Comparative Immunology, Microbiology and Infectious Diseases 66 (2019) 101322

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Seroepidemiological study of Q fever, brucellosis and tularemia in butchers and slaughterhouses workers in Lorestan, western of Iran

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ARTICLE INFO

Keywords:
Q fever
Tularemia
Brucelloses
Zoonoses
Seroprevalence
Lorestan province
Iran

ABSTRACT

Most zoonoses are occupational diseases. Q fever, brucellosis and tularemia are major zoonotic diseases for butchers and slaughterhouse workers. However, little information is available about these infectious diseases in such professional populations in western of Iran. The aim of this study was to investigate the seroprevalence and risk factors associated with these three zoonoses among butchers and slaughterhouse workers in the Lorestan province of Iran. In 2017, 289 individuals (144 butchers or slaughterhouse workers, and 145 people from the general population) were enrolled in 11 different counties of this province. Collected serum samples were tested by ELISA for detection of IgG antibodies against *Coxiella burnetii*, *Brucella* spp. or *Francisella tularensis* antigens. The seroprevalence of Q fever, brucellosis and tularemia among all participants were 23.5%, 31.8% and 3.8%, respectively. The seroprevalence of brucellosis and Q fever among butchers and slaughterhouse workers (43.7% and 29.8%, respectively) were significantly higher ($p < 0.05$) than those of the general population (20% and 17.2%, respectively). A contact history with small ruminants (sheep and goats) was associated with a higher risk of positive serology for all three studied zoonoses. The high seroprevalence for Q fever and brucellosis we found among butchers and slaughterhouse workers suggests that both diseases are common in these populations of the Lorestan province. Since these two infectious diseases are clinically unspecific, they must be systematically included in the etiological diagnosis of infectious diseases occurring in these at-risk populations. In addition, we recommend specific training programs as well as the use of personal protective equipment in these occupational groups to reduce the occurrence of these zoonotic diseases.

1. Introduction

Emerging and reemerging infectious diseases represent significant health threats and challenges worldwide, more than half of them being zoonoses [1]. Most zoonoses are occupational diseases often transmitted to workers through direct contact with animals and their carcasses. The risk of transmission of zoonotic pathogens from animals to humans during occupational activities depends on several factors, including the animal health situation, the type of work performed, the need for frequent contact with either domestic or wildlife animals, and their tissues or carcasses, and the level of knowledge of at-risk situations of the exposed individuals and their use of personal protection equipment [2].

Brucellosis is a worldwide zoonosis caused by bacteria of the genus *Brucella* [3]. It remains one of the most frequent zoonoses, affecting wildlife animals, livestock and humans. The major risk factors for human brucellosis include contact with infected animals (mainly livestock) or their tissues and body fluids (blood, urine, vaginal discharge),

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https://doi.org/10.1016/j.cimid.2019.06.003
Received 12 April 2019; Received in revised form 8 June 2019; Accepted 10 June 2019

and consumption of unpasteurized dairy products. Thus, brucellosis is usually more common in slaughterhouse workers, animal farmers, and veterinarians compared to the general population [4]. The annual incidence of brucellosis in Iran is estimated at 0.001% [5]. However, the prevalence of this disease varies between different regions of Iran depending on the climatic conditions, the raised livestock species and their health situation, and the access or not to pasteurized dairy products [6].

Q fever is a widespread zoonosis caused by the bacterium *Coxiella burnetii*. Human infections may correspond to acute or chronic diseases, the most severe form being endocarditis [7]. Like in other Middle East countries, Q fever is endemic in Iran [8]. This disease was first reported in this country in 1951. Subsequently, *C. burnetii* infection was detected by culture or serology in humans, sheep, cattle, goats, camels, equines, rodents, birds, ticks, and even eggs. Q fever infection is mainly associated with contact with livestock, but also exposure to contaminated environments where C. burnetii can survive for months. Dairy products may be contaminated with *C. burnetii* but are rarely involved in human transmission [9].

Tularemia is caused by *Francisella tularensis*. The infectious dose of this bacterium is considered very low since 10–50 bacteria are enough to cause infection in humans and animals [10]. *F. tularensis* can infect a significant number of wildlife species as well as domestic livestock. This pathogen may be transmitted to humans in many ways, including through direct contact with contaminated animals or their tissues, consumption of undercooked contaminated meat or contaminated drinking water, bites or scratches from animals, arthropod bites, and environmental exposures such as breathing aerosols from contaminated dust [11]. In Iran, human cases of tularemia were firstly reported in 1980, although positive serological tests were reported in livestock several years earlier (1973) [12]. Then, various studies have shown the persistence of *F. tularensis* circulation in different parts of Iran [1,11–15].

Slaughterhouse workers and butchers are at high risk of developing these three diseases through direct contact with infected animals and their tissues. Our study aimed at evaluating the seroprevalence of brucellosis, Q fever and tularemia among these occupational groups in the Lorestan province of Iran. Given that specific antibodies directed against these three pathogens can persist for years after infection [16–20], serological tests can provide appropriate information about the history of exposure of individuals to the corresponding pathogens.

