

**Incidence of Early and Late Adverse Reactions to Antivenom in Snakebite Patients: A
Prospective Cohort Study in Southeastern Iran (2017–2018)**

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Abstract:

Introduction: Despite being a lifesaving treatment, antivenom administration can be associated with adverse reactions, including immediate hypersensitivity reactions and delayed serum sickness. The purpose of this cohort study was to assess prospectively the incidence of serum sickness and adverse drug reaction associated with the use of polyvalent equine F(ab')₂ antivenom.

Material and Methods: This prospective cohort study was carried out among 295 snakebite victims in the Sistan and Baluchestan over 2017 and 2018. Information on demographics, treatment, and adverse reactions was obtained through an accurate questionnaire. Patients were also closely followed up one month after discharge to detect delayed reactions, mostly serum sickness.

Results: Most were male patients (62.7%), mean age was 29.2 ± 19.3 years. Adverse reactions were noted in 48 patients (16.3%), of which most were early reactions. Pyrogenic reactions were most common (8.1%), followed by skin manifestations (2.7%), and anaphylactic reactions (2%).

Conclusion: All acute reactions were successfully handled. In 8 participants, clinical symptoms suggesting serum sickness was detected while none of them were definitely confirmed to have serum sickness. Razi Polyvalent Antivenom has a favorable safety profile with a low incidence of both immediate and delayed reactions, specifically serum sickness reactions.

Key words: Adverse drug reactions; Coagulopathy; Equine F(ab')₂ antivenom; Hypersensitivity; Venom-induced complications

1- Introduction

The World Health Organization (WHO) recognizes snake bite as one of the neglected tropical diseases with high priority because it has a massive morbidity and mortality in the poor communities[1]. In the Middle East, snakebite is one of the heavily burdened diseases in Iran, especially in the Sistan and Baluchestan Province [2, 3].

In Iran, the polyvalent equine F(ab')₂ antivenom produced by the Razi Vaccine and Serum Research Institute is used as the primary therapeutic agent for neutralization of venom from medically important local species, including vipers[4]. Antivenom plays a vital role in envenomation management and counteracting systemic envenomation, although it is not free from complications. Administration of heterologous proteins can result in various adverse events (immediate or delayed) ranging from anaphylaxis to serum sickness [5-7].

Immediate adverse drug reactions, including fever, hives, and anaphylaxis, usually happens within hours of infusion. In contrast, serum sickness, which involves fever, rash, joint pain, and swollen lymph nodes, typically appears 5 to 14 days after administration [8]. The rate of these adverse events varies widely around the world. This variation is influenced by factors like the purity of the antivenom, the manufacturing process, the dose given, and individual patient sensitivity [5, 9]. Even though modern purification methods have greatly decreased the foreign protein load and lowered the immunogenic potential of antivenoms, the risk of adverse reactions is still a clinical concern [10].

Despite the important role of Razi antivenom in the Iranian healthcare system, there are few thorough, prospective studies from endemic areas like Sistan and Baluchestan. Most existing studies focus on clinical effectiveness or immediate reactions, while delayed events such as serum sickness are often underreported, partly due to a lack of follow-up after patients leave the hospital. This study aims to prospectively assess the adverse drug reactions and incidence of serum sickness and other adverse events after the administration of Razi polyvalent antivenom to snakebite victims in major hospitals in Sistan and Baluchestan Province between 2017 and 2018. By using a structured follow-up protocol over one month, this research seeks to provide a clearer view of the safety profile of this crucial medication. This, in turn, will help inform clinical practice and enhance patient care in this high-risk group.

2- Material and Methods

2.1 Study design

This study utilized a prospective cohort design. The protocol was initiated after obtaining official coordination and necessary approvals from the Iranian CDC, University of Medical Sciences in the study region. Following the development and validation of a comprehensive data collection questionnaire, all snakebite patients admitted to the hospital, who received antivenom and provided oral consent were enrolled in the cohort. The study incorporated an active follow-up component for one month after discharge to monitor for the delayed onset of serum sickness.

2.2 Setting and Participants

2.2.1 Setting

This study was conducted on snakebite patients admitted to hospitals affiliated with the University of Medical Sciences in Sistan and Baluchestan Province, Iran (see Fig 2). This region is a known endemic area for snakebites. Zabol city was selected as the recruitment site regarding the high incidence of the snakebite cases. The primary treatment centers for snakebite victims changed during the study period. In 2017, patients were admitted to Imam Khomeini Hospital (Zabol) and Shohadaye Hirmand Hospital (for children). In 2018, Amir al-Momenin Hospital of Zabol became the main referral center.

