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IMMUNE RESPONSE AND COST ANALYSIS OF INTRADERMAL RABIES VACCINATION FOR POST-EXPOSURE PROPHYLAXIS REGIMEN IN HUMAN

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Background

The outbreak of rabies in human in Bali-Indonesia is causing an extraordinary pressure for the government in providing adequate doses of anti-rabies vaccine for post-exposure prophylaxis (PEP). Here, we directly compare the immune response and benefit of the intradermal (ID) protocol for rabies vaccine delivery with the intramuscular (IM) route. **Methods:** Sixty health workers who were willing to participate in this study have been randomly selected and grouped into ID, IM, and control groups, each with 20 volunteers. The Thai Red Cross ID- and Zangreb IM-protocols have been applied to the respective group. The sera of the volunteers were collected at day 0, week 1, week 3, week 4, month 3, month 6, month 9, and month 12 after the first vaccination. Anti-rabies virus IgG was detected using Platelia™ Rabies II Kit (Bio-Rad). **Results:** Anti-rabies IgG could be detected in the ID-group at one week. The ID-vaccine delivery induced a slightly higher maximum antibody titer compared to IM, though not statistically significant ($p>0.05$). ID vaccination caused less adverse reactions and produces longer lasting protective immune response. Cost minimization analysis (CMA) on the provincial and national PEP data in 2009-2011 shows that the ID-delivery will reduce the total cost for a completed regimen by USD 28.5, and would have saved the Indonesian government budget approximately USD 3.6 and 4.3 million for complete regimens in Bali and Indonesia, respectively. **Conclusion:** The ID administration of anti-rabies vaccine induces a similar immune response compared to that of intramuscular injection. It also produces longer lasting protective immune response. It offers additional advantages of potential net cost savings as well as decreasing the pressure on vaccine availability due to the high number of dog bite cases.

Keywords: anti-rabies; vaccine; intra-dermal; intramuscular; immune-response

INTRODUCTION

Rabies is a fatal neuro-pathogenic disease which caused by rabies virus, a member of *Lyssavirus* Genus of *Rhabdoviridae* Family, in warm-blooded animals and human.[1] Formerly rabies free, the island province of Bali, Indonesia has been experiencing the rabies outbreak since November 2008.[2]

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This local outbreak may also have a global implication if the world health authorities might have to release new standards for pre-exposure prophylaxis (PrEP) for travel to the main tourist destinations like Bali where rabies is uncontrollable in the foreseeable future. Information from traveler demonstrates that many had indeed had PrEP before going to Bali or directly asked for PEP as they come home.[3-5] Travellers are indeed the largest group in the general population to receive rabies PrEP.[6]

The risk that Bali might become a rabies endemic area is obvious. Bali is a densely

population province with 3.6 million inhabitants in 5600 Km^2. The dog population, the world's most common rabies carrier, is remarkably high. Although the exact number is not known, incomplete data show that number of dogs compared to human is 1 to 6.5 (unpublished data) with an estimated dog population of 550,000. The dog density then is around 100 dogs per Km^2. This estimation is much higher than in Tanzania as an example, which has a density of 9.38 dogs/km^2.[7] Simply after the beginning of the outbreak, the number of dog bites was more than 21,000 in 2009 and increased dramatically to 62.000 in 2010 with higher awareness and reporting. The average of more than a hundred dog bite per day was causing an extraordinary pressure for the government in providing adequate doses of anti-rabies vaccine for PEP.

The existing standard practice for rabies post-exposure treatment in Indonesia is wound cleansing and intramuscular (IM) injection of double doses anti-rabies vaccine at day 0, followed by a single dose injection at day 7 and 21 or 28.[8] Dependent on the severity and risk level of the bite, anti-rabies immunoglobulin (RIG) should be given at day 0. However, the RIG is often not available at the local hospitals. The current Zagreb protocol is costly. It includes IM injection of 4 doses of 0.5 mL of vaccine for a completed PEP treatment. Based on the number of dog bites described above, more than 280,000 doses of vaccine have been used for PEP in Bali, in 2009-2010 alone.