2. Materials and methods

2.1. Study area

This cross-sectional study was carried out in 2017, in the Lorestan province in western Iran. This province has a total area of 28,308 km^2 and a population of more than 1,760,000 people. Khorramabad is the capital of the province. Lorestan is located in the middle of the Zagros Mountains. The study areas included all the Lorestan province counties: Aligudarz, Azna, Nurabad, Borujerd, Doroud, Khorramabad, Poldokhtar, Rumeshkhan, Kuhdasht, Alashtar, and Dowreh (Fig. 1, part I).

2.2. Sampling

Patients enrolled in the study included butchers and slaughterhouse workers (high-risk group), and people from the general population (control group). We used a stratified random sampling method (based on the county) to select individuals at high-risk for zoonotic diseases. For each county, the number of selected individuals was calculated according to the total population and the proportion of butchers and slaughterhouse workers. The control group included men over the age of 18 who visited the diagnostic laboratories for routine laboratory tests (e.g., blood glucose test). The same numbers of people were included in each of the two groups.

After obtaining informed consent from the participants, 6 ml of blood was collected form each person. Sera were extracted and kept at −20 C until they were transferred to the Pasteur Institute of Iran for serological testing.

The following information was collected for each participant: employment history; level of job satisfaction; contact with livestock species; perceived level of risk of exposure to zoonoses; observance of individual protection measures against zoonotic diseases; and level of knowledge concerning tularemia, brucellosis, and Q fever. The level of personal protective measures was assessed based on criteria such as wearing gloves and boots, and performing practices such as disinfecting hands and tools after work. In the statistical analysis, the borderline serological results for the three diseases evaluated (i.e. suspected cases) were considered negative.

This research was approved by the Ethics Committee of Lorestan University of Medical Sciences (IR.LUMS.REC.1397.008).

2.3. Serology

For detection of IgG antibodies against *F. tularensis*, serum samples were tested using the Tularemia ELISA kit (Virion\Serion GmbH, Germany) according to the manufacturer's instructions. Sera were tested with the commercial Q fever ELISA kit (Virion\Serion GmbH, Germany) for detection Phase II IgG antibodies against *C. burnetii*. For detection of IgG antibodies against *Brucella*, sera were tested with the commercial Brucellosis ELISA kit (IBL, Germany). For all three diseases, the results were classified into positive, borderline (suspected cases), or negative categories according to the ODs results, and based on manufacturer's instructions.

2.4. Statistical analysis

Statistical analyses were performed using SPSS software version 23. Descriptive analysis was performed for quantitative variables such as mean, standard deviation, median, and qualitative data such as frequency and percent. For inferential analysis, chi-square, Fisher's exact, and logistic regression tests were used. A p value less than 0.05 was considered statistically significant.

3. Results

In this study, 289 individuals (144 butcher and slaughterhouse workers, and 145 people from the general population) were enrolled from 11 counties of Lorestan province. All the participants were male. There was no significant difference in mean age (± SD) between the general population group (40.02 ± 13.43) and the butchers and slaughterers group (39.93 ± 10.01) (P = 0.94).

The overall seroprevalence of Q fever, brucellosis, and tularemia were 23.53%, 31.83%, and 3.81%, respectively (Table 1). The seroprevalence of Q fever among butchers and slaughterhouse workers (29.86%) was significantly higher than that of the general population (17.24%) (OR: 2.04, 95% CI: 1.17–3.58, P = 0.01). The seroprevalence of brucellosis was also higher in the butchers and slaughterhouse workers (43.75%) compared to the general population (20.00%) (OR: 3.11, 95% CI: 1.84–5.25, P = 0.001). In contrast, no significant difference was found between the two groups for the seroprevalence of tularemia (OR: 0.56, 95% CI: 0.16–1.97, P = 0.36).

For tularemia, there was no significant difference (P = 0.81) between the seroprevalence observed in the different counties of Lorestan province (Fig. 1, part II, A). The highest tularemia seroprevalence was observed in Aligudarz, Azna and Khorramabad counties. In contrast, there was a significant difference between counties regarding the seroprevalence of brucellosis and Q fever (P < 0.001). The highest seroprevalence for both diseases were found in Aligudarz and Khorramabad counties (Fig. 1, part II, B and C). The brucellosis seroprevalence