2.2.2 Participants:

The study cohort consisted of all snakebite patients who received antivenom therapy and provided oral consent to participate. Patients were actively followed for a period of one month after hospital discharge to monitor for the occurrence of delayed adverse events, particularly serum sickness. The form 3 was designed to document the clinical and para-clinical symptoms of suspected serum sickness patients. The serum sickness was confirmed according to the overall symptoms and the final diagnosis of the specialist (Fig 1).

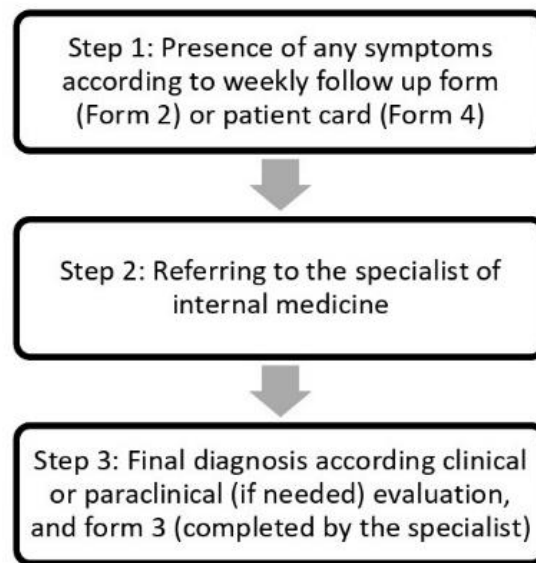


Figure 1. Schematic diagram of the diagnostic workflow from symptom detection to final diagnosis

2.2.3 Inclusion criteria

All snakebite patients admitted to the hospital who received antivenom therapy and provided oral consent to participate in the study. Because we did not have any further intervention from the standard protocol for snakebite victims treatment and regarding the emergency situation of the participants we did not take the written consent from the patients.

2.2.4 Exclusion criteria

Patients with incomplete medical records regarding snakebite were excluded. Lack of verbal consent and no requirement for antivenom serum were other exclusion criteria. Factors such as hypersensitivity or pregnancy were not considered as exclusion criteria and all patients were included regardless of these factors.

2.3 Exposure

2.3.1 Source and Type of Antivenom:

The antivenom used in this study was the polyvalent equine antivenom produced by the Razi Vaccine and Serum Research Institute. This product is prepared through the purification and concentration of hyperimmune horse plasma immunized with the venom of the following 5 snake species including, Levantine viper (*Vipera lebetina*), Zigzag mountain viper (*Vipera albicornuta*), Saw-scaled viper (*Echis carinatus*), Persian horned viper (*Pseudocerastes persicus*), Caucasian pit viper (*Agkistrodon halys*). The active components of this antivenom consist of F(ab')₂ fragments of immunoglobulins against the snake venoms. Each milliliter has the capacity to neutralize more than 50 LD-50 of the venom from each of the mentioned

snakes, based on lethal toxicity testing in mice. This product contains a maximum of 0.25% (w/v) phenol as a preservative.

2.3.2 Administration Protocol:

1. Patient Identification: Every snakebite patient who arrived at the hospital and met the criteria for antivenom therapy was eligible.

2. Informed Consent: After providing oral consent, the patient was formally enrolled in the study.

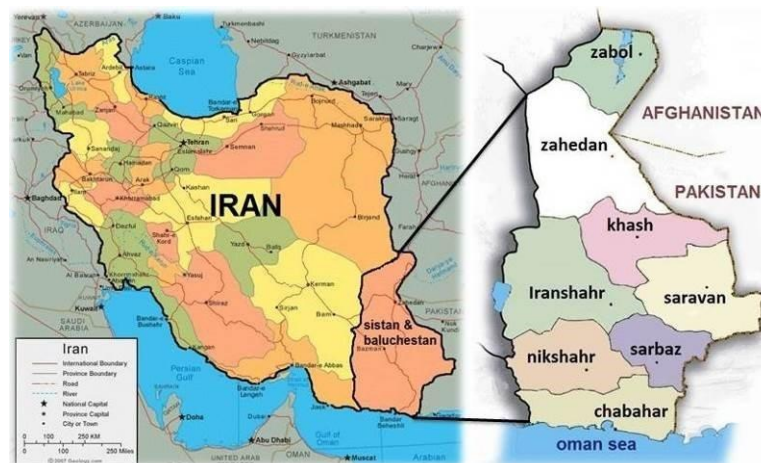


Figure 2. The location of the study setting (Sistan and baluchestan province); source [11]

3. Administration: The antivenom was administered via slow intravenous infusion, as directed by Iranian health minister guideline [12].

