



Investigation And Testing Rabies Virologically In Bali, Nusa Tenggara Barat (NTB) and Nusa Tenggara Timur (NTT) Province, Indonesia

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Abstract

Background: Rabies is still endemic in the working area of the Denpasar Veterinary Center. For this reason, it is still necessary to conduct ongoing rabies surveillance activities aimed at: detecting the presence of rabies virus in dogs at risk of contracting rabies which related to efforts of liberation rabies in Bali Province, detecting the possibility of rabies virus presence in dogs in NTB Province so that this area remains rabies-free, detected rabies virus in dogs in the Flores Island and surrounding areas related to rabies control activities in NTT Province. Method: Rabies surveillance in dogs is mainly carried out by taking samples of dog brains that are at risk of transmitting rabies. The sample examined by the Fluorescent Antibody Test (FAT) method. Results: In 2016, the number of animal brain samples examined by the Denpasar Veterinary Center was 2,066. In Bali Province, the number of animal brain samples examined was 1,480 samples, 206 / 1,480 (13.92%) samples were positive for rabies. Positive cases of rabies came from dogs 205/206 (99.51%) samples and cats 1/206 (0.49%) samples. The average number of rabies positive cases per month is 17 cases. This number decreased sharply compared to 2015 there were 44 cases, per month. The most common rabies cases were found in Buleleng Regency as many as 41 cases, caused by dogs that have not vaccinated. The number of dog brain samples from districts/cities in NTB Province was 417 samples; there was no rabies positive. While animal brain samples from districts/cities on Flores Island and Lembata, NTT Province were examined as many as 169 samples, 45/169 (26.63%) samples were positive for rabies. Positive cases of rabies came from 44/45 dogs (97.78%) samples and 1/45 goats (2.22%) samples. Conclusion: The results of this surveillance show that rabies is still endemic in the Province of Bali and islands around the island of Flores, NTT. Mass vaccination programs, a collaboration between government agencies, communication, information and education about rabies to the community still need to be improved. Until now the NTB Province is still free of rabies. Control of rabies infectious animal traffic to the NTB Province and rabies-free areas in NTT is still needed.

Keywords: *Brucellosis, BPT, CFT, Bali, NTB, NTT.*

Interodution

The working area of the Denpasar Veterinary Center covers three provinces: Bali, Nusa Tenggara Barat (NTB) and Nusa Tenggara Timur (NTT) which two of the three provinces are endemic rabies areas. The NTT province, particularly Flores and Lembata islands, has been declared infected by rabies since 1997, while Bali Province has been diagnosed with rabies since the end of 2008 [1] and until now positive cases of rabies are still frequently found, and there is a tendency to increase case. In the Bali Province, since the simultaneous mass vaccination, Rabies cases have decreased dramatically. In 2008 the number of positive cases was 17.31%, in 2009 (25.17%), in 2010 (10.87%) in 2011

(13.29%), in 2012 (14.83%). In 2013 of the 992 samples examined, 41/992 (4.13%) tested positive for rabies with a total of 3.42 rabies cases per month. The most common rabies cases found in Bangli District (12) cases. However, in 2014 the number of cases increased dramatically. Of 1,258 dog brain samples examined, 126 / 1,258 (10.02%) were positive for rabies. The average number of cases per month is 10.5 cases. Rabies cases are more common in Karangasem District (25) cases and mostly occur in dogs that have never vaccinated against rabies [2]. Geographically, NTB Province (which is still free of rabies) but has the potential to be infected with rabies because it limited by two

provinces infected with rabies namely the Bali Province and the island of Flores, NTT. The results of the 2014 Denpasar Veterinary Center surveillance in NTB province, all of 452 dog brain samples were rabies negative. In NTT, particularly Flores and Lembata Islands, rabies tends to be endemic.

In 2013 from 20 dog brain samples examined, 7/20 (35.00%) tested positive for rabies, while in 2014 there was a decrease in the number of cases, out of 77 dog brain samples examined, 24/77 (31.17%) tested positive for rabies. Under these conditions, as one of the technical service units of the Animal Health Directorate, the Directorate General of Animal Husbandry and Animal Health, the Ministry of Agriculture, which is in charge of animal health, it is an obligation for

Denpasar Veterinary Center to assist local governments in overcoming rabies in infected areas and maintain regions/provinces that are still declared free of rabies. For that reason in 2016, Denpasar Veterinary Center

will conduct virological surveillance of rabies in the Bali, NTB and NTT province.

Output

The expected output from Rabies disease surveillance activities is the availability of data and information about the presence of the rabies virus in dogs from the Bali, NTB and NTT province.

Disease Risk Analysis

Risk analysis of the transmission of rabies in Bali, NTB and NTT province includes:

- Hazard Identification (Hazard Identification),
- Risk Assessment,
- Consequence Assessment, the surveillance, and monitoring of rabies in the working area of the Denpasar Veterinary Center in the 2016 fiscal year can look in Tables 1, 2, 3.

