Rabies control strategy - use of lab data to optimize vaccination and zoning

Dong-Kun Yang/OIE expert for rabies and JE
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What is main problems raised by stray dogs?

- The number of stray dogs is estimated to be over 200 million in the world
- Transmission of zoonotic diseases (i.e rabies)
- Canine distemper and parvoviral diseases are transmitted by stray dogs
- Road accidents
- Noise and panic to people
- Predation on wild species – impact on the environment
Rabies vaccines for dogs

- WHO and OIE recommend that cell culture produced, inactivated, adjuvanted, combined vaccines would be useful with other antigens.
- Potency: 1.0 IU/ dose
- Quality controls have to be performed to guarantee.
- Safety, stability (during long storage)
- Potency test: NIH test for vet use
- Immunogenicity on 35 animals (serological survey and challenge study).
- Vaccines should confer protective immunity for at least 1 year.
Central vaccination

- Intended for domestic dogs which have affiliations (community dogs)
- Considered as the most effective strategy
- Requires owner’s participation
- Requires engagement of local authorities and public awareness
- Vaccination of dogs against rabies and other diseases
- Possible identification of dogs (insertion of chip)
- Issue dog vaccination card/certificate

The dog fight with rabid raccoon dog but was still alive.
Current rabies situation in Asia

- >90% human cases from dogs
- Anti-ABLV and the newly discovered lyssavirus antibodies have been found in bats in Thailand.
- Detection of RV antibodies in bats in China.
- Ferret badger transmits RABV in Taiwan.
- Raccoon dogs spread RABV in Korea and fight with pet and domestic animals.
Defining the strategy of control

- In order to minimize public health risk due to rabies
- Assessment of the current epidemiological situation- check a program for the management of stray dog populations
- Parenteral vaccination of dogs, cats and ferrets.
- Person responsible of vaccination plan
- Mass vaccination campaign, set up rabies risk region, oral vaccination, dog identification
- Vaccine used and cold storage places
- Teams involved and training for vaccinators and dog catchers
- Material use (syringes, needles, muzzle etc)
- Period of time and frequency of vaccination campaigns
- Practical organization in the fields
Characterization of dog rabies (1)
Epidemiological parameter estimates in canine

Figure 2. Observed Frequency Distributions of Important Epidemiological Parameters

- Incubation period: 22.3 days
- Infectious period: 3.1 days
- Mean transmission distance: 0.88 km
When \( R_0 < 1 \), the infection will die out in the long run.
But if \( R_0 > 1 \), the infection will be able to spread in a population
RO range was 1.2, indicating that rabies is to transmit to animals.
## Classification: Genus *Lyssavirus*

<table>
<thead>
<tr>
<th>Gt</th>
<th>Strain Name</th>
<th>Distribution</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RABV</td>
<td>World-wide</td>
<td>Dogs, wild carnivores, bats, livestock, man</td>
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<tr>
<td>2</td>
<td>Lagos Bat (LBV)</td>
<td>Nigeria, C. Africa</td>
<td>Fruit bats, cats, dogs</td>
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<tr>
<td>3</td>
<td>Mokola (MOKV)</td>
<td>Nigeria</td>
<td>Shrews, rodents, cats, dogs, man</td>
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<tr>
<td>4</td>
<td>Duvenhage (DUV)</td>
<td>S. Africa, Zimbabwe</td>
<td>Insectivorous bats, man</td>
</tr>
<tr>
<td>5</td>
<td>EBLV-1</td>
<td>Europe</td>
<td>Insectivorous bats (Serotine), man</td>
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<td>6</td>
<td>EBLV-2</td>
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<td>7</td>
<td>ABLV</td>
<td>Australia</td>
<td>Insectivorous &amp; frugiverous bats, man</td>
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Only RABV genotype 1 of lyssavirus has been identified in Asian countries.
Diversity of the Lyssavirus genus

Current Rabies vaccine can protect infection against GT1 of phylogroup I. Most anti-rabies virus antisera do not neutralize the phylogroup 2.
The genetic diversity in Lyssavirus

Bayesian phylogenetic analysis of glycoprotein ectodomain sequences from representative lyssavirus species, implemented in BEAST (v1.4.8).
RABVs in China divided into six lineages and most isolates were grouped into two China I and II. Control program by dog culling in the late 1980s resulted in changing China II into I.
The RABV affecting the Taiwan ferret badgers (RABV-TWFB) is a distinct lineage within the group of lineages from Asia and that it has been differentiated from its closest lineages, China I (including isolates from Chinese ferret badgers) and the Philippines, 158–210 years ago, indicating that multiple RABV colonization events in the ferretbadger probably occurred and has been circulating in TWFB for 100 years.
Based on phylogenetic analysis, all rabies viruses in Asian counties belong to Phylogroup 1 and genotype 1.
Characterization of Korean raccoon dogs

