

## Original Article

# A Comparative Study on the Adverse Reactions of Purified Chick Embryo Cell Vaccine (PCECV) and Purified Vero Cell Rabies Vaccine (PVRV)

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## Abstract

**Objective:** Human rabies is preventable by prompt application of post-exposure prophylaxis (PEP). The aim of this study was to compare the adverse reactions of purified vero cell rabies vaccine (PVRV) with purified chick embryo cell vaccine (PCECV) vaccination for the PEP.

**Methods:** In this double blind clinical trial study, 1449 people bitten by animals (279 females), were recruited from 9 different cities of Iran, and randomly assigned to receive intramuscular injections of the PVRV (n = 702) and PCECV (n = 747) vaccines in 5-dose regimen. The local and systemic adverse reactions were compared between two groups.

**Results:** The mean age was 26.8 years (SD, ± 13.1 years) and 27.4 years (SD, ±13.9 years) in PVRV and PCECV group, respectively. Bites were most often located on the lower extremities in both groups. The most common local adverse reaction in both groups was pain at the injection site (4%). Most of the reported systemic adverse reactions were headache (2.5%) and fever (1.9%) in PCECV and PVRV group, respectively. The incidence of itching was higher in the PVRV group compared to the PCECV group (1% vs. 0.1%) ( $P < 0.05$ ). There was no significant difference between two groups regarding systemic adverse reactions.

**Conclusion:** The results of the present study indicated that PCECV vaccination was associated with fewer itching at the injection site. There was no significant difference between PCECV and PVRV vaccine regarding local and systemic adverse reactions. Therefore, the PCECV vaccine can be administered instead of PVRV, when our country encounters serious challenges in PVRV vaccine supply.

**Keywords:** Clinical trial, PCECV, PVRV, rabies, vaccines

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## Introduction

Rabies with a mortality rate of nearly 100% is a major public health problem in most of the developing countries.<sup>1,2</sup> Although effective vaccines for the post-exposure prophylaxis (PEP) of rabies are available,<sup>3</sup> there are still about 50,000 to 60,000 human deaths annually.<sup>1,4</sup> The rabies virus (genus *Lyssavirus*, family *Rhabdoviridae* of the order *Mononegavirales*) is present in the saliva of infected mammals and most commonly is transmitted via a biting incident.<sup>5,6</sup> Rabies is commonly diagnosed after the onset of neurological symptoms, but infection can be prevented by proper wound care, administration of rabies immune globulin (RIG), and post-exposure administration of anti rabies vaccination, which are 100% successful in inhibiting human rabies following disclosure.<sup>7</sup> Different epidemiological studies conducted in different parts of Iran (Ilam, Rafsanjan, Birjand and Golestan) have shown that the incidence of animal bites in our country has been increasing in

recent years.<sup>8-10</sup> However, currently the disease is being controlled if we compare the situation with 40 years ago.<sup>8,11</sup> The first vaccine was developed on the nerve tissue by Pasteur in 1885, but the production of this vaccine has been discontinued as it causes neuro-paralytic complications in some individuals.<sup>12</sup> At present, cell culture vaccines and embryonated egg vaccines have replaced nerve tissue vaccines in industrialized countries and are recommended by WHO.<sup>13</sup> They are considered safe and well tolerated, which are largely used for animal and human use with varying degree of safety and efficacy in most parts of the world.<sup>14</sup> Purified vero cell rabies vaccine (PVRV) is available in Iran. So far, no report has been published to confirm any specific adverse reactions to this vaccine in Iran,<sup>15</sup> but because this vaccine is produced by a single source provider, an Iranian national committee of rabies control approved the using of other recommended vaccine by WHO. Therefore, purified chick embryo cell vaccine (PCECV) with the brand name of Rabipur has been supplied and is available for administration in our country. There are many different types of studies comparing the safety, immunogenicity and adverse reactions of rabies vaccines in the world. However, according to available evidences, no study has been conducted on the assessment of adverse reactions of rabies vaccines at the national level in Iran. Therefore, the present study is aimed to compare the adverse reactions of PVRV with PCECV vaccination for the PEP. This study was a double blind, randomized clinical trial and conducted in 9 cities of Iran.