Table 1: Rabies Risk Analysis in Bali Province 2016 Fiscal Year

Location	Identification Danger	Assessment Risk	Assessment The consequences	Targeted
All Regencies / Cities in Bali Province	<ol style="list-style-type: none"> 2015 case data HPR Traffic Population System maintenance Vaccination/group immunity status (based on the results of serological surveillance in 2014) 	<ol style="list-style-type: none"> A total of 516 out of 3,061 (16.86%) samples diagnosed with FAT rabies HPR traffic from one area to another is challenging to control. Estimated dog population reaches 500,000 In general, the proportion of dog ownership in Bali is 95% owned dogs (61% are released and 34% caged or bound), and 5% are non-proprietary dogs 320 of 677 (47.30%) positive samples of rabies serology (<70%) 	<ol style="list-style-type: none"> Rabies cases are still quite high, and diseases are circulating in the field. The spread of disease is difficult to control. The high population of HPR is a high contact rate. The maintenance system that is left behind makes it difficult to monitor and administer vaccinations to HPR. Half of the population is sensitive to animals. 	Based on the formula for calculating the number of samples, in the Bali region, the minimum sample target is 665.

Table 2: Rabies Risk Analysis in Mataram and West Lombok, NTB

Location	Identification Risk	Assessment Risk	Assessment The consequences	Targeted
Lombok West and Mataram	<ol style="list-style-type: none"> HPR traffic through people's ports (traditional fishermen) Hunting culture Animal vaccination status System maintenance 	<ol style="list-style-type: none"> There are still many fishermen from outside the island of Lombok, especially from endemic rabies areas that bring dogs on a cruise. Hunting culture increases the risk of rabies transmission through wild animal bites (game) and the movement of animals to other areas. Free areas such as NTB do not carry out rabies vaccination. Most maintenance of HPR is wil. West Lombok is released 	<ol style="list-style-type: none"> HPR from endemic areas has the potential to transmit rabies. Wild animals (prey) is very potential to transmit rabies through bites, and hunting areas to outside the area further increase the potential for the spread of rabies. 	The number of samples is following the calculation of sample size, so the target sample in NTB is at least 420 samples.

Table 3: Rabies Risk Analysis on Flores Island, NTT Fiscal Year 2016.

Location	Identification Danger	Assessment Risk	Assessment The consequences	Targeted
All regencies in mainland Flores	<ol style="list-style-type: none"> 2015 case data HPR Traffic Population System maintenance .Vaccination / group immunity status (based on the results of serological surveillance in 2015) 	<ol style="list-style-type: none"> 14 out of 99 (14.14%) samples diagnosed with FAT rabies HPR traffic from one area to another is challenging to control. Estimated dog population reaches 300,000 In general, the proportion of dog ownership in Flores is quite high, with a maintenance system released as a house/garden guard and hunting. A total of 137 of the 500 (27.40%) samples that tested positive for rabies serology (<70%) 	<ol style="list-style-type: none"> Rabies cases are still quite high, and diseases are circulating in the field. The spread of disease is difficult to control. High population of HPR is high contact rate. The maintenance system that is left behind makes it difficult to monitor and administer vaccinations to HPR. Nearly 70% of the population is sensitive animals. 	Following the calculation of the number of samples and the estimated achievement of sample targets, the total number of samples taken in Flores is 350 samples.

Materials and Methods

Theory

Rabies surveillance and monitoring activities are carried out by taking a dog's brain sample with the following criteria:

- Dogs which have a risk of transmitting rabies (dogs that suddenly bite people and or other animals).
- Dogs that show clinical symptoms of rabies and show changes in behaviour.
- Wild Dogs will eliminate by local service officials.
- Dogs served as dishes from a dog meat restaurant.
- Died dogs due to being hit by vehicles on the highway. It is a consideration because in general dogs infected with rabies will experience changes in behaviour and tend to lose their instinct to avoid vehicle traffic.
- Dogs are originating from infected areas.

Method

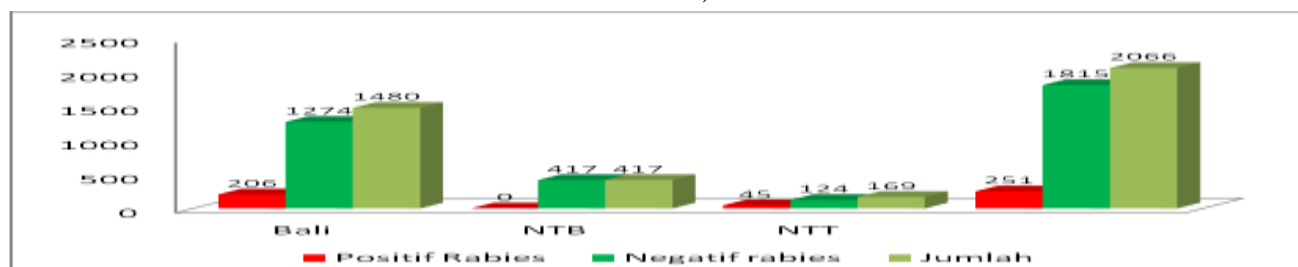
Dog brain samples were fresh, fresh frozen or 50% glycerin preservative then tested in the Fluorescent Antibody Test. Samples made into thin cloth preparations on glass objects, aerated at room temperature, then fixed with

cold acetone for 30 minutes. The preparations dripped with the fluorescein isothiocyanate (FITC) conjugate (Bio-Rad) incubated in 37 °C incubators for 30 minutes, rinsed with PBS, covered with a glass cover containing 10% glycerin, then examined under a fluorescent microscope.