- Weigh: 4~6kg in summer, 6~10kg in winter
- Body length: 60 ~ 80cm
- Tail length: 13 ~ 25cm
- Color of fur: brown – dark black
- Has short legs
- Activity periods: 18:00 – 06:00
- Home range: 0.58~0.98㎢
- Use shared toilet
- Average life span: 7-8 years
- Natural enemy: badger, wolf
- Do not bark and hibernate in winter
- Cause of death: Demodex, parasite, rabies etc
A number of rabies cases in Taiwan were reported to OIE in 2013.

Dog-to-dog transmission of rabies in Taiwan was eliminated in 1961. The Chinese ferret-badger, also known as the small-toothed ferret-badger (*Melogale moschata*), is widely distributed in Southeast Asia. Over 370 Rabies cases in ferret-badger have occurred in Taiwan since 2013.
A black puppy appears weak Sept. 10, 2013 after being infected with rabies by a wild ferret badger in Taitung County. Taiwan informed the OIE of another case of rabies, in which a man in the eastern county of Taitung was bitten by a rabies-infected Formosan ferret-badger. The OIE said it released reports about the two incidents worldwide. Currently, only 10 countries and regions in the world are listed as rabies-free: Japan, Singapore, Iceland, New Zealand, the United Kingdom, Sweden, Norway (excluding Svalbard Islands), Australia, Hawaii, and Guam.
Control of rabies in France

Oral vaccination of wild life against rabies has proven to be a powerful Method for controlling sylvatic rabies in several European counties.

SAG2 bait (Virbac Laboratories)

VRG bait (Merial Laboratories)

Bait = vaccine + biological marker (tetracycline)
Oral vaccination of dogs

Field trial – China
CAV-E3Δ-CGS

CHALLENGES:

- Oral vaccine
- Single dose
- Long-lasting immunity
- Cheap vaccine
- Manufactured locally

- 96 ‘street’ dogs were vaccinated orally using CAV-E3Δ-CGS baits
- Uptake rate 93.8%
- 87.8% VNA
- 80% VNA > 2 years
- 10 challenged- all survived


Oral vaccination of dogs using a bait containing the recombinant rabies-human adenovirus type-5 vaccine confers also long-lasting immunity against rabies.
• Prevention of rabies in the recent outbreak region
Rabies virus circulating in Gangwon province jumped into new region. Korean Veterinary Authorities ordered person in charge of the region to inoculate vaccines to all animals raised in newly designated rabies risk county. Large amount of bait vaccines were distributed in the surrounding region (pink color). Several meetings were held to control rabies.
Analysis of glycoprotein gene (partial) collected from animal rabies of Korea
Regular prevention system on rabies in Korea

• Annual vaccination of dogs all around of Korea has been conducted.
• Obligatory vaccination of cattle in rabies risk area.
• 1.4 million doses of rabies vaccine are inoculated into dogs
• Sero-surveillance: testing randomly selected dogs and cattle residing in rabies risk region annually
• Distribution of bait vaccine: about 960,000 doses have been distributed in two provinces since 2000 and check intake rate.
• Quarantine: strengthening animal quarantine to block influx from foreign countries.
• Education: person in charge of prevention and national people
Prevention system in outbreak of rabies

- **Report** outbreak of rabies to MAFRA and OIE
- **Issued an emergency order of vaccinating** all dogs, cats and cattle to the farm and the surrounding region.
- Prevent wild animals from gaining access to the infected farm
- **Distribution of bait vaccine** to the surrounding region, 10km.
- **Sero-surveillance** of randomly selected animals residing in the surrounding region after primary vaccination and re-directed vaccination to the regions showing lower seropositive rate.
- **Promote the use of mass media** and send test message to national people
- Conduct epidemiological survey about origin
- **Quarantine**: strengthening animal quarantine to block influx.
Antibody response in dogs inoculated with rabies vaccine

In order to support controlling rabies in rabies risk region, dog’s sera were collected and tested by virus neutralizing assay. Even though owners or person in charge of prevention of disease said all dogs were inoculated with vaccines, results tell us that booster vaccine is needed immediately.
Antibody response in cattle inoculated with rabies vaccine

In case of cattle, results tell us that there were various sero-positive rates according to the regions, suggesting that booster vaccination to cattle is required.
Annual sero-surveillance of rabies in Gyonggi-do

Percentage of rabies virus seropositive samples from dogs and cattle between 2002 to 2013 except during the period 2010-2011.

