Criteria for the use of parenteral and oral immunization of dogs

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Introduction

Parenteral and oral vaccination

Conclusions and recommendations
Introduction

Central-point campaigns in northern Tanzania - Dog registration, vaccination, fitting of temporary collars and issue of vaccination certificates for each vaccinated dog

Advertising of campaigns in northern Tanzania
Vaccination is a highly effective method for preventing rabies

- Many national rabies vaccination programs started after WW2 have allowed to eliminate canine rabies from entire countries and even continents:
  - Japan, Taiwan, Singapore (in the 1950s)
  - Europe and the USA (by 1970)

- Wildlife rabies prevalence has been reduced in North America and large parts of Western Europe have been declared free of terrestrial wildlife rabies following years of large scale application oral vaccination:

- Canine mass vaccination programs in countries still affected by canine rabies have significantly reduced the number of animal and human rabies cases in many countries of Latin America, Asia and Africa

- Other programs are less advanced and many struggle with logistics and implementation and thus, tragically, canine rabies continues to claim the majority of the 55,000 human lives each year
The components of a successful canine rabies control program are known:

- Knowledge about rabies and its epidemiology
- Clear governance and coordinated mandates among national agencies and stakeholders
- Adequate legislation, sustainable infrastructure and funds available
- Appropriate surveillance and knowledge about rabies epidemiology
- Knowledge of the dog population
- Appropriately trained personnel and accurately used equipment
- Community awareness and involvement
- Sufficient quantities of appropriate supplies and materials

**Blueprint for Rabies Prevention and Control** ([www.rabiesblueprint.com](http://www.rabiesblueprint.com)).

Comprehensive guidelines developed over the last 50 years by experts panels of the WHO, CDC, GARC, and the OIE are available for most aspects of dog rabies control.
A key factor cited regularly for the success of dog rabies control program is the use of potent canine rabies vaccines.

However, depending on

- which type of vaccine is used
- how the vaccines are produced and stored
- how they are handled and administered
- which type of animal is targeted
- the frequency of vaccination campaigns
- the number and distribution of animals that are reached
- The immune response generated in individuals and the population
- and how campaign success is measured

dog rabies vaccination may produce highly variable results.
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Collection of registration fees in Bohol as part of community fund schemes

Castration and vaccination in Thailand

Community education: Courtesy of STELLA MARIE D. LAPIZ, Bohol Rabies Elimination project
Parenteral vaccines for the immunization of dogs against rabies

**First generation rabies vaccines (low potency, safety concerns)**
Infected nerve tissue brain homogenates containing rabies virus inactivated by drying, phenol or formalin treatment (CNS reactions)

**Modified live modified virus vaccines (risk of rabies infection)**
Attenuated virus strains grown of chick embryos (Flury, Kelev) or hamster kidney and porcine kidney cell culture (SAD, ERA)

**Live recombinant rabies glycoprotein vaccines**
Canary- or adenovirus vectors expressing ERA glycoprotein (no adjuvants)

**Parental inactivated (killed) cell culture vaccine (safe, more concentrated)**
Produced on BHK or Vero tissue culture using fixed rabies virus strains (CVS, Pasteur virus, Pitmann Moore, Vnukovo etc)
Inactivated beta-propiolactone (phenol and formaldehyde no longer authorized)
Adjuvated with aluminum hydroxide, aluminum phosphate, saponine, mineral oils or other immune-modulating substances

**Indications/Standard claims:**
Active s.c. or i.m. immunization of dogs and cats (and others species) against rabies
Minimum titer of rabies antigen 1IU/ml
Minimum age at vaccination 3 months (interference with maternal immunity)
Duration of immunity 1-3 years (depending on passing regulatory challenge study)

* WHO recommended
In order to produce vaccines that are safe, pure and of sufficient potency, the vaccine production and control process needs to be highly standardized.
Clear differences exist in the regulations ruling rabies vaccines in Europe, the USA and other parts of the world. But even vaccines produced under the same regimen may be markedly different. Vaccines produced without strict governance are often of lower quality and may lack batch-to-batch consistency. Counterfeit vaccines have become a major issue in recent years.