Intra-dermal (ID) administration of rabies vaccine is an attractive option for Indonesia. In Thailand, ID rabies vaccine administration was begun in 1984. In this regimen, doses of 0.1 mL of vaccine per ID site are injected at two sites per visit on days 0, 3, 7 and 28 (2-2-2-0-2).[9] The protocol is found to reduce the cost of vaccination by 68%.[10] This regimen has been implemented in various countries such as India,[10] Bangladesh,[11] and others. It has been recommended by The World Health Organization[12] to be implemented world wide. However, this economical ID protocol has not been adopted in Indonesia and other countries. This might due to the lack of complete confidence in the method.[13] Therefore, evidences are needed to promote the use of ID protocol, so the pressure in providing adequate doses of anti-rabies vaccine for PEP lessens, and human mortality reduces. Here, we describe the immune response and cost analysis of rabies vaccine ID-delivery in Bali.

PATIENTS AND METHODS

Volunteer enrolment

The volunteers were selected from health personnel who were willing to be involved in the study. The age and sex of the volunteers were statistically homogenous (data not shown). Ethical clearance for this study has been approved by Ethic Commission of Faculty of Medicine Udayana University. All participants provided written informed consent. Vaccine-related complaints from each volunteer after vaccine injection were recorded.

Study design and serum collection

The volunteers were randomly grouped into ID, IM, and control groups. The ID-administration was based on the Modified Thai-Red Cross (TRC) as described above. The IM group members were vaccinated using the current Indonesia's standard,[8] based on Zagreb protocol.[14] The control group members received no vaccine trough out the experiment but were offered ID vaccination at the end of the trail. The vaccine used in this study was VERORAB™ (Sanofi Pasteur France).

The sera were collected at day 0, week 1, week 3, week 4, month 3, month 6, month 9, and month 12 after the first vaccine administration. Whole venous blood samples were drawn using 3 mL disposable syringes and collected in plain glass tubes without anticoagulant. The sera were separated after incubation at room temperature and centrifuged in 1000 rpm for 10 minutes. The sera were preserved in -80°C before use. The complete number of sera could be collected up to 6 months after the first vaccination. Due to the mobility of the vounteers, only 14 and 15 sera could be collected at the month 9th and 12th of both IM and ID groups, respectively. The sera from control groups were collected up to the month 6th ofter from the begin of the study.

Enzyme link immunosorbent assays (ELISA)

The Platelia™ Rabies II Kit (Bio-Rad) was used in this study. The ELISA procedure was conducted in accordance with manufacturer's brochure. Briefly, the microplate was adjusted to room temperature (26^0C) in its protective packaging. Control and sample sera were diluted into 1:100 with diluent provided in the kit. Quantification standards were prepared by diluting the S6 standard in two-fold dilutions. Diluted samples, serum controls, and quantification standards were pipetted at the volume of 100 µL into each well of the microplate. The microplate was then incubated in 37°C for 60 minutes. After a washing step, 100 µL conjugate solution was added to each well of microplate and incubated in 37°C for 60 minutes. The washing step was repeated five times. Thereafter, an enzymatic development solution was added to each well, and the microplate was incubated in a dark room for 30 minutes. The reaction was stopped by adding 100 µL stop solution. The optical density was determined using stat fax spectrophotometer with dual wave length of 450-620 nm. The OD values were converted into Equivalent Unit (EU) following a standard curve

generated with the OD and EU values of the quantification standards.

Cost analysis

Cost minimization analysis (CMA) was calculated based on the WHO recommendations[15] that included the direct cost for consumables for injection and unit vaccine dose, indirect cost related to transportation and income lost for PEP visit, and additional training costs at provincial and national levels. The calculation of the potential net cost (saving) of ID immunization was done using existing data of the number of complete PEP regimens given in Bali and Indonesia in 2009-2011.

Data analysis

The EU values of the antibody titer at day 0, week 1, week 3, week 4, month , month 6, month 9, and month 12 were statistically analyzed using the student T test in the SPSS version 15.0 software.