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## Patients and Methods

### Subjects

This study was a double blind randomized clinical trial, performed in healthcare centers in 9 cities of Iran (Ghaemshahr, Sari, Qom, Gonbad, Aqqala, Gorgan, Kerman, Sirjan and Bam). Cities were selected by the disease management center (Ministry of Health, Treatment and Medical Education, Tehran, Iran) based on the population size, facilities, the number of health care centers and the total number of registered animal bite cases for the years 2010.

The Committee of Rabies Control, Ministry of Health, as well as Treatment and Medical Education of Iran approved this study. Written informed consent was obtained from subjects or subjects' parents/legal representative of underage subjects. Individuals were included in the study, if they were between 5 and 55 years of age, with a category II or III exposure (as defined by WHO),<sup>5</sup> and those who were volunteered to participate. Individuals were excluded from the study, if they had a history of previous animal bites; had received immunization against rabies previously; had a significant acute or chronic infectious disease; receiving transfusion with blood or blood products within the past month; were concomitantly receiving corticosteroids or immunosuppressive drug therapy; had an axillary temperature  $\geq 37.5^{\circ}\text{C}$  before injection; or were pregnant (Figure 1). The study was started simultaneously at all healthcare centers and conducted from 2011 to 2012 for one year.

### Study design

The sample size calculation was based on the rate of reported adverse reactions to vaccination with PCECV and PVRV, which was 3.2% and 1.1%, respectively.<sup>16-18</sup> With an alpha risk of 5% and a test power of 80%, 744 participants would be necessary in each group. Randomization was performed using the balanced block randomization; for 1:1 randomization of 2 groups and the blocks size of 4. Since the vaccines were visually different, they were administered by someone, who was not responsible for trial evaluations. In addition, patients were unaware of the vaccine given to them (double blind). Extensive information on addresses and phone calls was collected at baseline to enhance the ability to track the patients. One day before the expected visit, a trained nurse contacted the patient to remind him/her.

### Vaccines and regimens

PCECV (Rabipur®, Novartis, Germany) and PVRV (Verorab®, Mérieux Institute, France) were used in this study. PCECV is a sterile lyophilized vaccine obtained by growing the fixed rabies virus strain Flury LEP-25 in primary cultures of chick fibroblasts. The virus is inactivated with  $\beta$ -propiolactone, purified and concentrated by zonal centrifugation. PVRV is a sterile, stable, freeze-dried suspension of rabies virus prepared from strain PM-1503-3M obtained from the Wistar Institute grown on vero cell cultures. These are inactivated by  $\beta$ -propiolactone and purified by ultracentrifugation.<sup>13</sup> Each enrolled subject was randomly assigned to receive immunization with either PCECV or PVRV administered inter muscularly into the deltoid muscle with 5 doses on days 0, 3, 7, 14, and 28.

### Assessment of adverse reactions

Immediate systemic and local adverse reactions to the vaccines were monitored by the the physicians who were blinded to the vaccines type within 30 minutes after each injection. Local and

systemic reactions were evaluated and recorded daily for 3 days after each injection by patients on a special form. The solicited local reactions were evaluated at the injection sites, including: pain, erythema, itching, lymphadenopathy, abscesses, swelling and bruising. The solicited systemic reactions monitored, included headache, fever, weakness, muscle aches, nausea or vomiting, dizziness, sweating, stomach ache, urticaria, hypotension, lymph node swelling, shortness of breath, sore throat, shock-like state, sensory processing disorders, guillain-barré syndrome, seizures, encephalopathy, optic neuritis and joint pain. Subjects with adverse reactions were referred to the healthcare center and reported that reaction was validated by a physician from the investigational team. All additional information was collected 2 weeks after the last injection by telephone call to patients or their parents and recorded on forms.