A warning was given to the county showing low seropositive rate in animals.
Annual sero-surveillance of rabies in Gangwon-do

Percentage of rabies virus seropositive samples from dogs and cattle since 1999 except during the period 2000-2001.

A warning was also given to the county showing low seropositive rate in animals.
• Vaccination against rabies with injectable vaccines induces a humoral response with the production of rabies neutralising antibody.

In cats and dogs, the peak of rabies neutralising antibodies is generally reached between 4 to 6 weeks after first antigenic stimulation.
Immune response of rabies vaccines in cattle

Cattle inoculated with several kinds of rabies vaccines showed high antibody Titer post vaccination 30 days.
The reasons given for failure to vaccinate domestic dogs were in Sri Lanka:

- Dog could not be handled: 28%
- Dog owner absent: 20%
- No knowledge about campaign: 13%
- Vaccination point too far: 11%
- Owner refused vaccination: 9%
- Dog was pregnant: 6%
- Other reason: 6%
How to increase vaccination rate?

• To control the latent social risk of disease, the government spreads accurate information and attempts to improve the public’s attitude toward adopting prevention.

• However, these methods with the knowledge, attitudes and practices do not always work.

• People with better attitudes, stronger subjective norms, and perceptive behavioral control have stronger behavioral intention.
Concomitant administration of GonaCon™ and rabies vaccine in female dogs in Mexico. _Vaccine_. 2013

• **Pregnant women**: Rabies PEP is not counterindicated for pregnant women.

• **No reported risk of abortion and no other harm to the fetus.**

• No adverse effects of simultaneous vaccination with the immunocontraceptive GonaCon and a commercial rabies vaccine on rabies virus neutralizing antibody production in dogs.

• **No limping or prostration was observed.**

• **Minor muscle atrophy in the left hind leg of dogs were observed.**
In countries where dog rabies is endemic, OIE recommends implementation of mass vaccination for dogs by parenteral route. The coverage of parenteral vaccination is often below the necessary level (over 70 - 80%). Because some dogs are sick, in pregnant or below 3 month age. In fact, young dogs whelped by rabies immunized bitches have low titers of maternal antibodies that may be disturbing vaccination.
Humoral response of young dogs to rabies virus after mass vaccination in Tunisia

No interference was observed between maternal antibodies and active immunization of young dogs, and the efficacy of very early vaccination against is demonstrated. The vaccine, Rabirabta is produced by the Vet Res Ins of Tunis. The puppies responded to rabies vaccination whether or not they had maternal antibodies.
Humoral response of young foxes after oral vaccination with a VRG vaccine

One to three month old fox cubs whelped by vixens that have been vaccinated or not against rabies respond to oral vaccination. There is no interference between passive and active immunity.
Rabies control strategy - use of lab data to optimize vaccination and zoning - 2

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• Application of TVR program in rabies risk region
Incidence of rabies in Korea since 1970

1993-2013

2012-2013

Sylvatic rabies
Trap-vaccination-release (TVR) program had been performed for 7 years. The Ontario state was declared with rabies free region at October 2008. Concentric rings around the location of a rabid animal represent vector population reduction (PR), TVR and ORV zones.
In 2009, an outbreak of raccoon rabies in Central Park in New York City, New York, USA, infected 133 raccoons. Five persons and 2 dogs were exposed but did not become infected. A trap-vaccinate-release program vaccinated ≈500 raccoons and contributed to the end of the epizootic.
TVR program

- Trap-vaccination-release program has been described.
- The program involved setting 100 live traps baited with food 10 km$^2$ for 8 night over 2 week period.
- Captured animals were restrained and received an IM injection of rabies vaccine.
- After vaccination and ear tagging (chip), captured animals were released at the site of capture.
- The TVR program was proven to be effective in controlling rabies in Ontario of Canada and New York city.
Strategy to control sylvatic rabies in Korea

- **ORV** (oral rabies vaccination)
  - 10-40 bait/km²
- **TVR** (trap-vaccination-release program)
- **PR** (population reduction)
  - Vector reduction

**Estimated cost**

<table>
<thead>
<tr>
<th>Unit ($/km²)</th>
<th>ORV</th>
<th>TVR</th>
<th>PR</th>
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<tr>
<td>50</td>
<td>1000</td>
<td>500</td>
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Strategy to control rabies in new region

Buffer zones to block the transmission of rabies should be set up in the Newly occurred regions.
Antibody response in Korean raccoon dogs inoculated with inactivated rabies vaccines

Rabies specific antibody titers were measured by fluorescent antibody virus neutralization test at 14 days post immunization and 28 days. Most of raccoon dogs inoculated with inactivated rabies vaccine except one showed VN antibody titers ranging from 0.5 to 13.77 IU/ml.
Distribution of bait vaccine in Korea