**9CFR 113.209 Rabies Vaccine Killed Virus:**

For registration: Immunogenicity of vaccine prepared with virus at the highest passage from the Master Seed shall be established by challenge in each species for which the vaccine is recommended:

22 of 25 (88%) or 26 of 30 (86.6%) of uniform and sero-negative animals vaccinated with vaccine formulated at minimum protective dose (MPD) should survive a challenge applied 1 year after vaccination, to which 8 of 10 unvaccinated controls succumb. Species other than carnivores can be tested by serology.

Each batch destined for commercial use needs to be tested for inactivation (absence of residual virus), purity, safety (3 animals) and potency (NIH).

**European Pharmacopeia 6.1: Rabies Vaccine (inactivated), for Veterinary Use (04/2008:0451):**

23 of 25 (92%) animals of minimum age vaccinated with a vaccine of minimum protective dose should survive the challenge to which 8 of 10 unvaccinated controls succumb (antigen confirmed in the brain)

Potency for species other than carnivores can be tested by serology. The test is considered valid if the mean titer of 20 animals is 0.5IU/ml for the observation period and less than 10% have antibody titers less than 0.1IU/ml.

Batch release tests include: Identification (specificity), sterility, absence of residual animals, potency (Pharmacopeia test in mice by challenge and serology)
The quality of a vaccine and handling and storage conditions do matter

Vaccines are a mixture of proteins, lipids, water, minerals, adjuvants and stabilizers

An adequate and lasting response requires that antigen content levels are elevated and virus particles remain intact and properly adjuvated throughout shelf life

Handling and storage conditions (see below), which lead to the dis-integration of virus particles or the formulation will inevitably reduce the potency of vaccines

This effect is accentuated for vaccines formulated at lower pay load

**high temperatures**

**direct sunlight**

**temperature fluctuations**

**microbiological contaminants**

**pH changes**

**vibrations**

**chemicals**

Minke J & Bouvet J, unpublished data 2011
Kinetics of parenteral rabies vaccines in dogs

In Swedish pet dogs

After primo-vaccination, virus neutralizing antibodies appear in dogs older than 3 months within 7 to 14 days. Following a booster, antibody titers generally increase within 7 days.

On average, VNA titers of lab beagles peak at 14 days, European pets between day 21 and 30 and mixed local breeds in rabies endemic countries between 30 to 60 days. Individual differences exist between dogs, cats usually react better and maintain antibodies longer than dogs.

Neutralizing Antibody titres gradually decline and significant numbers of animals may drop titers below the threshold considered protective (> 0.5IU) after day 60.

In the literature protective antibody levels range from 1-2 months to several years P.V.

Minke et al., Veterinary Microbiology 133 (2009) 283–286
## Ability of rabies vaccines produced in Europe to induce at least 0.5IU/ml of VNA

<table>
<thead>
<tr>
<th>Reference</th>
<th>Test method</th>
<th>Sample and sampling period</th>
<th>Key Findings</th>
</tr>
</thead>
</table>
| Cliquet et al. Rev. Sci. Tech Off. Int. Epiz 2003, 22(3) 857-866 | RFFIT and FAVN | 25 000 sera (17693 dogs) 1993 to 2002 | -92.8% of dog- and 98.1% of cat sera had titres above 0.5IU  
- Peak titers were observed @ 4-5 weeks  
- Dogs vaccinated twice reach higher titers  
- Titters drop rapidly after 5 months  
- very young and older dogs respond less strongly  
- significant differences between vaccines  
- monovalent vaccine induce higher titers than multivalent vaccines |
| Mansfield et al. Vet Record 2004, 154, 423-426 | FAVN | > 17 000 16073 dog sera tested | - 88-96% of dogs reach titres above 0.5IU/ml  
- titers peak @ 4 weeks, rapidly drop thereafter  
- age and origin (haplotypes) of dog matters  
- multiple vaccinations induce higher response  
- marked differences between vaccines |
| Zanoni et al. Schweiz Arch. Tierheilk. 2010, 152 (52), 561-568 | RFFIT | 13 469 sera, (10999 dogs) 1997 to 2009 | - marked differences between vaccines to reach 0.5IU (78-98.5%)  
- better in dogs 6 month and older  
- multiple injection needed to sustain titers beyond 1 year  
- test failure rate greater in sera collected > 4 months p.v.  
- Results different depending on dog age and size/breed |
| Berndtson et al. Acta Vet. Scandinav. 2011, 53:22 | FAVN | 6789 sera 2005 | - 91.9% of dogs had titers above 0.5IU/ml up to 120 d P.V.  
-Results significantly different depending on dog age and size  
- Significant differences depending on vaccine brand, number of vaccine injections and interval between vaccination and testing |
## Observations from community-based vaccination campaigns

