventive vaccination cordon (cordon sanitaire) along the Rhine River in November 2004 (Fig. 1). Unfortunately,



**FIGURE 1:** Rabies situation in Germany in 2004 with rabies cases (dots) and ORV areas in Hesse (HE), Baden-Wuerttemberg (BW), and Rhineland-Palatinate (RP) indicated (shaded).

**TABLE 1:** Number and time points of ORV campaigns and number of baits distributed in Rhineland-Palatinate in 2005 (cw – calendar week)

Vaccination campaign	Time point	Size of vaccination area in km <sup>2</sup>	No. of baits for Aerial distribution	No. of baits for hand distribution
1	09.03.2005 (10 cw)	6800	177 600	4800
2	18.04.2005 (16 cw)	6250	186 400	11 200
3	30.05.2005 (22 cw)	6250	186 400	9 600
4	11.07.2005 (28 cw)	6250	186 400	15 200
5	19.09.2005 (38 cw)	6250	186 400	15 200
6	07.11.2005 (45 cw)	238	-	4000

this countermeasure could not prevent re-infection of the rabies-free fox population, and after seven years of absence the first rabid foxes were found in RP near the Rhine River in January 2005 (Müller et al., 2005). Until April 2005 a total of 18 rabies cases were confirmed in the south-eastern part of RP and the further rapid spread westwards suggested that rabies challenged an almost unprotected fox population as the before established cordon sanitaire obviously was not able to induce a sufficient herd immunity. Considering the high fox population density at the time compared to previous years (DJV, 2004) this resembled an emergency situation.

The drastic increase of rabies incidence in RP and the resulting westward spread of the disease increased national and international concerns and required swift response planning and corrective actions by veterinary authorities. Since WHO and EU recommendations on ORV did not cover such emergency situations, an alternative and efficient ORV strategy had to be developed and implemented within a very short period of time under the auspices of the national reference laboratory for rabies (NRL) at the Friedrich-Loeffler-Institute (FLI).

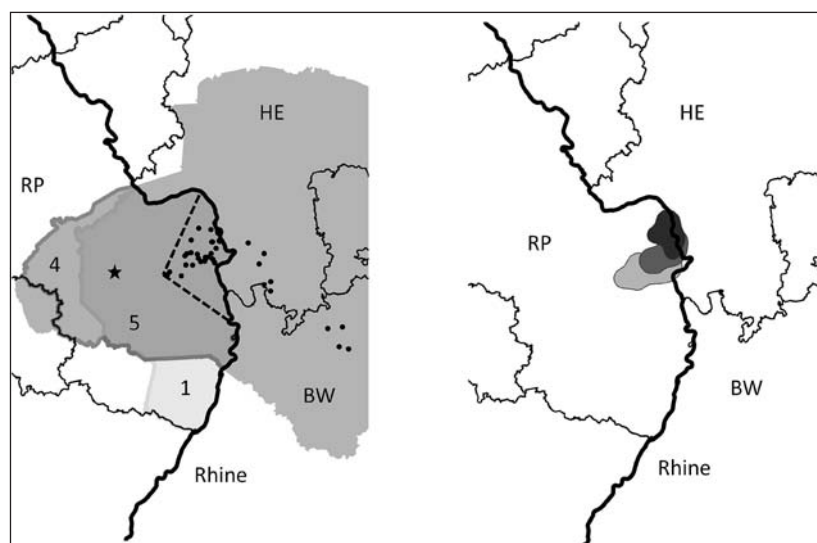
### Emergency vaccination strategy and corrective actions

A vaccination approach used after re-infection of Carinthia, Austria in 2002 (Vogl, 2002) was used as a template to modify the basic ORV strategy in such a way that it could exert its full potential. In a first step the future vaccination area in RP was pinpointed by buffering cumulated rabies cases in RP and neighbouring HE and BW of the past two years with a radius of 40 kilometres using GIS software (ArcView) (Eisinger et al., 2005). The resulting areas were merged into a single area representing the total vaccination area to be treated in RP in 2005. As new rabies cases were detected the vaccination areas were redefined and enlarged accordingly in subsequent ORV campaigns if necessary. The principle item of the new emergency strategy in RP was an increased frequency of ORV campaigns at a reduced interval. Considering the earliest possible time period without freezing temperatures, the first ORV campaign was scheduled for the 10<sup>th</sup> calendar week (CW) in March followed by consecutive large-scale vaccination campaigns in a 6 week interval using a bait density of 30 baits per km<sup>2</sup> until the rabies outbreak had been brought under control (Tab. 1) (European Commission, 2002).