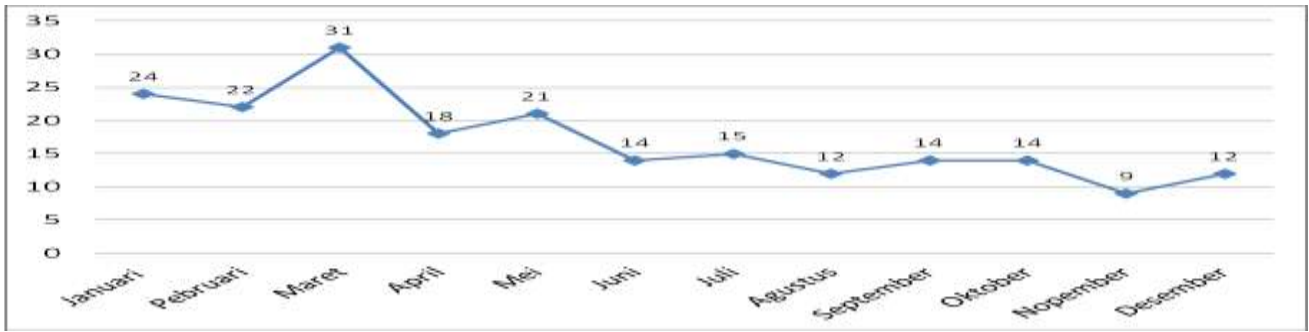
Results

In 2016, the Denpasar Veterinary Center received 2,066 samples for rabies testing from various animals, every 1,480 samples from Bali Province, 417 samples from NTB Province and 169 samples from NTT Province (Graph 1). The number of rabies cases in animals in Bali Province in 2016 dropped sharply compared to 2015 in line with the decline in the number of positive rabies cases in dogs (Graph 2).

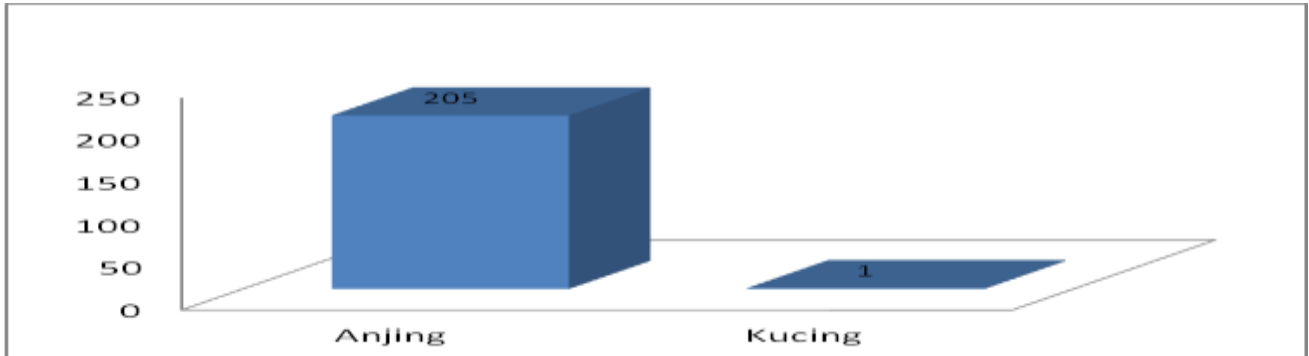
Positive cases of rabies have also attacked cats in Karangasem Regency (Graph 3). The average number of rabies positive cases per month in the Province of Bali is 17 cases as presented in Graph 2. The most common rabies cases were found in Buleleng regency as many as 41 cases (Graph 4). Rabies positive cases were more prevalent in dogs that were not vaccinated 177/205 (86.34%) (Graph 5), in dogs with 149/205 (72.68%) (Graph 6), and mostly occurred in dogs aged between 1 -3 months 49/205 (23.90%) (Graph 7).