### Data analysis

Comparison of characteristics between two groups was done using student's *t*-test for continuous variables. Chi-square and Fisher exact test were used to compare adverse reaction rates between two groups. IBM SPSS 20 was used for statistical analysis and a *P*-value of  $< 0.05$  was considered statistically significant.

## Results

Overall, 1,449 subjects participated in the study. The number of subjects was less than the calculated sample size (1488), because during the study period there were not sufficient eligible samples in some cities. In addition, since this study was a short term one, no patient was lost to follow-up over the study period. Out of 1,449 subjects, 702 were in PVRV group. The mean age was 26.8 years (SD,  $\pm 13.1$  years) and 27.4 years (SD,  $\pm 13.9$  years) in PVRV and PCECV group, respectively. There were 148 (21.1%) and 131 (17.5%) females in PVRV and PCECV group, respectively. There was no significant difference in mean ages and sex ratios between two groups ( $P > 0.05$ ). Table 1 shows the distribution of subjects in two groups by the residential area. Qom and Gorgan had the lowest and Aqqala had the highest number of participants, respectively. There was no significant difference in distribution of participants across cities, between two groups. Table 2 shows the category of exposure in two groups. In both groups, category III was slightly more than category II; there was no significant difference between two groups regarding the category of exposure. Also, the statistical analysis showed that there was no significant relation between category of exposure and observed adverse reactions in both groups. No significant differences were observed in the location of the bite between two groups (Table 3). Type of local reactions for the two vaccines is shown in Table 4. The most frequently reported local reaction was pain at the injection site (3.8% of PCECV recipients, 3.9% of PVRV recipients). There was only a significant difference in itching between two groups ( $P < 0.05$ ); the incidence of itching was higher in the PVRV group compared to the PCECV group (1% vs. 0.1%). The reported systemic reactions are shown in Table 5. There was no significant difference in systemic reactions between two groups. Overall, adverse systemic reactions were infrequently reported in this study and were similar between two groups.