4. Baseline Assessment & Monitoring: Prior to the study commencement, the physician and nurse of each shift were trained by the principal investigator on the study procedures and the method of completing the Case Report Forms (CRFs). Form No. 1 was completed by the treating physician and nurse. This form recorded the patient's medical history, signs and symptoms upon arrival, and laboratory results before treatment. Potential adverse events following the infusion and any subsequent therapeutic actions were exactly recorded in the same form.

5. Follow up: In the city of Zabol, a head coordinator was appointed at the Disease Management Center. Upon a patient's discharge, a form containing their phone number, address, and personal details was completed and delivered to this coordinator (Form 2). At the end of each week, the coordinator's representative visited the centers to collect the forms. Weekly follow-up calls were made by the head person to monitor for symptoms. If the patient reported any of the symptoms listed on the form, they were referred to a hospital physician for evaluation to rule out serum sickness (Form 3).

Additionally, after discharging from hospital, each patient was given an information form describing the symptoms of serum sickness, instructing them to contact the referral hospital if any of those symptoms occurred (Form 4).

2.4 Outcomes

2.4.1 Primary Outcome

1. Severe hypersensitivity within first few hours of antivenom therapy: Severe hypersensitivity was defined as Cyanosis or $SpO_2 \leq 92\%$ at any stage, hypotension (systolic blood pressure, < 90 mmHg in adults), confusion, collapse, loss of consciousness, or incontinence [13]
2. Serum sickness recorded during the one-month follow-up period defined as fever, nausea, vomiting, diarrhoea, itching, recurrent urticaria, arthralgia, myalgia, lymphadenopathy, periarticular swellings, mononeuritis multiplex, proteinuria, immune complex nephritis, rarely encephalopathy [14]. The incidence of serum sickness, defined as the onset of symptoms occurring 5 to 30 days after antivenom administration. The diagnosis was confirmed during the active weekly follow-up period or upon the patient's return to the hospital.
3. Pyrogenic reactions defined as pyrogenic (endotoxin) reactions (within 1-2hours) involve rigors, fever (risk of febrile convulsions in children), vasodilatation, hypotension and a fall in blood pressure [14].

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188 2.4.2 Secondary Outcomes

- 189 1. Mild hypersensitivity, occurring within the first few hours of antivenom infusion,
190 recorded in the hospital defined as generalized erythema, urticaria, periorbitaloedema,
191 or angioedema [13]
- 192 2. Moderate hypersensitivity, occurring within the first few hours of antivenom infusion,
193 recorded in the hospital defined as Dyspnoea, stridor, wheeze, nausea, vomiting,
194 dizziness (presyncope), diaphoresis, chest or throat tightness, or abdominal pain [13]
- 195 3. Any other adverse events recorded during the one-month follow-up period.

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197 2.5 Data collection

198 Data were collected using a multi-stage, multi-source process to ensure comprehensive
199 capture of both immediate and delayed outcomes.

200

201 2.5.1 Data Collection Instrument

202 A comprehensive and validated questionnaire was used, adapted from the standard form of
203 the Razi Vaccine and Serum Research Institute. The instrument consisted of the following
204 sections:

205 Section 1: Patient Medical and Drug History.

206 Section 2: Signs and Symptoms upon Hospital Admission (pre-treatment).

207 Section 3: Laboratory Results before initiation of antivenom therapy.

208 Section 4: Record of Potential Adverse Events and Therapeutic Measures taken during
209 hospitalization.

210 Section 5: Post-Discharge Follow-up for the emergence of "Serum Sickness" symptoms.

211 Section 6: Patient Identification Card.

212

213 2.5.2 Data Collection Procedure

214 a. Hospital Phase (Initial Data):

215 For every enrolled snakebite patient, Sections 1-4 of the form 1 were completed at the
216 hospital by the treating physician and nurse.