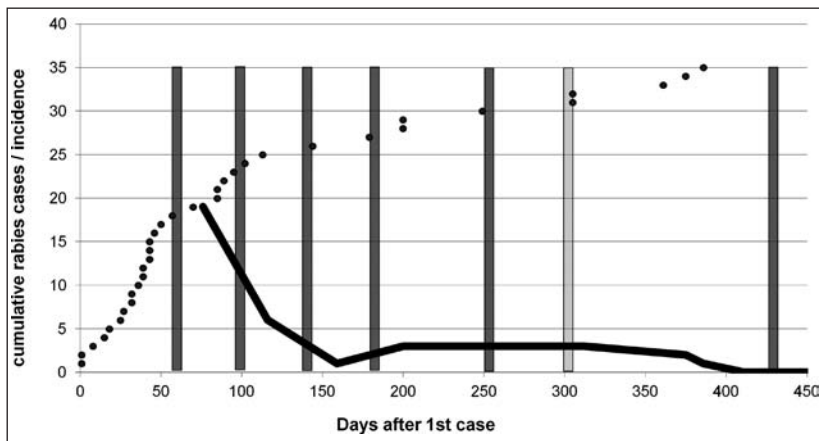
Vaccine baits were distributed using fixed-wing aircraft equipped with a satellite navigated and computer-supported fully automatic dropping device (SURVIS, Vos et al., 2001; Müller et al., 2012b) using a flight line distance of 500 meters. Coordinates on the precise location of vaccine bait drops were transmitted on-line on a daily basis by the flight services to the NRL for quality assessment of aerial distribution and identification of areas with suboptimal bait density (< 25 baits per km<sup>2</sup>) and none-flying zones (urban settlements and military areas) using GIS software (ArcView) as described (Mulatti et al., 2011; Müller et al., 2012b). Maps showing the calculated bait density on the ground were sent electronically within two days both to district veterinary authorities and flight services for immediate organization of complementary hand distribution of baits at a local level and optimization of aerial distribution in consecutive campaigns, respectively. Hunters were briefed in regular meetings and workshops to increase awareness, to enhance surveillance and sample submission of ORV monitoring, and to prepare hand distribution of vaccine baits in urban areas and none-flying zones. Because aerial distribution of baits was scheduled up to five times (Tab. 1), high risk spots within the ORV area were defined as 1 km<sup>2</sup> grid cells in ArcView that did exhibit no bait drops in four out of five consecutive ORV campaigns and where herd immunity was considered low. In those high-risk areas an additional hand distribution campaign of baits was planned (Tab. 1).

### Monitoring of ORV campaigns

Stability of the bait/vaccine (Fuchsoral®, SAD-B19) was tested by regulatory authorities at the Paul-Ehrlich-Institute (PEI) following international recommendations before and after distribution in the field. Rabies surveil-



**FIGURE 2:** A: rabies cases (dots) and ORVs (shaded) in 2005, in Rhineland-Palatinate (RP) the number of campaigns is given, a possible vaccine-associated case (star) is indicated. B: Infected area in Rhineland-Palatinate (RP) as calculated using home range estimation (threshold 90%). Three different time periods were calculated, with light-gray (day 1–70), gray (71–144) and dark-gray (145–386).