RESULTS

Anti-anti rabies IgG could be detected in ID-group at week 1, although the titer was mostly under protective of 0.5 IU[8] (see Table 1 and Fig. 1). The ID-group showed a slightly higher maximum antibody titer compare to IM-group, although this was not statistically significant (p>0.05) (Table 1 and Figure 1).

Table 1
Average of Anti-rabies IgG antibody titer of volunteers and the proportion of volunteers with a protective IgG titer (>0.5 EU) after intradermal, intramuscular, and control.

Period	Vaccine administration		
	intramuscular	intradermal	control
D_0	0.00 (±0.00) {0.0%}	0.00 (±0.00) {0.0%}	0.00(±0.00) {0.0%}
W_1	0.00 (±0.00) {0.0%}	0.28 (±0.13) {5.0%}	0.00(±0.00) {0.0%}
W_2	1.56 (±0.93) {95.0%}	2.26 (±1.12) {95.0%}	0.00(±0.00) {0.0%}
W_4	2.87 (±1.09) {100.0%}	2.29 (±1.00) {100.0%}	0.00(±0.00) {0.0%}
M_3	1.54 (±1.09) {80.0%}	3.23 (±1.12) {100.0%}	0.00(±0.00) {0.0%}
M_6	1.14 (±1.16) {70.0%}	1.56 (±1.09) {100.0%}	0.00(±0.00) {0.0%}
M_9	1.39 (±1.17) {78.6%}	0.86 (±1.10) {57.1%}	0.00(±0.00) {0.0%}
M_{12}	1.07 (±1.06) {46.7%}	0.87 (±1.36) {46.7%}	0.00(±0.00) {0.0%}

D_0, week 1 (W_1), week 2 (W_2), week 4 (W_4), month 3 (M_3), month 6 (M_6), month 9 (M_9), and month 12 (M_{12}) after the first vaccine administration. The value is in equivalent unit (EU). The standard error of each value is in parenthesis. The proportion of volunteers with protective IgG titer is in brace.

None of the control group members had a detectable anti-rabies IgG. The maximum proportion of volunteers with a protective IgG titer was reached equally four weeks in IM and ID groups, however the latest group maintained 100% proportion of IgG protective level up to six months after the first vaccine administration. The proportion of volunteers with protective IgG decreased after three months in IM groups (Table 1).

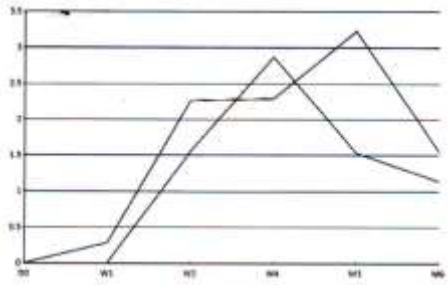

Figure 1
Average of Anti-RV antibody titer (EU/ml) of volunteer after intramuscular (black colomn) and intradermal (gray colomn) at Day 0 (D_0), week 1 (D_7), week 2 (D_{14}), week 4 (D_{28}), month 3 (M_3), month 6 (M_6), month 9 (M_9), and month 12 (M_{12}) after the first vaccine administration. Standard errors are shown in bars.

Adverse reactions after intramuscular and intradermal administration of rabies vaccine in human are presented in Table 2. The ID group complained of pain, itching, erythema, and induration at the administration sites ranging from 5% to15%. The IM-group members complained of myalgia (25%), pain at the injection sites (15%), and headache (15%).

The ID protocol will reduce the total cost for a completed regimen from USD 75.32 to 46.77. The potential net cost savings of ID administration in Bali and Indonesia, in 2009-2011, would have been USD 3,624,446 and 4,247,503 respectively for Bali and the country. The analysis is presented in Table 3.