217 b. Discharge and Active Follow-up Phase (Outcome Data):

218 A list of discharged patients, including precise addresses and contact numbers, was provided
219 to a dedicated follow-up officer from the Zabol University of Medical Sciences.

This officer then conducted active weekly follow-ups for one month using a follow up form (form 2). If a patient reported or was found to have symptoms, they were referred back to the hospital for confirmation and treatment, and the outcome was recorded (form 3).

Simultaneously, upon discharge, patients were given a patient identification card (form 4) and instructed to return to the hospital if any symptoms listed on the card (indicative of serum sickness) appeared (form 3).

2.5.3 Data Management and Quality Control

Completed questionnaires were sent to the principal investigators for initial review. Any deficiencies or missing data were noted, and the forms were returned to the relevant officer for correction by cross-referencing with available hospital records. The data from the finalized forms were then double-entered into a computer database for verification before statistical analysis.

2.6 Statistical analysis

Data analysis was performed using SPSS software (version 22.0, IBM). Descriptive statistics were used to summarize the demographic and clinical characteristics of the study population. Continuous variables with normal distribution are presented as mean $\pm$ standard deviation (SD), while non-normally distributed data are presented as median and interquartile range (IQR). Categorical variables are presented as frequencies and percentages.

For inferential statistics, the Chi-square test (or Fisher's exact test where appropriate) was employed to examine associations between categorical variables, such as the relationship between the number of antivenom ampoules administered (categorized if necessary) and the incidence of adverse reactions. Because the minimum number of ampoules received in the first round was 5 per protocol, so number of ampoules was considered as an ordinal variable and was categorized as (<5, 5-10, >10).

A p-value of less than 0.05 was considered statistically significant for all tests.

2.7 Ethical considerations

This study was conducted after obtaining the necessary official approvals and coordination with the University of Medical Sciences in the study region. Oral informed consent was obtained from all patients or their guardians prior to their enrollment in the study. To protect patient confidentiality, all collected data were anonymized and handled securely throughout the research process. This project was approved by research community of Razi Vaccine and Serum Research Institute (RVSRI) with 2-18-18-95102 project number. Given that no intervention was carried out at the implementing university, verbal consent was considered sufficient, that permission for telephone calls and subsequent follow-ups was obtained from the patient themselves or their companion.

3- Results

3.1 Patient Characteristics and Demographics

A total of 295 snakebite victims who received antivenom were included in this study between June 2017 and October 2018. The cohort was predominantly male (62.7%, n=185) with a mean age of 29.2 ± 19.3 years (range: 9 months to 80 years). The majority of patients (94.2%) had no significant documented past medical history or recent use of medications that could confound the assessment of adverse drug reactions. Among the pre-existing conditions, hypertension was the most common, noted in 14 patients (4.7%). The epidemiological characteristics are summarized in Table 1.

Table 1. Epidemiological and Clinical Profile of Snakebite Victims (N=295)

Characteristic	Value
Sex, n (%)	
Male	185 (62.7%)
Female	110 (37.3%)
Age (years)	
Mean $\pm$ SD	29.2 ± 19.3
Range	9 month – 80 year
Season of Bite, n (%)	
Summer	221 (75.0%)
Spring	57 (19.3%)
Autumn	17 (5.8%)
Winter	0 (0%)
Time of Bite, n (%)	
12:00 PM – 6:00 AM (Lowest)	25 (8.8%)
6:00 AM – 12:00 AM	65 (22.9%)
12:00 AM – 6:00 PM	59 (20.8%)
6:00 PM – 12:00PM (Peak)	135 (47.5%)
Bite Location, n (%)	
Lower Limbs	151 (51.2%)
Fingers or Toes	97 (32.9%)
Upper Extremities	45 (15.6%)
Other (Head/Torso)	2 (0.6%)

3.2 Antivenom treatment and utilization

A total of 2,384 ampules of antivenom were administered to the 295 patients. The median number of vialampules used per patient was 7.

The mean duration of hospitalization was 3.2 ± 1.84 days, with a median of 3 days. The total cumulative hospital stay for all patients was 832 days.

3.3 Incidence and Spectrum of Adverse Reactions to Antivenom

Among the 295 patients who received antivenom, 48 (16.3%) experienced adverse reactions. These reactions were categorized based on their time of onset into immediate (early) and delayed (late) reactions.

3.3.1 Early-onset reactions

Among the 295 patients who received antivenom, 48 individuals (16.3%) experienced at least one adverse reaction. The spectrum of these reactions is detailed in Fig 3.