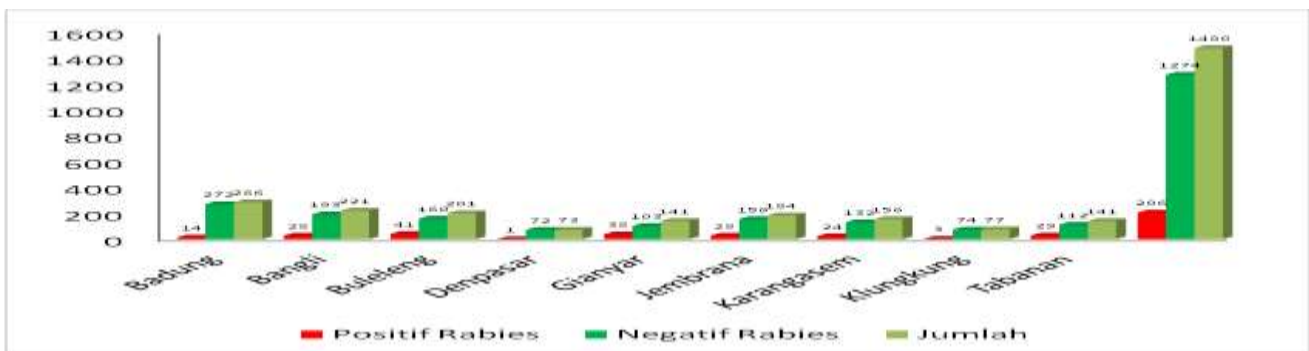
Graph 1: The number of samples examined at the Denpasar Veterinary Center for Rabies testing originating from the Provinces of Bali, NTB and NTT, 2016. (N = 2,066) samples



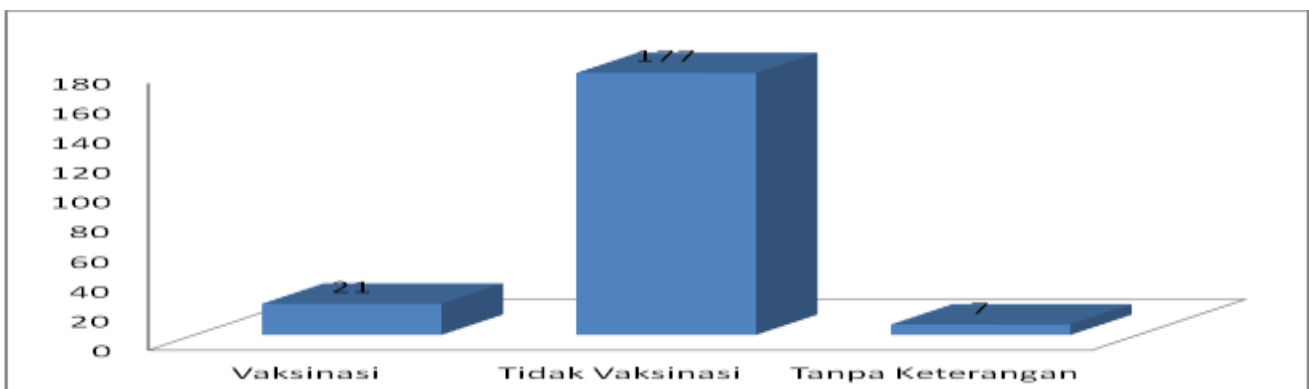
Graph 2: The number of rabies cases per month in Bali Province in 2016



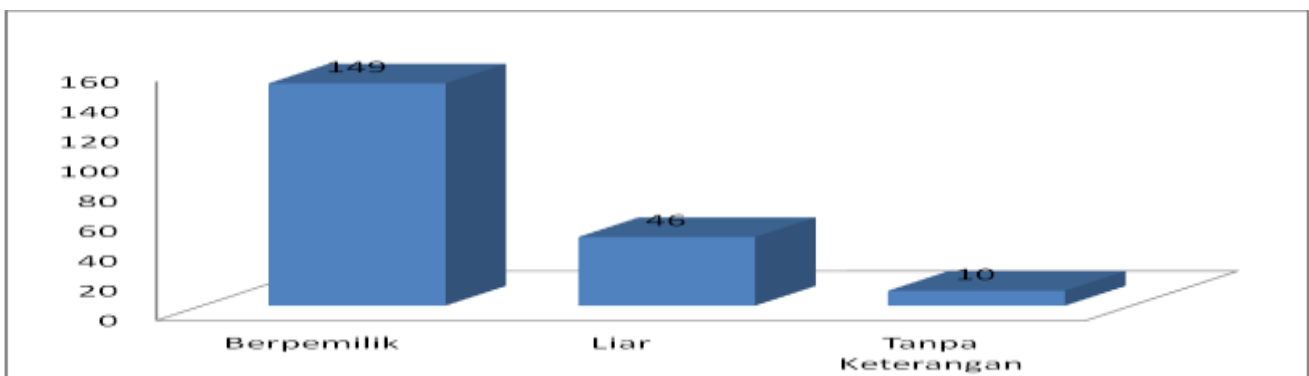
Graph 3: The number of positive cases of rabies in animals in Bali Province in 2016