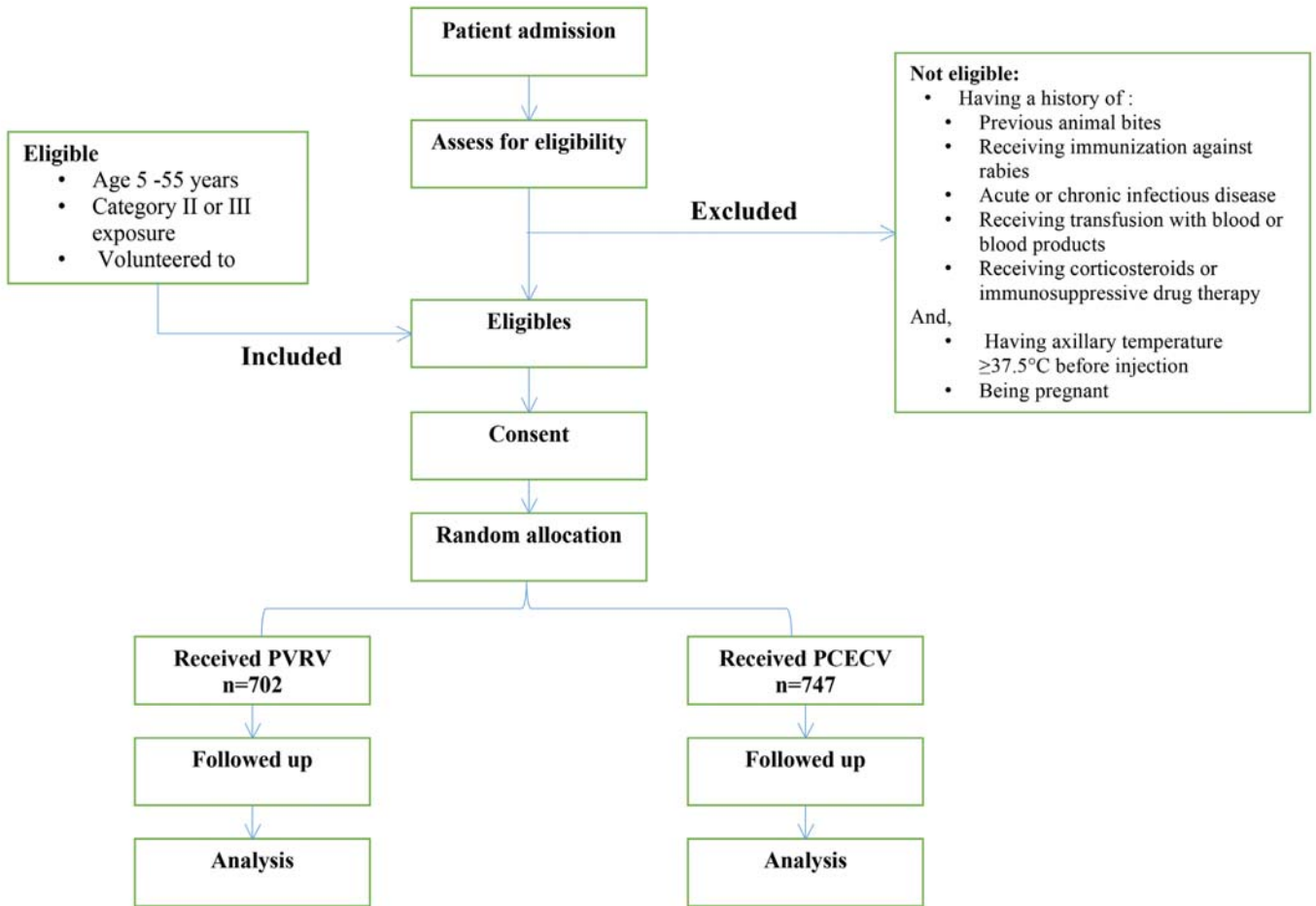


Figure 1. Flow chart of study design

Table 1. Distribution of subjects in two groups by the residential area

City	PCECV (n = 747)	PVRV (n = 702)	Total
Ghaemshahr (n%)	98 (50.5)*	96 (49.5)	194 (100)
Sari (n%)	101 (49.8)	102 (50.2)	203 (100)
Qom (n%)	11 (50.0)	11 (50.0)	22 (100)
Gonbad (n%)	99 (50.0)	99 (50.0)	198 (100)
Aqqala (n%)	138 (59.0)	96 (41.0)	234 (100)
Gorgan (n%)	10 (50.0)	10 (50.0)	20 (100)
Kerman (n%)	91 (50.0)	91 (50.0)	182 (100)
Sirjan (n%)	98 (50.0)	98 (50.0)	196 (100)
Bam (n%)	101 (50.5)	99 (49.5)	200 (100)

\*Data are presented as number (%); PVRV: Purified vero cell rabies vaccine; PCECV: Purified chick embryo cell vaccine

Table 2. Distribution of subjects in two groups by the category of exposure

Category of exposure	PVRV (n = 702)	PCECV (n = 747)	P-value
Category II	341 (48.6)*	354 (47.4)	0.674
Category III	361 (51.4)	393 (52.6)	

\*Data are presented as number (%); PVRV: Purified vero cell rabies vaccine; PCECV: Purified chick embryo cell vaccine

**Table 3.** Locations of the bite in two groups

Bite location	PVRV (n = 702)	PCECV (n = 747)	P-value
Head and neck (n%)	4 (30.8)*	9 (69.2)	0.205
Chest and abdomen (n%)	9 (52.9)	8 (47.1)	0.698
Back (n%)	12 (50.0)	12 (50.0)	0.864
Upper extremity (n%)	246 (49.3)	253 (50.7)	0.567
Lower extremity (n%)	396 (47.5)	438 (52.5)	0.328
Multiple location (n%)	35 (56.5)	27 (43.5)	0.187

\*Data are presented as number (%); PVRV: Purified vero cell rabies vaccine; PCECV: Purified chick embryo cell vaccine