The most frequent type of reaction was pyrogenic reactions (8.1%), followed by cutaneous and mucosal manifestations such as urticaria and rash (2.7%). Anaphylaxis was reported in 6 patients (2.0%), with cases categorized as severe (n=3, 1.0%) and moderate (n=3, 1.0%). Isolated symptoms, including headache (with or without dizziness and vomiting) and nausea (with or without vomiting), were observed in 8 (2.7%) and 6 (2.0%) patients, respectively.

All cases of anaphylaxis and other acute reactions were successfully managed with supportive care and antihistamines, with no fatalities recorded. Furthermore, statistical analysis using the Chi-square test indicated no significant association between the number of antivenom ampules administered and the incidence of adverse reactions ($P > 0.05$). The mean age of people with adverse reaction was 25.01 (SD=20.42) compared with 29.7 (SD=19.2) in the people with no adverse reaction group ($P=0.175$).

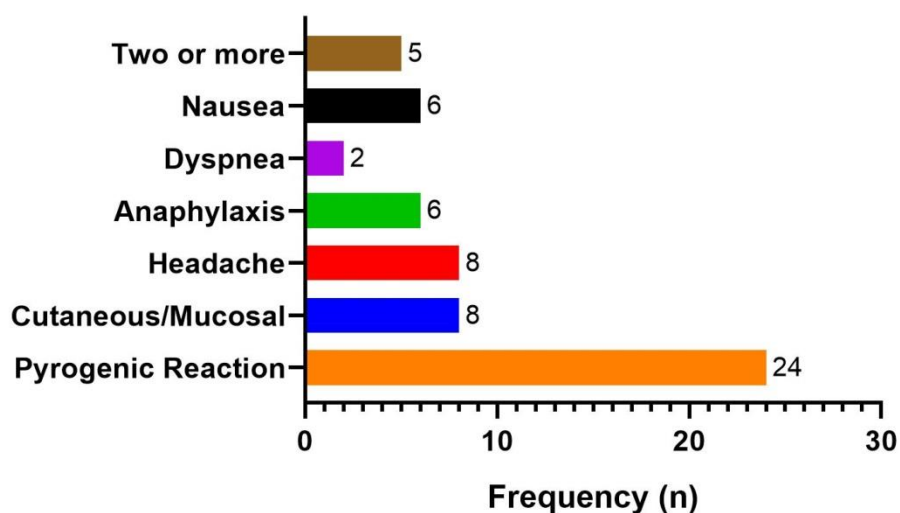


Figure 3. Frequency and Types of Adverse Reactions Following Antivenom Administration (data of 48 patients with adverse events)

The analysis of adverse reaction rates, standardized per 1000 administered ampules of Razi Institute antivenom, revealed the following incidence: Pyrogenic reactions were the most frequent, with an incidence of 10 per 1000 ampules. Anaphylactic reactions occurred at a rate of 2.5 per 1000 ampules. Cutaneous and mucosal manifestations were observed at a rate of 3.4 per 1000 ampules. Other specified reactions were reported at a rate of 5.9 per 1000 ampules.

3.3.2 Late-Onset Reactions (Serum Sickness)

During the 4-week follow-up period, 8 out of 295 patients (2.7%) presented with symptoms suggestive of serum sickness (table 2). The onset of symptoms typically occurred within the first two weeks after antivenom administration, with the majority of cases (5 out of 8) appearing in the first week.

315 Table2. Status report of the injured who showed symptoms suspicious of serum sickness
 316 during the 4-week follow-ups after the snakebite.

Row	Gender	Age	Number of Doses Received (in ampoules)	Time of Symptom Onset				Symptoms	Actions Taken	Final Diagnosis
				Week 1	Week 2	Week 3	Week 4			
1	Male	3	15	✓	--	--	--	Fever & chills - Joint pain - Swelling of each joint – Muscle pain	No specific intervention was documented; however, symptoms resolved during follow-up.	-----
2	Female	66	6	--	✓	--	--	Fever & chills - Severe headache	Referred to the focal point physician and investigated.	Serum sickness ruled out. Diagnosed with diabetes, hospitalized and treated.
3	Female	50	6	--	✓	--	--	Muscle pain	Referred to the focal point physician and investigated.	Serum sickness ruled out.
4	Female	75	37	✓	--	--	--	Pain in the upper abdomen - Change in urine color	History of kidney failure. Transferred to Zahedan.	Died due to hepatic and renal failure.
5	Female	27	8	✓	--	--	--	Loss of appetite - Nausea - Pain in the upper abdomen - Severe headache	Referred to the focal point physician and investigated.	No signs of arthritis, bleeding, or arthralgia. Treated as outpatient with anti- nausea medication, symptoms resolved.
6	Male	73	9	✓	--	--	--	Change in urine color	Referred to a urologist due to	Diagnosed with kidney stones. Serum