<table>
<thead>
<tr>
<th>Reference</th>
<th>Vaccine</th>
<th>Nº of dogs (Test method)</th>
<th>Key Findings</th>
</tr>
</thead>
</table>
| Tepsumethanon et al. 1991, Vaccine Vol. 9 627-630 | TCO | 54 Thai owned dogs RFFIT | - VNA positive: 96% (D14), 88% (D60), 74% (D180)  
- Single dose s.c. unable to maintain VNA for 1 year  
- Antibodies re-appear 14 days after booster  
Except for D30, no significant difference in dogs vaccinated one or multiple times  
No impact of parasites, anemia, age, breed, |
| Seghaier et al. 1999 Am. J. Trop. Med Hyg. 61(6), 879-854 | TCO | 301 rural Tunisian mongrels RFFIT | - VNA > 0.5IU/ml: 32% (D0), 73% (D30), 38% (M7), 36% (M12), 95% (M13) after booster  
- puppies do sero-covert and should be vaccinated  
- 11.5 % of total population impossible to vaccinate |
| Matter et al. | TCO | mongrel dogs in Tunisia and Turkey | - dogs under 3 months (5-20% in Tunisia)  
- dogs accessible to vaccination by mobile vaccination (Tunisia 75-85% and Turkey 50-60%) and door-to-door (Tunisia 75-85% and Turkey 57%)  
- Ownerless dogs account for 6-13% in Tunisia and 8% in Turkey |
| Kayali et al. 2003, Bull WHO 81 (10) | TCO | > 1300 owned village dogs Tschad | High vaccination coverage achieved, 88%, or 87% taking into account small # of unowned dogs  
Main difficulties: maintenance of cold chain, lack of time, transport of dogs to vaccination point, dogs too old, too young, sick, escaped |
Reasons cited why insufficient numbers of dogs are vaccinated

**Campaign logistics**
- In appropriate period of the year, month or day selected to conduct campaign
- Insufficient government attention or funds available or diverted to higher priority diseases
- Not enough vehicles, gasoline, materials, or trained staff

**Information campaign**
- Information campaign did not reach human population targeted
- Information material not clear

**Owner related**
- Owner not available during vaccination campaign
- Lack of knowledge or interest by owner
- Distance to vaccination stations too far
- Fear that vaccination interferes with ability to fulfill dog’s role

**Dog related**
- Dog ecology not full understood, resulting in insufficient and/or inhomogeneous vaccine coverage
- Vaccination coverage too low: Dogs not present during campaign, dogs escape, too young, cannot be transported to vaccination point, cannot be handled

**Vaccine related**
- Vaccine not available or not in sufficient quantities
- Cold room not available or working
- Difficult to maintain cold chain during the day
- Vaccine of insufficient or limited potency
Oral vaccines for the immunization of dogs against rabies

Modified live oral vaccines for dogs derived from SAD Berne

- **SAD B19**: attenuated rabies virus, extensive field use in wildlife in Europe, good laboratory immunity, field trials in Turkey and the Philippines, hand-made baits, good uptake and vaccine delivery, non-negligible risk of rabies for humans

- **SAG2, double deletion variant***: commercially used for wildlife control in several European countries, fully protective in dogs as of $10^7$TCID$_{50}$ *per os*, more attenuated than SAD B19, negligible risk of inducing rabies, field trials in South Africa and Mexico, currently the only commercially registered product for dogs (India)

- **Reverse genetics mutants SAD B19: SN10-333, SPBN-GA, SPBN-GA-GA**: experimental vaccines, fully protective in dogs as of $\sim10^{8.5}$TCID$_{50}$ *per os*, SPBN GAS-GAS considered for commercial development.