**Table 4.** Types of local reactions in two groups

Local reactions	PVRV (n = 702)	PCECV (n = 747)	P-value <sup>†</sup>
Pain (n%)	27 (3.9)*	28 (3.8)	0.915 <sup>†</sup>
Erythema (n%)	9 (1.3)	8 (1.1)	0.705 <sup>†</sup>
Itching (n%)	7 (1.0)	1 (0.1)	0.033 <sup>‡</sup>
Swelling (n%)	4 (0.6)	2 (0.3)	0.438 <sup>‡</sup>
Bruising (n%)	0 (0.0)	4 (0.5)	0.125 <sup>‡</sup>

\*Data are presented as number (%); <sup>†</sup>Chi-square test for the comparison of the percent of local reactions in the PVRV group versus the PCECV group; <sup>‡</sup>Fisher exact test; PVRV: Purified vero cell rabies vaccine; PCECV: Purified chick embryo cell vaccine.

**Table 5.** Types of systemic reactions in two groups

Systemic reactions	PVRV (n=702)	PCECV (n=747)	P-value <sup>†</sup>
Headache (n%)	8 (1.4)*	16 (2.5)	0.154 <sup>†</sup>
Fever (n%)	11 (1.9)	10 (1.6)	0.697 <sup>†</sup>
Weakness (n%)	5 (0.8)	11 (1.7)	0.212 <sup>†</sup>
Muscle Aches (n%)	6 (1.0)	5 (0.8)	0.669 <sup>†</sup>
Nausea or Vomiting (n%)	4 (0.7)	7 (1.1)	0.550 <sup>‡</sup>
Dizziness (n%)	5 (0.8)	6 (0.9)	0.860 <sup>†</sup>
Sweating (n%)	3 (0.5)	4 (0.6)	1.000 <sup>‡</sup>
Stomach Ache (n%)	4 (0.7)	3 (0.5)	0.717 <sup>‡</sup>
Urticaria (n%)	0 (0.0)	1 (0.2)	1.000 <sup>‡</sup>
Hypotension (n%)	0 (0.0)	1 (0.2)	1.000 <sup>‡</sup>
Joint Pain (n%)	6 (1.0)	3 (0.5)	0.327 <sup>‡</sup>

\*Data are presented as number (%); <sup>†</sup>Chi-square test for the comparison of the percent of systemic reactions in the PVRV group versus the PCECV group; <sup>‡</sup>Fisher exact test; PVRV: Purified vero cell rabies vaccine; PCECV: Purified chick embryo cell vaccine

## Discussion

Vaccination is the mainstay for both pre- and post-exposure prophylaxis against rabies. Therefore, greater attention to prevention and control of animal rabies will increase demand for the various safe and potent rabies vaccines. In Iran, the PVRV vaccine has been used for many years, and serious adverse reactions caused by vaccination have not been reported.<sup>15</sup> However, in recent years, the PCECV has been added to the rabies PEP regimen to optimize vaccine availability and avoid risks of providing rabies vaccine by a single source provider. To our knowledge, this is the first clinical trial in Iran, which attempts to evaluate immediate or delayed adverse reactions of these two anti rabies vaccines. In the present study, we selected the PVRV as our reference vaccines. This study was multicenter and included people from different parts of both urban and rural areas of Iran.

Results of the present study showed that there was no significant difference in systemic reactions between two groups. Observations

of local reactions in the two groups showed the higher rate of itching in PVRV group compared to PCECV (about 10 times). Results also showed that pain at the injection site was the most commonly observed local reaction to both vaccines (about 4%). A few local and systemic reactions were observed with more or less similar rates in both groups. There are many studies, which have evaluated the safety and immunogenicity of PCECV and PVRV in different populations. One similar study was conducted on 152 people bitten by dogs and other animals from 4 different centers in India. They were randomly assigned to receive Vaxirab, PCEC, and PVRV. Subjects in all three groups had neutralizing antibody titers by day 14. Adverse reactions were observed in some subjects in all the three groups. Mild pain at the site of injection was the common adverse reaction, which was observed soon after the injection. Other reactions observed were itching, which was noted after 5 – 10 min, nausea after 30 – 40 min and weakness after 15 – 20 min. In all, 15.4% of people receiving Vaxirab, 14% subjects receiving PVRV and 10% subjects receiving PCECV, complained