									hematuria.	sickness ruled out.
7	Male	17	6	--	--	--	✓	Fever & chills - Muscle pain - Severe headache	Referred to the focal point physician and investigated.	Serum sickness ruled out.
8	Female	44	8	✓	--	--	--	Joint pain - Muscle pain - Severe headache	Referred to the focal point physician and investigated.	Discharged as outpatient with Indometacin. In follow-ups, symptoms were resolved.

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319 The most frequently reported symptoms among these patients were fever and chills, severe
320 headache, myalgia (muscle pain), and arthralgia (joint pain).

321 No confirmed serum sickness was detected. In one patient (Case 1) serum sickness was not
322 ruled out due to data inadequacy. It was presenting with the classic triad of fever, arthralgia,
323 and myalgia. The symptoms resolved with supportive care during subsequent follow-ups. In 5
324 cases (Cases 2, 3, 6, 7, 8), the diagnosis of serum sickness was ruled out following specialist
325 evaluation, with alternative diagnoses including diabetes and renal calculi. One patient (Case
326 4) with a history of renal failure and who received 37 ampules of antivenom died due to
327 hepatorenal failure. While the temporal relationship to antivenom administration was noted,
328 the contribution of serum sickness to this outcome could not be definitively established
329 amidst pre-existing organ dysfunction.

330 One patient (Case 5) with non-specific symptoms (nausea, headache) was treated
331 symptomatically as an outpatient, with subsequent resolution of symptoms.

332 In summary, while symptoms raised suspicion for serum sickness in 8 patients, the diagnosis
333 was not confirmed in any cases, indicating a low incidence of this delayed complication in
334 our study population.

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340 4- Discussion

341 This study reaffirms the good safety profile and proven efficacy of the pentavalent antivenom
342 produced by the Razi Institute in Iranian snake bite patients. The demographic data considers
343 a male preponderance of 62.7% and an average age of 29.2 years, which correspond to those
344 expected in either rural or agricultural work patterns in Iran and the Iranian peninsula, and are
345 consistent with the Iranian data on snake bites in Asia[15, 16]. The seasonal clustering in
346 summer aligns with the increased snake activity in warmer months, further stressing the
347 environmental risk components of snakebite epidemiology.

348 Concerning the treatment regimen, the administration of a median of seven vials per patient
349 matches Ministry of Health guidelines of 5-10 ampules depending on the degree of clinical
350 severity [3]. The total average hospital stay of three days, as well as the absence of direct
351 correlation between the doses and side effects, further confirms the rational usage and
352 harmless nature of Razi's antivenom.

353 The incidence of adverse event outcomes was 16.3% that primarily consisted of mild
354 pyrogenic reactions (with an incidence of 10 per 1000 ampules), though only 2% had
355 anaphylaxis (a rate of 2.5 per 1000 ampules). Cutaneous and mucosal manifestations were
356 detected at a rate of 3.4, and other specified reactions were reported at a rate of 5.9 per 1000
357 ampules. The lack of severe pyrogenic reactions indicates that GMP compliance was high at
358 the Razi Institute, thus reducing the risk of contamination with the endotoxin, which causes
359 febrile reactions via TLR4 receptor activation and consequent cytokines (IL-1 β , IL-6, and
360 TNF- α) [17]. All severe cases were effectively managed using standard pharmacological
361 prophylaxis and supportive care, highlighting the importance of clinician preparedness and
362 early intervention.