Recombinant oral vaccines for dogs

- **Vaccinia Copenhagen recombinant V-RG encoding ERA-G***: extensively used for wildlife rabies control in Europe, the USA, Israel and South-Korea, fully protective in dogs as of $10^9$TCID$_{50}$ *per os*, three reports of human vaccinia infection after dog bait limit potential for use in dogs

- **E3-deleted Canine Adeno Type 2**: experimental vaccine proposed for pet vaccination in China, fully protective intra-nasally and *per os* in dogs as of $\sim10^6$ and $\sim10^7$TCID50 respectively, back-bone immunity in dog population may be an issue

Other recombinant candidates (AdRab GP, NYVacc 879 RG, AdRab E1-deleted) have failed to immunize dogs by the oral route

Generally, at least 1 log higher titers are required when using baits to immunize dogs. The high titers required, the complex bait packaging process and high shipment expenses translates into high average sales prices of 2USD and higher per dose

* WHO recommended
### Bait acceptance trials in dogs

<table>
<thead>
<tr>
<th>Reference</th>
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<th>Location (Test model)</th>
<th>Key Findings</th>
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</table>
| Linhart et al. 1997 64(2):114-24 | Several different baits incl. FMP/DMP | USA, Mexico, Egypt | Bait acceptance:  
- Confined pets and beagles: 65-91%  
- Household dogs: 71-96%  
- Farm dogs: 32-88% |
- sachets swallowed or punctured 23-84% |
| Yakobson et al. Dev Biol 2008, 131 151-156 | Coated sachet & Fish Meal Polymer | Israel, Hand fed dog packs | 28% max uptake, bait acceptance disturbed:  
- Dominant animals consuming multiple baits  
- baits swallowed intact when competition  
- ORV unsuitable to vaccinate packs |
| Bergmann et al, 2008, Dev Biol 131:145-50 | Ontario Slim Coated sachet FMP/DMP | USA, Navajo/Hopi reservation | -Bait acceptance ranged from 30.7 to 77.8% (CS)  
- Behaviors observed: bait ignored, bait swallowed, vaccine bait and/or vaccine container discarded, Vaccine container intact or barely punctured |
# Experimental use of oral rabies vaccines in dogs

<table>
<thead>
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<th>Location (Test model)</th>
<th>Key Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ben Youssef et al. 1998 Am J Trop Med Hyg 58(-) 835-845</td>
<td>Freeze-dried/Parraffin coated prototype DBL2</td>
<td>Hand out model</td>
<td>381 baits distributed with acceptance of 87% 79% fully consumed, 98% of those dogs SDM + Refusal more often in older and less supervised dogs Baits presented with food in 30% of cases 25 unprotected human contacts (1.7% of population) Cost per vaccinated dog 1.7USD</td>
</tr>
<tr>
<td>Matter et al. 1998 Vaccine 16(7), 657-665</td>
<td>Freeze-dried/Parraffin coated prototype DBL2</td>
<td>Door-to-door (I) Transect-line (II)</td>
<td>- (I) 222 dogs tested, 33% considered free-roaming - 60% accepted bait at least partially - 41.3% of 482 baits distributed disappeared over night, overall 23.3% SDM+ - no unprotected human contact reported for I, compared to 32 for (II) (1.4% of all inhabitants) - (I) 34.3 person minutes &amp; 3.9USD, (II) 48 person minutes &amp; 19.2USD per dog accepting bait</td>
</tr>
<tr>
<td>Estrada et al. 2001, BMC Inf Dis 1:23</td>
<td>Hand made pig intestine bait</td>
<td>Philippines Hand fed to dogs in street</td>
<td>- of 126 dogs offered a bait, 96% accepted, however only 76% punctured container - No unintentional contact with non targets or humans</td>
</tr>
<tr>
<td>Perera et al. 2000, Cylon Med J. 45 (2)</td>
<td>FMP enhanced with fish oil</td>
<td>Sri Lanka Hand distribution</td>
<td>659/1242 or 53% of dogs accepted bait with vaccine released into the oral cavity</td>
</tr>
</tbody>
</table>
Baits for the oral immunization of dogs against rabies