About 960 thousand doses were distributed in 2014.
The amount of bait vaccine has been regulated according to the incidence of region.
The time of distribution is spring and autumn twice a year.
Sero-epidemiology of rabies in wild raccoon dogs

In total, 94 Korean raccoon dogs’ blood were taken in 2012
In total, 144 raccoon dogs were caught in two years. There was no rabies case in the region where TVR program was applied in 2013.
Selecting locations for capturing raccoon dogs and installing traps
Several kinds of baits (chick, raw chicken, fried chicken and fish: salury)
The captured animals in trap
(wild cat, stray cat and raccoon dogs)
The captured animals in the trap
(Dead raccoon dog was found in a sultry weather)
Instrument for inserting microchip and reader
• Which is raccoon dog’s favorite bait?

The results indicate that Korean raccoon dogs preferred to eat raw fish.
Immunological response to vaccination in wild animals

- Vaccination against rabies via IM or oral route induces a high neutralizing antibody titer in raccoon dogs.
- In raccoon dogs, the peak of rabies neutralizing antibodies is reached at 4 weeks after first vaccination.
• Rabies vaccines
Immune response to vaccine

• Microorganisms are recognized through Toll like receptors.
• Early signaling events lead to the production of type 1 IFN-α, β, IL6, chemokines, which allow the initiation of the inflammatory and antiviral responses.
• Type 1 IFNs are effective in promoting survival and activation of dendritic cells, the cells that present antigens to lymphocytes to build an adaptive immune response.
• The CD4+ lymphocytes recognize foreign and vaccine specific antigens, they have been processed through the MHC class II exogenous presentation pathway.
• Inactivated antigen or peptides are expected to trigger an CD4+ T cell and a humoral B cells response.
• Live vaccine is expected to trigger an additional strong CD8+ cell response (cytotoxic T cell).
Depletion of CD4+ T cells did not allow vaccinated mice to resist a peripheral challenge.

The high antibody responders are more resistant to peripheral virus challenge, whereas the low antibody responders are susceptible.

CD8+ T cells do not play a primary role in immunoprotection.

The most important role of rabies vaccine is in the induction of a sustained antibody response with the help of CD4+ T cell activation.

The inactivated vaccines that induce mainly B cell activation with the help of CD4+ T cells are the most appropriate choice to preserve integrity of the nervous system.
Response to immunization

- At one week after primary vaccination, there was a significant rise in the level of IgM.
- At two weeks after vaccination, there was a rise in the level of IgG1, IgG3 and IgA.
- After a booster vaccination, the level of IgG increased faster.
- Overall, IgG1 is the major IgG subclass after 1st and 2nd vaccination.
Live attenuated rabies vaccines

- **Minimum requirements** for live rabies vaccine are as follows:
  - Non-pathogenic, ability to propagate high virus titer in cells, ability to induce protective immunity after administration and thermal and genetic stability.
  - Flury or SAD origin strains cause rabies in vaccinated animals.
  - WHO and OIE recommended stopping MLV rabies vaccines for parenteral inoculation in animals in 2004, 2014 respectively.
  - But, the ERA333 strain is a live rabies virus vaccine expressing a manipulated rabies virus glycoprotein and has been used in EU.
Inactivated rabies vaccines

- Inactivated rabies vaccines require that high RABV titers be produced in tissues or cells.
- Nerve tissue vaccine (NTV) produced from RABV infected brain tissue of sheep, goats and mice were developed about 100 years ago.
- In 2005, the WHO recommended that NTVs be replaced by cell culture rabies vaccine because of adverse reactions and the OIE in 2013.
- CVS-11, Pittman-Moore-NIL2, RC-HL and PV strains have been used for producing inactivated rabies vaccines worldwide.
- The rabies vaccine strains are grown in culture systems with BHK and Vero cells and inactivated with beta propiolactone (BPL).
- Phenol and formaldehyde are no longer recommended for virus inactivation.
- The most common adjuvants are aluminum hydroxide and aluminum phosphate.
Rabies Vaccine and Vaccination

- **Parenteral injection**: Domestic animals (most Asian countries)
- Recombinant and inactivated virus vaccines’
- Primary vaccination (e.g. for animal movement/trade).
- Annual boosters.
- Monitor vaccination coverage in the population.

- **Oral Vaccination**: Stray or wild animals (Korea, Taiwan, Nepal etc).
- Mainly distribution of bait vaccine.
- Modified live virus or recombinant vaccines (VRG and SAG2).
- Monitoring the impact of oral vaccination campaigns in the field.
- Try to apply TVR program.
Let’s go together to achieve the goal, eradication of rabies in Asia by 2020