363 The fact that there was not a single reported case of serum sickness in this series is especially
364 interesting, especially in light of the mean global incidence of 3-6% [18]. This diminished
365 immunogenicity can presumably be attributed to major advances made in downstream
366 processing, namely through the use of caprylic acid fractionation and enzymatic purification
367 (for example, pepsin digestion to yield F(ab')₂ fragments). Such steps reduce not only the
368 actual quantity of exogenous proteins but also reduce the quantity of high molecular weight
369 aggregates. Furthermore, such purification steps eliminate the anticomplementary activator
370 regions (namely, the Fc portion) from IgG class antibodies. As reviewed by León et al.
371 (2013) [17], although there has been controversy regarding whether anticomplementary
372 reactivity (ACA) accounts for early reactions, such steps collectively account for production
373 of a compound with inherently reduced immunogenic properties. The absence, therefore, of
374 manifestations suggestive of delayed (type III hypersensitivity) reactions fits with current
375 pathophysiologies, whereby true associations between dose-dependent exposure to foreign
376 proteins and immune complex-mediated pathophysiologies can be established. The reduced,
377 "purified" antivenom, therefore, likely presents a decreased antigenic stimulus, thereby
378 obviating this risk.

379 The lower immunogenic potential of the current formulation of the Razi Institute may be
380 attributed to enhancement in refining and enzymatic purification, lower level of recovery, and
381 lower activation of the complement and foreign proteins [10]. Several similar studies have

been conducted in different regions. The reported adverse event rate was higher in these studies.

In the study conducted in Chilaw Hospital in Sri Lanka between the years 2007 & 2009, all patients above the age of 14 years who underwent antivenom infusion were considered for the study. Early side effects were assessed, and it was concluded that the incidence of typical hypersensitivity reactions was found in 77 out of 120 patients (64%), anaphylaxis in 48% (57/120), and pyrogenic reactions occurred in 37 out of 120 patients (27%) [19].

Another study among 93 patients from the years 2000 & 2004 at the Phoenix Children's Hospital and the Banner Good Samaritan Medical center reported the incidence of acute side effects of hypersensitivity as 5 out of 93 patients or 5.4%; mild side effects occurred in 4 patients. In this study, the side effects were lower than what was reported earlier [20]. In the study conducted in the years 2012-2013, 50 antiserum-treated snakebite patients were assessed: initial fever & chills occurred in 23.53%, initial hypotension in 21.57%, and initial anaphylaxis in 52.94% of the patients. Adverse events tended to occur in 43.1%. Mild side effects were found in 70.59% & severe side effects in 5.88% patients. In this study, the side effects tended to be higher than what was reported by references [21]. The adverse reaction rate in our study (16.3%) was substantially lower than the 43.1% reported by Deshmukh et al. (2014) in India. This marked difference can be primarily attributed to the superior purification technology employed by the Razi Institute, particularly the caprylic acid fractionation and pepsin digestion processes that yield highly purified F(ab')₂ fragments with significantly reduced foreign protein load and eliminated Fc-mediated complement activation (10, 17). Additionally, geographic and ethnic variations in patient populations may contribute to differing immunoreactivity profiles, as genetic factors can influence susceptibility to hypersensitivity reactions. Differences in clinical protocols, including antivenom administration rates, premedication practices, and criteria for adverse reaction reporting, might also partially explain the discrepancy. Nevertheless, the substantially lower reaction rate observed in our cohort, despite similar snake species and clinical settings, strongly supports the favorable safety profile of Razi polyvalent antivenom and highlights the success of its advanced manufacturing process in minimizing adverse events compared to less purified products used in other regions.

A project in Australia was conducted on 195 patients who underwent antivenom. This multi-center cohort study revealed that the majority, i.e., 74% underwent antivenom due to coagulopathy, followed by non-specific side effects in 12% and neurotoxicity in 5%. Myotoxicity occurred in only 4% of the patients while the dose used was mean of 4 ampules of antivenom. Of them, 48 patients or 25% had initial type IV hypersensitivity reaction; anaphylaxis occurred in 21 patients; symptoms were moderate in 11, severe in 10, hypotension was seen in 9, and skin symptoms alone occurred in 27. Among them, adrenaline was successful in administering in the remaining 26 patients who had side effects due to antivenom [13]. In Australia, the above-mentioned cohort study was conducted from November 2012 until March 2014 in which the early acute side effects & the incidence of serum sickness were assessed in antivenom-treated patients. This study taken in the 100 hospitals on a total of 279 patients showed all of them were patients bitten by snakes & were followed by the researcher for 6 weeks after acute encephalitis after taking antivenom; out of them, all 138 patients who received antivenom showed an incidence of antivenom-related side effects like serum sickness in 29%, and acute side effects of hypersensitivity in 23%. In this study, serum sickness proved to be the adverse effect that occurred most often, though no relation could be established as regards the incidence and the venom dose. There is evidence

from early research conducted by Trinca that out of 100 patients given antivenom injections, serum sickness occurred in 6 (7%) patients. However, this study does not involve follow-up of patients. In another survey at Port Moresby Hospital, serum sickness occurred in 7% of 28 patients. The symptoms proved to be drowsiness and headache, joint pain and fever and chills in this investigation. The other symptoms are fewer in this study. A literature review in the United States, conducted by the author of this article, showed in 5 to 23% cases of serum sickness [22].