**FIGURE 3:** Cumulative number of rabies cases in Rhineland-Palatinate (dots) displayed on a time-axis (days after 1<sup>st</sup> case). Rabies incidence (black line) was calculated as number of new cases until three weeks after the start of ORV campaigns (dark columns: using aerial distribution, light gray: manual distribution).

lance followed EU recommendations using a sample size of eight foxes/100 km<sup>2</sup>/year with priority on animals showing abnormal behaviour suggestive of rabies, e. g. animals found dead, road kills and animals having had exposure to humans (European Commission, 2002). Rabies surveillance was additionally increased in a 10 km wide zone adjacent to vaccination areas to track a possible spread of rabies beyond vaccination areas. Herd immunity in foxes was determined using the rapid fluorescent focus inhibition test (RFFIT) (Cox and Schneider, 1976; Schaarschmidt et al., 2002). Rabies virus isolates from vaccination areas were characterized in a cascading approach using first a panel of ten anti-nucleocapsid monoclonal antibodies (MAb) (Schneider et al., 1985), partial sequencing of the N gene, or even full genome sequencing (Müller et al., 2009).

### Impact of the emergency vaccination strategy

Within 50 days after the incursion of rabies into RP on the 14<sup>th</sup> of January 2005 15 cases were reported in that area at a fairly stable frequency with a tendency of a westward spread (Fig. 2A). This prompted veterinary authorities to shift the spring ORV campaign to the earliest possible date followed by consecutive six weeks interval vaccination campaigns till the outbreak was brought under control (Tab. 1). Because of the detection of a possible vaccine associated rabies case in a fox at the edge of the first ORV area the size had to be re-adjusted. By CW 45 the core area of 4900 km<sup>2</sup> had been vaccinated five times, and an area of 6250 km<sup>2</sup> and 550 km<sup>2</sup> four times and one time, respectively (Fig. 2B).

In the small area vaccinated in November 2004 (Fig. 1) the average herd immunity in foxes (virus neutralising antibody titres > 0.5 IU/mL) was 37.5% (N = 88) in March 2005. After three and five ORV campaigns in 2005 the seroconversion increased to 48.5% (N = 299) and 57.8% (N = 199), respectively, taking into account samples from the entire vaccination area (Fig. 2A). Already after the second ORV campaign, rabies cases were reported only sporadically, with intervals of up to 50 days between detection of rabid animals (Fig. 3). Due to this drastic

effect it was decided already in summer 2005 to stop the six weeks interval bait distribution and to switch to the standard ORV scheme of distributing baits in autumn and spring. The emergency vaccination strategy not only had significantly reduced the rabies incidence (Fig. 3), it also resulted in an abrupt halt of spatial spread. The assumed infected area had its greatest expansion westward early 2005 but then decreased in size, and eventually shrank close to the Rhine River (Fig. 2B). An epidemiological assessment, i. e. taking into account previous rabies cases, mode of bait distribution, serology data, and topographical landscape features identified high risk spots in this area, in which in fact rabies continued to be sporadically reported. In those high-risk areas located south of the city of Mainz an additional hand distribution campaign of baits was conducted in November 2005 comprising about 238 km<sup>2</sup> (Tab. 1).

In January 2006, one year after the first detected case, three more cases were reported from this area. However, on February 2<sup>nd</sup> 2006 the last case of rabies was found, 386 days after the onset (Fig. 3). After subsequent ORV campaigns the average herd immunity in foxes remained at a high level with 49% (N = 618) in 2006 but decreased to 37% (N = 712) in 2007.

### Discussion

With this emergency vaccination strategy rabies could be eliminated in RP within 13 months after re-infection of an almost unprotected fox population (Fig. 3). In contrast to the Austrian approach that considered enlargement of vaccination areas within a short period of time to avoid further spread of the disease (Vogl, 2002) our approach aimed at a consecutive vaccination of one and the same area using reduced intervals. The six week interval of ORV campaigns (Tab. 1, Fig. 2A) was chosen considering the incubation period and the development of the immune response in foxes after vaccination assuming that all foxes incubating rabies would die within six weeks anyway. This approach proved to be highly efficient to build sufficiently high herd immunity within a short period of time (18 weeks). The herd immunity remained stable for the following year even when the standard ORV scheme was used. Generally, the measures taken resulted in an abrupt halt of the fox epidemic both in terms of the number of animals infected and the size of the area affected. In fact, rabies cases never spread beyond the initial core of 1300 km<sup>2</sup> area preventively vaccinated in November 2004 (Fig. 2B). Based on German legislation, the detection of a possible vaccine associated rabies case forced veterinary authorities to enlarge the vaccination area although this case did not have any epidemiological consequence (Müller et al. 2009). In the long-term vaccinated neighbouring regions of HE and BW rabies also disappeared in 2005 but using the standard ORV strategy.