of adverse reactions. All these reactions subsided spontaneously and did not require any medication. When statistically analyzed, the difference in the incidence of reactions was not significant.<sup>17</sup> Another study was conducted in India to assess safety and immunogenicity of PVRV and PCECV vaccines for pre-exposure vaccination in children. Two vaccines administered intramuscular with a three-dose regimen on days 0, 7 and 28 in 175 healthy schoolchildren by a nurse who was blinded to the vaccines type. Virus neutralizing antibody (RVNA) concentrations were measured on day 49. Pain after vaccination was reported in 2 to 12% of subjects, and fever was reported in 2% to 5%. However, this difference was not statistically significant. Nounexpected or serious adverse event was reported during the study. This study showed that PCECV and PVRV are safe and immunogenic when administered intramuscularly for pre-exposure prophylaxis of rabies in children.<sup>19</sup> PCECV is approved for pre- and post-exposure prophylaxis, either by the intramuscular or intradermal administration. In more than 25 years of use, PCECV has been widely used for pre-exposure prophylaxis in children.<sup>19,20</sup> According to a review study, PCECV has been administered to more than 1,200 children in clinical trials, from toddlers to those in elementary school, using intramuscular and intradermal schedules, demonstrating safety and immunogenicity.<sup>20</sup>

A recent study in China compared the safety and immunogenicity between PVRV and PCECV in patients with WHO category II animal exposure, in different age groups. Information collected for the demographic and adverse reactions and RVNA titers for 387 patients after vaccination with PVRV or PCECV. The results showed no significant differences of safety and immunogenicity between PVRV and PCECV. However, when compared with other age groups, most systemic adverse reactions (36/61), occurred in patients aged < 5 years, and < 5-year-old patients, had a significant lower RVNA titer in both groups.<sup>21</sup>

Current studies show that many approved vaccines with different components such as PVRV, PCECV, and Human diploid cell vaccine (HDCV) and many regimens with different vaccination schedules (Zagreb, Essen) are being used in the world. However, many host related factors may influence the vaccine potency or host immune responses such as: sex, age, hormonal and genetic factors, acute and chronic diseases, stress, and nutritional deficiency.<sup>22,23</sup>

In the present study, participants were randomized to receive the PVRV or PCECV vaccine. There were no significant differences between the two groups regarding age, sex and residential area. Therefore, a possible confounding effect of these variables was excluded by randomization. Furthermore there was no significant difference between two groups regarding category of exposures. This can remove the effect of post-traumatic stress induced by animal biting.

The strength of our study was its large sample size, including participants from different part of Iran. Also, no participant lost to follow up.

There are some limitations in our study. First, we only assessed adverse reactions of two anti-rabies vaccines in a short time period; further follow-up studies are needed to determine immunogenicity of vaccines, persistence of immunity, type of regimens and vaccination schedules. Second, we did not include young children (aged < 5 years) in our study. As more than 50% of human rabies deaths are seen in children under the age of 15, future studies should focus on finding the most optimal vaccine

and vaccination schedule to ensure sufficient levels of immune responses and minimize adverse effects in this group.

In conclusion, both PCECV and PVRV were well tolerated by all subjects. There were no deaths, serious adverse events or other adverse events leading to hospitalization of the subjects. All subjects were available for observation throughout the study period. Also, they were alive and healthy at the end of the observation period (32 day). This study shows that PCECV is as safe as PVRV. Therefore, it can be used as an alternative when PVRV is not accessible.

**Conflict of Interests:** None declared

**Funding:** None

## Registration

The study protocol has been registered in the Iranian Registry of Clinical Trials (IRCT) ([www.irct.ir](http://www.irct.ir)) with reference number of (IRCT2015102124470N1).

## Ethical approval

This study was approved by the Committee of Rabies Control, Ministry of Health, Treatment and Medical Education of Iran.

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