The zero incidence of confirmed serum sickness in our cohort, despite a global reported range of 3-6% (18), is noteworthy and can be primarily explained by the advanced purification process of Razi antivenom, including caprylic acid fractionation and pepsin digestion, which yields highly purified F(ab')₂ fragments with minimal immunogenicity (10, 17). Although our active one-month follow-up protocol was robust, we acknowledge the possibility of mild or subclinical cases being underreported as a potential limitation. However, among the eight patients who presented with suspicious symptoms, thorough specialist evaluations ruled out serum sickness in all but one case, confirming alternative diagnoses. While the lack of confirmed cases may partly reflect underdiagnosis of very mild symptoms, the inherently low immunogenic potential of this purified antivenom remains the most plausible explanation. We therefore believe that the true incidence of serum sickness following Razi polyvalent antivenom administration is substantially lower than the global average.

One fatal case occurred in a 75-year-old female with a history of renal failure who received 37 ampules of antivenom and died due to hepatorenal failure. Although the temporal relationship to antivenom administration was noted, we believe this fatality was primarily attributable to the patient's pre-existing severe comorbidities and venom-induced complications, particularly acute kidney injury, rather than a direct adverse effect of the antivenom itself. The high number of ampules administered reflects the severity of envenomation rather than a causative factor for mortality. Notably, no signs of anaphylaxis or serum sickness were documented in this patient, and the hepatorenal failure is a well-recognized complication of severe snakebite envenomation, especially in elderly patients with underlying organ dysfunction. Therefore, we conclude that this death was most likely due to the natural history of severe envenomation compounded by baseline renal impairment, and not directly attributable to antivenom therapy. It should also be noted that a high dose was administered for this patient according to the national protocol (12), which recommends dose titration based on clinical response and laboratory parameters; in this case, severe envenomation with progressive coagulopathy and deteriorating renal function necessitated repeated dosing.

Relating to the international norms, the product is comparable to the WHO criteria for the strength of the product, with low adverse reaction rates ($\leq 2.5/1000$ ampules). These results confirm the WHO criteria that with the effective administration of antivenoms, including pharmacological premedication, the mortality rate would be reduced even with the existing rural health facilities [23].

5- Conclusion

Nevertheless, host variability, delay of treatment, and insufficient follow-up data remain potential confounding variables. Taken cumulatively, these data further establish that Razi Institute antivenom is both safe and efficacious, yielding rapid normalization of coagulopathies and a correspondingly low hypersensitivity risk. Ongoing research aimed at enhancing antivenom purity, extending pharmacovigilance, and incorporating biotech and humanized antibody approaches may further propel antivenom safety and competitiveness within the global context of Iranian snake venoms. The favorable safety profile of Razi Institute's polyvalent antivenom, particularly the absence of confirmed serum sickness,

supports its continued use as the first-line treatment for snakebite envenomation in Iran.
Ongoing pharmacovigilance and efforts to further enhance purity are recommended.

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Authors' Contributions:

FA: Study concept and design, Statistical analysis, Drafting of the manuscript.

LM: Study concept and design, Conducting the Project, statistical analysis, critical revision of the manuscript.

MT: Study concept and design, administrative and technical support.

SMZ: Acquisition of data, administrative support.

DS: Acquisition of data, administrative and technical support.

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Conflict of Interest:

Three of the authors (Fereshteh Ansari, Ladan Mokhberalsafa, and Majid Tebianian) are employees of the Razi Vaccine and Serum Research Institute, which is the manufacturer of the antivenom evaluated in this study. The remaining authors (Seyed Mohsen Zahraei, Amir Bassi shad, Dadkhoda Sofi, and Javad Akbarizadeh) declare no competing interests. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. All authors have completed the ICMJE Form for Disclosure of Potential Conflicts of Interest.

Data Availability:

510 The datasets generated and analyzed during the current study are available from the
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Preprint

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