In RP, after initial increase the seroconversion rates remained stable and later dropped by 12% in 2007, even though ORV was performed with increased bait density. While the reason for this remains elusive, seroconversion rates in wildlife may always be biased by non-homo-

genous sampling. The fact that the last rabies case was detected less than one year after the beginning of the emergency campaigns proved the success of this strategy and underlines that the incidence of rabies is the crucial figure for evaluating rabies control efforts (Cliquet et al., 2010). Also, it was theoretically shown that rabies elimination is possible with vaccination coverage lower than the gold standard of 70% (Thulke and Eisinger, 2008). As this strategic approach turned out to be successful it remains open whether a reduction in the number of ORV campaigns or a longer interval would have been as successful in RP at the time. Basic preconditions for such counteraction are the availability of financial resources on the one hand and guaranteed supply of sufficient vaccine baits on the other hand. Fortunately, both were given and allowed a swift action within a short period of time.

How crucial this immediate response is was seen in Italy, where fox rabies re-emerged in October 2008 (de Benedictis et al., 2008). Initial control started early 2009 with three emergency oral vaccinations on a small scale using manual distribution (de Benedictis et al., 2009). However, fox rabies spread westward to Veneto region and reached the autonomous provinces of Trento and Bolzano in spring 2010, necessitating corrective actions. Aerial emergency oral fox vaccination was implemented in the winter 2009/10 using a similar strategy as in Rhineland-Palatinate, Germany, but fewer campaigns to contain the epidemic (Capello et al., 2010). The changes to a large-scale vaccination led to a drastic decrease in rabies incidence and eventual elimination (Mulatti et al., 2011; de Benedictis et al., 2009) indicating that even a smaller number of ORV campaigns might work as well. In Italy, the Alpine Mountains proved to be another challenge for rabies control (Mulatti et al., 2011), underscoring the need for epidemiological counseling and evaluation and eventual adaptation of the strategy.

The applied strategy was successful in the concrete situation in RP in Germany, and it is likely that it can be successfully applied in similar situations. Since now large parts of Western Europe are considered free from rabies, it is necessary to prepare for emergency situations due to re-introduction of rabies. Although the EU has implemented strong regulations concerning animal health requirements applicable to the non-commercial movement of pet animals with respect to rabies (998/2003 EEC), a risk of a punctual re-introduction of rabies cannot completely be ruled out as shown by recent reports of illegally imported rabid dogs into the European Union and Switzerland (Johnson et al., 2011). Therefore, this strategy should be taken into account for contingency planning but would require the establishment of a vaccine bank at national or European level or a guaranteed immediate supply by manufacturers.

Ideally such emergency vaccination should aim at containing and eventually eliminating the rabies epidemic in the core area (Thulke et al., 2008). Although admittedly the emergency ORV strategy described here requires allocation of sufficient high financial resources it is likely that money will be saved in the long run by the rapid elimination of rabies.

A similar approach could also be used not in an emergency situation but in a nationwide fox rabies control program. Considering the results in Germany as well as in Italy it seems feasible that fox rabies can be eliminated in less than two years using high density interval baiting.

## Acknowledgement

The authors would like to thank Anke Kliemt and Kathrin Teske for excellent technical support. Gratefully acknowledged is the financial support by the German Federal Ministry of Food, Agriculture and Consumer Protection.

Conflict of interest: Adriaan Vos is employed by IDT Biologika GmbH, the manufacturer of the oral vaccine used.

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