DISCUSSION

Rabies is a largely preventable disease in human if the standard protocol for PEP, such as wound cleansing, rabies vaccine, and RIG, is completed.[16,17] Vaccines based on cell-culture (CCV) enhances the effectiveness of PEP. The number of vaccination failures reported using CCV are remarkably few, and nearly all occurred in developing countries due to incomplete administration of the WHO recommended protocol.[18,19] This has also occurred in Bali. Only small proportion of rabies victims sought medical help and received post-exposure vaccination with or without RIG. Frequently, vaccination regimens were not completed because of the short incubation period following a high risk bite.[2] A failure of

Table 2
Adverse reactions after intramuscular and intradermal administration of rabies vaccine in human in Bali

Adverse Reaction	Vaccine Administration			
	Intramuscular (n=20)		Intradermal (n=20)	
	N	%	N	%
Pain	3	15	1	5
Itchy	0	0	1	5
Erythema	0	0	3	15
Induration	0	0	1	5
Fever	0	0	0	0
Myalgia	5	25	0	0
Headache	3	15	0	0

Table 3
Potential cost minimizing analysis (CMA) of ID regimen and annual PEP burden in Bali and Indonesia 2009-2011 (in USD)

Direct Cost		IM	ID
Material cost per injection (includes needles, syringes, etc)		0.01	0.20
Vaccine cost per dose		14,78	2.96
Number of visit per patient (time)		3	4
Number of injection per patient (time)		4	8
Indirect cost			
Transport cost per person per visit		2,69	2,69
Half day income lost per person per PEP visit		2,69	2,69
Total cost for a completed regimen		75,32	46,77
Potential Net Cost (Saving) of ID per patient			28,55
Additional investment			
Assumed cost for training of trainer for ID			10,000
Assumed cost for training of health worker in Bali			25,000
Assumed cost for training of health worker in Indonesia			50,000
PEP Burden in Bali 2009-2011	Number of PEP Regimen	IM	ID
2009	18,865	1,420,960	882,395
2010	57,435	4,326,152	2,686,476
2011	51,884	3,908,037	2,426,832
Total Bali	128,184	9,655,150	5,995,703
Potential Net Cost (Saving) of ID in Bali			3,624,446
PEP Burden in Indonesia 2009-2011			
2009	35,316	2,660,092	1,651,877
2010	63,334	4,770,480	2,962,397
2011*	51,884	3,908,037	2,426,832
Total Indonesia	150,534	11,338,609	7,041,106
Potential Net cost (Saving) of ID in Indonesia			4,247,503

* The total number of PEP regimens in Indonesia was not available in 2011. The number of PEP in Bali in the same year is used for the analysis. Source of data: Bali Province Health Office Denpasar and Directorate General of Disease Control and Environment Health, Indonesian Ministry of Health Jakarta.

preexposure prophylaxis (PrEP) has been also reported in a patient who had not received the recommended booster series.[20]

The Indonesian Ministry of Health guideline[8] describes the national standard for rabies PEP vaccine administration as four IM injections in the deltoid area of 0.5 ml per bite case. As the incidence of dog bites increased dramatically, vaccine stock was often limited. Dogs are reported to be the only source of human rabies and the associated fatalities in Bali.[2] The high density of both dog and human populations certainly contributed. Bali, 5,600 km^2 in land area, has almost 4 million inhabitants and and estimated 550,000 dogs. Dog keeping practices are similar in the various rabies endemic areas where dogs are mostly unleashed and free to wander as stray dogs.

With such high dog population density figures, it was certainly plausible that rabies would be difficult to control once introduced to Bali. Indeed, that is the case. In 2009-2010, there have been more than 83,000 dog bite incidents and over 100 human rabies cases.[2] The resulting community concern and demand for vaccination had to be met although the number of actual high-risk rabies exposures may have been less.