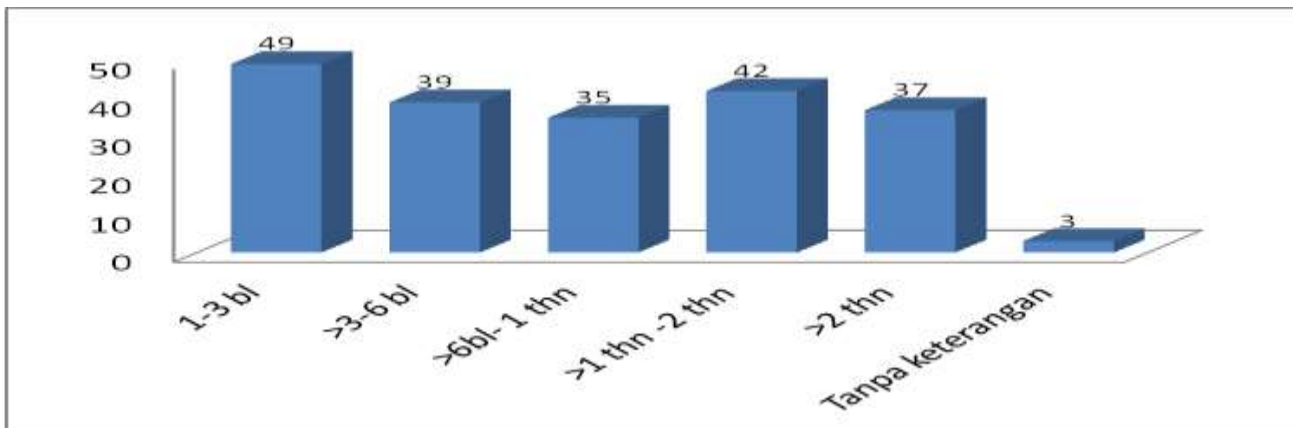
Graph 4: The number of rabies cases in each Regency/City in Bali Province in 2016



Grifik 5: The vaccination history of positive rabies dogs in Bali Province in 2016



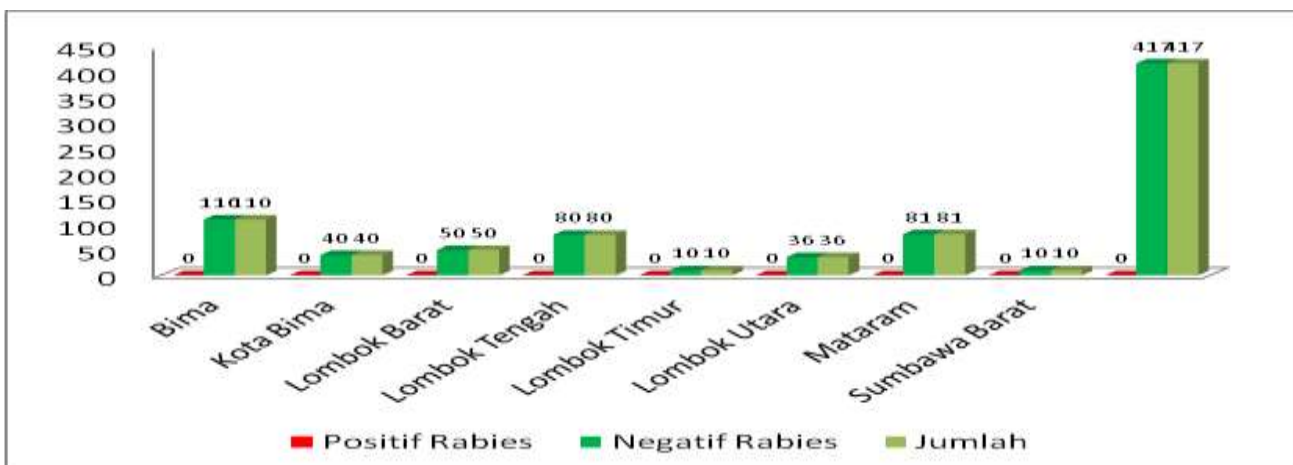
Graph 6: Status of positive rabies dog ownership in Bali Province in 2016



Graph 7: Age of rabies positive dogs in Bali Province in 2016

In NTB Province, the number of dog brain samples examined was 417 samples, most of which came from the results of elimination conducted by agencies that carry out animal husbandry functions in districts/cities in

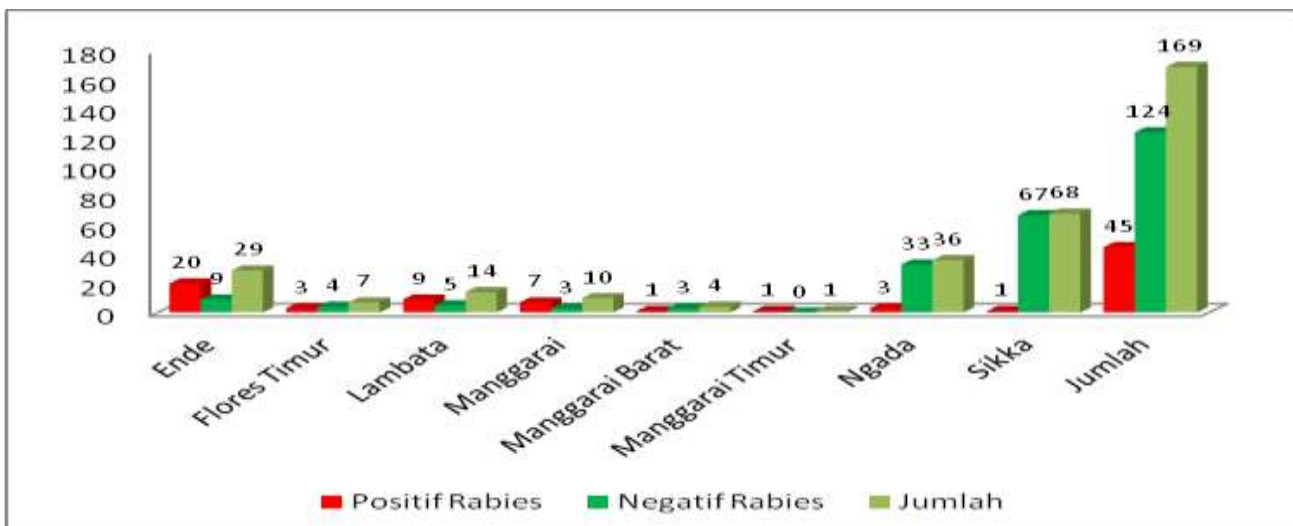
NTB Province in the context of early detection of rabies, so that NTB remains free from rabies. All samples tested negative for rabies (Graph 8).