- **Fishmeal polymer bait – V-RG**
- **Lyophilized, paraffin coated bait – SAG2**
- **Coated sachet – V-RG**
- **Dog consuming lyophilized, paraffin coated bait – SAG2**
- **Cotton-bait for CAV2 Courtesy of Dr. Hu, China**
Preliminary conclusions on oral rabies vaccination of dogs

Different models proposed for ORV but feasibility and compatibility with routine rabies control activities to be confirmed. Baits are expensive and use of collars also adds to expenses. How to measure success, what method for routine follow-up? How to mitigate the human health risk?

May add complexity to communication, human safety aspects and responsible dog ownership to be addressed.

Method perceived as intuitive and attractive, does promise hold in practice?: How to store, transport, distribute, hand baits to dog, how to reach all dogs, how to keep track of vaccination, how to avoid human contamination

Social behavior of dog packs not helpful to reach every animal.

When is a dog considered vaccinated? When bait consumed, vaccine container punctured, VNA detected? How to certify vaccination (collar, certificate)? Collared dog are accessible to parenteral vaccination

High titers and broad contact in pharyngeal mucosa required to achieve immunization. A high percentage of dogs does not seroconvert

Interference with efficacy if bait given with food/water

How stable are oral vaccines in tropical settings?
A community animal-health worker vaccinating a Maasai dog in northern Tanzania

Disposable dog collar for marking of vaccinated dogs

Children lining up with dogs, Serengeti Dog Vaccination project, Tanzania

K de Balogh, rabies pilot program Lusaka, Sambia

Learning to restrain dogs, WSPA Bohol

Emaciated street dog scavenging
Conclusions and recommendations

The Tierkel postulate, according to which canine and human rabies can be controlled if about 70% of the dog population is vaccinated, still appears to be valid.

Understanding the ecological parameters of the dog population is as fundamental to the design and implementation of successful, cost efficient and sustainable canine rabies control programs, as is proper diagnostics and adequate intervention follow-up.

Field observations confirm that a large majority of dogs is accessible to parenteral vaccination and should be vaccinated annually (including with 3Y DOI vaccines), regardless of their age, size, breed or gender, to avoid a drop below the herd immunity threshold due to rapid population turnover.

Effective parenteral vaccines against rabies are available, but differences in quality and characteristics exist between brands and depending on their origin. Deviations from recommended storage and handling conditions reduces vaccine potency and interferes with the vaccines’ ability to induce a strong and lasting immune response in most individuals. High potency rabies vaccines appear to resist and perform better under out-ofspecs conditions, although no direct comparisons have been made.

Oral vaccines have been postulated for years as a potential method to vaccinate feral, roaming, or otherwise difficult to handle dogs against rabies. So far, however, ORV has not yet passed the experimental stage and only one oral rabies vaccine has been registered for dogs so far.

Human safety remains an issue for most oral rabies vaccines. Other unresolved issues, such as social dog behavior, highly variable bait acceptance, variable and suboptimal seroconversion rates, potential interference with responsible dog ownership and dog registration, in addition to high cost of baits cast a doubt on the practicality of the method in the field. Before permitting to ORV to compete for the sparse resources available conventional dog rabies control, more research is required.

While limited removal of unwanted and high-risk dogs can be useful in the context of rabies control, non-selective elimination of dogs is not recommended as it may increase population turnover, decrease herd immunity and generate public opposition which may lead to overall campaign failure.