Intradermal administration is an alternative to overcome the limitation of vaccine stock and the increasing demand on vaccine. The protocol has been recommended by the World Health Organization.[12] Published results from several clinical trials have confirmed the immunogenicity and efficacy of the ID route for rabies PEP. This rabies vaccine delivery method is currently being used effectively in many Asian countries, including India, the Philippines, Sri Lanka and Thailand.[21] The ability of the ID route to induce an immunological response results from the fact that the skin is an effective immune organ and vaccine efficacy is enhanced when antigens are presented into the dermal layer.[22] The administration of antigens into the skin layer will facilitate their exposure to the numerous antigen-presenting cells, such as macrophages and dendritic cells, which are present in a high number.[23]

This study has proven that ID-administration of rabies vaccine induced serum antibody almost similar to the traditional IM administration. The new protocol produced even a slightly earlier and higher antibody titer, although this was statistically insignificant. Anti-anti rabies antibody could be detected in ID-group at week 1, although the titer was mostly under protective level of 0.5 IU [8]. The ID-delivery reached the maximum antibody titer after 3 months, whilst the IM was at week 4. This observation may not be significant given no sera were collected between week 4 and month 3 after vaccination. In addition, the ID-protocol has gained a slightly higher maximum antibody titer. Moreover, immune response following ID seems to last longer than IM. The ID administration maintained 100% proportion up to six months after the first vaccine administration, while it decreased after three months in IM groups.

ID vaccine delivery also caused fewer adverse reactions. The ID group members complained of mild discomfort, itch, erythema, and induration at the administration sites. The IM-volunteers complained of pain at the injection sites, myalgia and headache. All complaints were mild and did not require medication or treatment.

The immune response measured in this study should be equivalent to the protectivity against the natural virus challenge. The gold standard for protectivity of rabies antibody in humans is rapid fluorescent focus inhibition test (RFFIT).[24] The kit measures rabies glycoprotein antibody, a reliable indicator of the degree of immunity in people after PEP and PrEP.[25] The kit has been found to be simple, safe, rapid and can be considered as a useful alternative to the gold standard test. The ELISA has been proven to have a linear relationship to the RFFIT.[25] The ELISA is therefore recommended as an alternative method to measure the rabies-specific antibodies.[26]

The CMA findings suggest that an ID-protocol will cost much less than the IM-vaccination. The CMA methodology was selected given both vaccine deliveries have identical outcomes. Other methods such as cost-effectiveness analysis (CEA), cost-utility analysis (CUA) and cost-benefit analysis (CBA)[15] are inappropriate measures. Analysis of both vaccine protocols (Table 3) shows that the national fiscal burden for PEP occurred largely in Bali. This is clearly explained the magnitude of rabies outbreak in that province, which has caused more than 100 deaths during the first two years of the outbreak.[2] The ID protocol will reduce the total cost for a completed regimen by IDR 265,500 or USD 28.5. The cost savings of ID-vaccination if the protocol had been implemented since 2009 in Bali and Indonesia would be about USD 3.6 and 4.3 million. The other potential benefit of ID delivery is the dose-sparing protocol, which increases the availability of vaccines in cases where supply is limited.[22] The total doses that have been spent for PEP of about 128.000 regimens in Bali in 2009-2011 was 512.000 doses. If the ID delivery had been implemented, the total doses could had stretched for 320.000 regimens.

ID-administration of rabies vaccine for PrEP or PEP is highly recommended for Indonesia. The number of the rabid animal bites requiring PEP is far exceeding vaccine stock availability if IM administration is used. However, ID-delivery does have a disadvantage. It must be used within six hours after reconstitution or should be discarded.

To extend the economic advantage of ID-administration, manufacturers should provide smaller volumes of vaccine preparations.

CONCLUSION

In conclusion, the ID administration of anti-rabies vaccine induces a similar immune response compared to that of intramuscular injection. It also produces longer lasting protective immune response. It offers additional advantages of potential net cost savings as well as decreasing the pressure on vaccine availability due to the high number of dog bite cases.

Competing interests

The authors have no competing interests to declare.

Authors' contributions

Budayanti NS, Susilawathi NM, Sudewi AAR, Wignall FS, and Mahardika GNK were contributing the the conception and design of this work and in in analysis and interpretation of the data. Darwinata AE, Dwija P, Wirasandhi K, Subrata IK, NND Fatmawati and Susilarini NK have contributed in collection, analysis and interpretation of the data. All authors have been contributed in the writing of the manuscript.

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