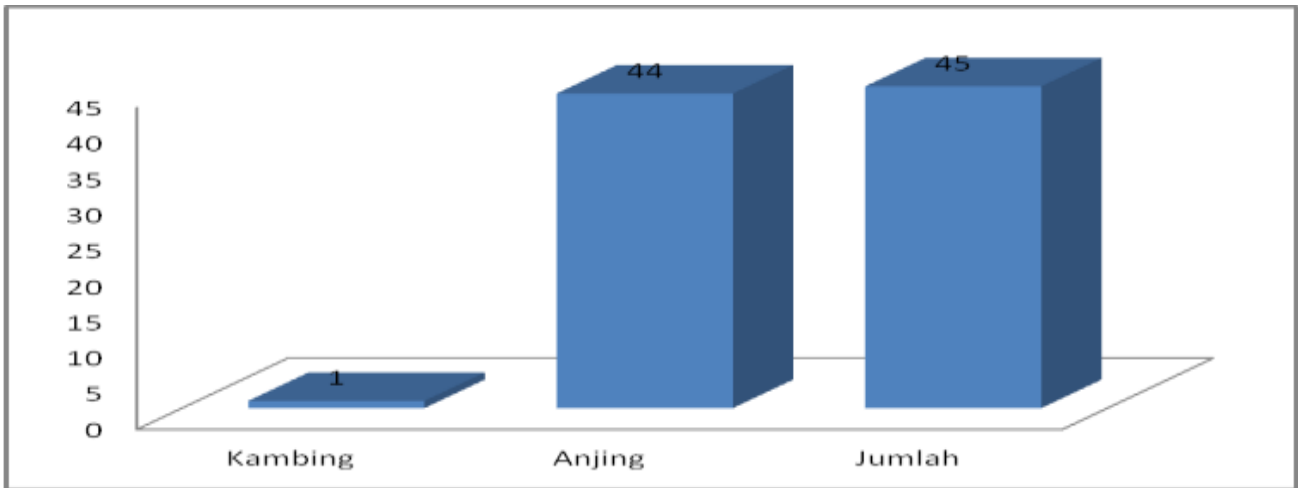
Graph 8: The number of dog brain samples examined at Denpasar Veterinary Center originating from districts/cities in NTB in 2016. (N = 417 samples)

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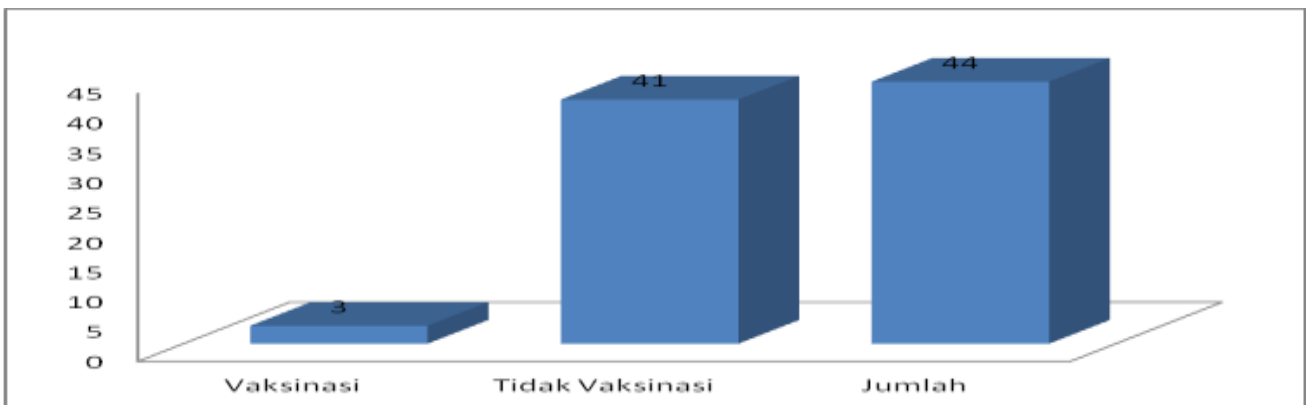
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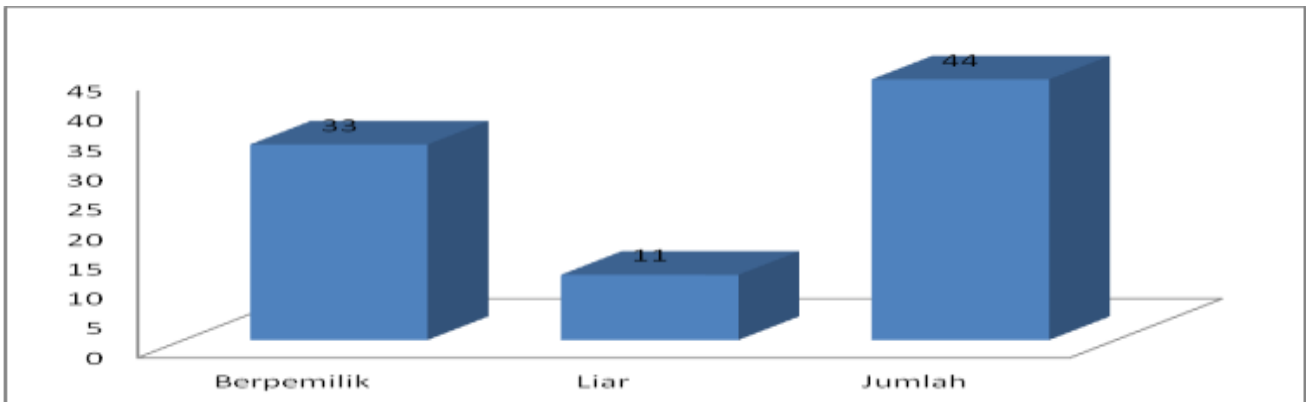
Graph 9: The number of animal brain samples examined at Denpasar Veterinary Center from various districts on Flores Island, NTT Province



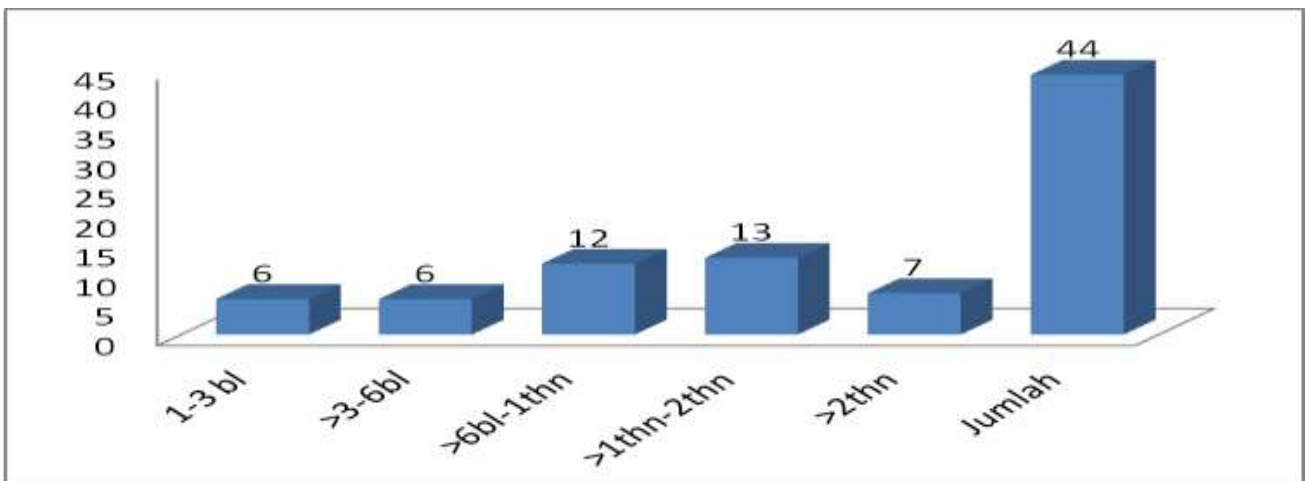
Graph 10: Types of positive rabies animals from districts on Flores Island, NTT Province, 2016



Graph 11: Vaccination status of rabies positive dogs from districts on Flores Island, and Lembata, NTT Province, 2016



Graph 12: Status of positive rabies dog ownership from districts on Flores Island, and Lembata, NTT Province, 2016



Graph 13: Age status of rabies positive dogs from districts on Flores Island, NTT Province, 2016

Discussion

Surveillance results in 2016 showed a decrease in the number of rabies cases in Bali Province compared to 2015. In 2015 the number of positive cases of rabies was 529 cases while in 2016, the number of positive cases of rabies was 206 cases. In 2015 rabies cases were not only found in dogs but also occurred in cats (6 cases), pigs (2 cases) and cattle (5 cases). In 2016 apart from dogs, rabies was also found in cats (1 case). The decrease in the number of rabies cases occurred simultaneously in all regencies/cities in Bali Province. It caused by the seventh year of massive and simultaneous mass vaccination activities in all regencies/cities in Bali whose activities began in April 2016. The highest rabies cases occurred in Buleleng regency which was 41 cases (Graph 4).

Rabies positive cases are more common in dogs that have not been vaccinated 177/205 (86.34%) cases, in dogs with 149/205 (72.68%) cases and most occur in dogs aged between 1-3 months 49/205 (23.90%) cases. The high dog population in the Province of Bali which estimated at 500,000 animals is a challenge in the context of liberating the Province of Bali from rabies. As many as 61% of the dog population are proprietary dogs released. The breed cycle of dogs is speedy resulting in new puppies not getting rabies vaccine at the time of mass vaccination so that many rabies cases infect dogs aged 1-3 months.

Community awareness and lack of awareness about the dangers of rabies causing them to let go of their dogs just like those who are very interested in transmitting the rabies virus. Vaccinating rabies in dogs that are left is not easy. The legitimacy target of stray dogs and those released by the government rejected by dog owners and non-government organisations through social media.

In NTB Province, the number of dog brain samples examined from January to December 2016 was 417 samples, coming from 8 districts/cities, all of which were negative for rabies (Graph 6). NTB Province is a rabies alert status area, bordered by two provinces infected with rabies, in the west bordering Bali Province and in the east with NTT Province. The traffic of goods/people crossing the NTB region through land, air, and sea is quite high. Efforts to introduce rabies-borne animals into the area by animal

lovers exist. Therefore tight control of the entry and exit of rabies-infected animals by animal quarantine institutions needs to be increased. Besides that structured surveillance, communication, information, and education about the dangers and prevention of rabies to the community in all regencies/cities in NTB Province needs to be improved.

In NTT Province, in 2016 the number of rabies cases increased by 45 cases compared to in 2015 there were 14 cases of the highest cases found in Ende District (20 cases) (Graph 9). In addition to dogs, positive rabies cases also found in one goat in Larantuka, East Flores Regency. On the island of Flores rabies tends to be endemic considering dogs have a high economic value. The price of an adult dog can reach one million per animal. However, the maintenance of dogs in this area is still mostly released. In Bali and NTT, most people keep dogs that function as housekeepers, gardens or for commercial purposes.

In Bali, dogs are usually used as a complement to religious ceremonies (*mecaru*), while in NTT dogs are usually cut for wedding ceremonies. Generally, their attention to the dog is very less. Dogs are allowed to roam around looking for food themselves going to landfills, markets or religious ceremonies, and breeding out of control. Stray dogs are challenging to catch or vaccinated.

The results of research conducted by Putra (2011) states that dogs that left with 81% potential as transmitters of rabies. Buying and selling dogs for commercial purposes in NTT and religious ceremonies in Bali also play an essential role in the spread of rabies in Bali and Flores. Rabies is a disease that is difficult to cure. One of the technical obstacles faced in controlling rabies is the number of stray dogs without owners or deliberately left and not taken care of by their owners.

Immunisation against stray dogs is technically very difficult, so vaccination coverage does not reach expectations. The absence of accurate data on the dog population is also a limiting factor in planning rabies control programs. As a material for planning vaccine needs, equipment, vaccinators and operational costs

in the field needed the proper dog population.

Mass rabies vaccination is believed to be an effective and economical way in terms of cost for rabies control. Vaccination failure is very complex, it caused by vaccine quality, vaccine handling is not proper, or the vaccine period has expired, the dog is in an incubation period.

Failure to control rabies is also caused by rabies vaccination coverage not reaching an adequate amount (70%), so that the rabies disease cycle, especially in deck dogs, cannot be interrupted. Not to mention other difficulties in terms of vaccinating on deck dogs, because these dogs are difficult to catch. The lack of facilities and infrastructure to support vaccination activities in Puskesmas, the availability of vaccines, the lack of funds for socialisation also play a role in the failure of rabies control.

Activity Risk Analysis

The target number of samples for surveillance and monitoring of rabies agents in the Bali, NTB and NTT province in 2016 was 1,435 samples, consisting of 665 samples from the Province of Bali, 420 samples from NTB and 350 samples from NTT. From the number of samples, Bali Province the

number of brain samples collected exceeded the target. Whereas from NTB and NTT Provinces the number of dog brain samples collected was 417 and 169 respectively. In NTB the number of sample targets had not been met because indeed the dog population in the District/City in NTB was not much. In NTT, dogs have a high economic value, so that eliminating dogs by the government is trying, besides catching dogs is very difficult especially without the support of the owner/community.

Conclusion

- Rabies is still endemic in Bali Province and several districts in Nusa Tenggara Timur Province.
- NTB Province is still free from rabies.
- In 2016 there was a high decrease in rabies cases in the Province of Bali.
- Positive cases of rabies in the Denpasar Veterinary Center working area are mostly caused by dogs that have never been vaccinated against rabies and come from proprietary dogs [3, 9].

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