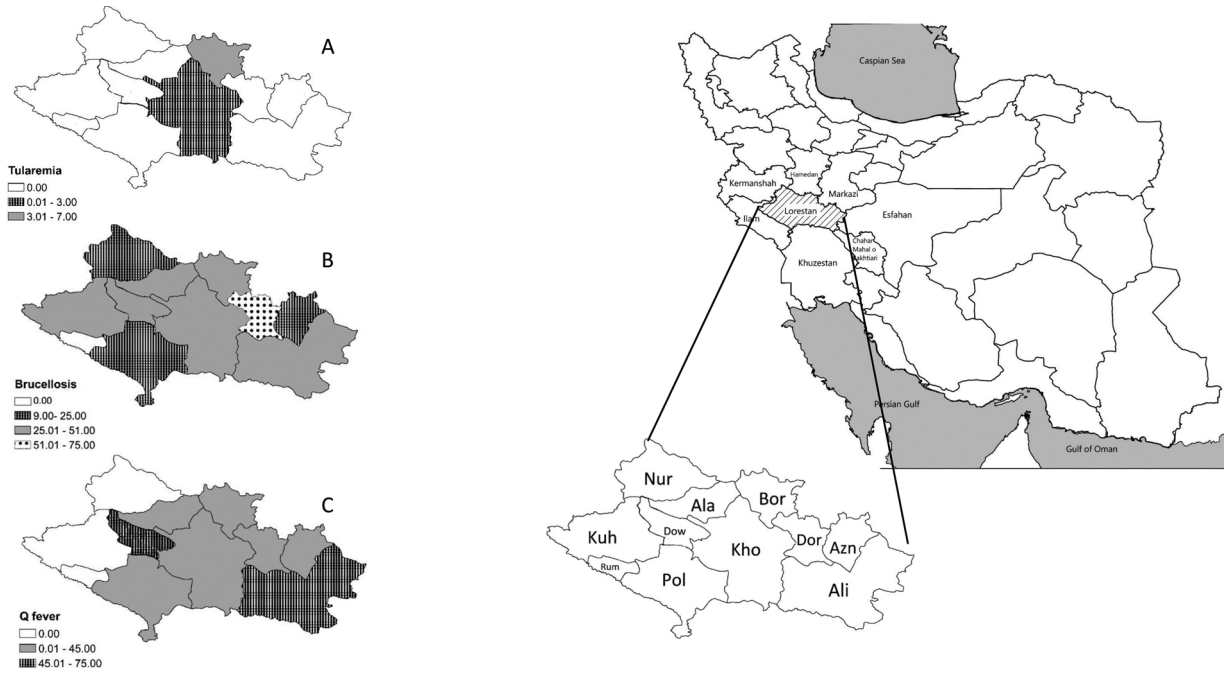

Fig. 1. Geographical location of Lorestan province and its counties in Iran. Nur: Nurabad; Kuh: Kuhdasht; Rum: Rumeshkhan; Pol: Poldokhtar; Dow: Dowreh; Ala: Alashtar; Bor: Borujerd; Dor: Doroud; Azn: Azna; and Kho: Khorramabad (I). Tularemia (part II A), brucellosis (part II B) and Q fever (Part II C) prevalence map.

was significantly higher in butchers and slaughterhouse worker in Borujerd, Doroud, and Alashtar counties (Table 2).

Having a work history of more than 15 years increased the risk of a positive serology for brucellosis (OR: 2.00, 95% CI: 1.02–3.90, p = 0.04). Contact with small ruminants (sheep and goats) increased the chance of seropositivity for all three diseases (Table 3).

4. Discussion

Butchers and slaughterhouse workers are particularly exposed to zoonotic agents due to their daily handling of animals and their derivatives. These occupational groups have a higher risk of exposure to zoonotic agents for which domestic animals are a major reservoir, such as brucellosis and Q fever. In the Lorestan province of Iran, we found significantly higher seroprevalences for these two diseases in these two occupational populations (43.75% and 29.86%, respectively) compared to the general population (20% and 17.24% respectively). These results likely indicate that Q fever and brucellosis are major occupational diseases in the Lorestan province of Iran.

The overall Q fever seroprevalence (23.53%) we found in the Lorestan province was inferior to that previously reported in a systematic review of cases observed in Iran (32.86%) [21]. The prevalence of Q fever in butchers and slaughterhouse workers (29.9%) was higher than that previously reported in Sistan-va-Baluchestan province (22.5%) [22], but inferior to those reported in Ilam (24.6%) [23], Kurdistan (38%) [24] and Kerman provinces (68%) [25] of Iran. Contact with small ruminants increased the risk of Q fever seropositivity.

During the large outbreak of Q fever occurring in the Netherlands, human infections were most frequently associated with dairy goats and sheep. It had been previously demonstrated that placenta and birth products from *C. burnetii*-infected goats and sheep contain high loads of bacteria [26]. According to our results, butchers and slaughterhouse workers are highly at risk of acquiring Q fever in the Lorestan province, and contact with infected sheep and goats is likely a major source of human infection. Therefore, control and prevention measures should be enhanced for individuals in contact with these animals, including by providing personal protective equipment at the workplace. Vaccination of sheep and goats can be a prevention method to reduce Q fever transmission by reducing the animal infection and abortion rates [27].

The seroprevalence of brucellosis among butchers and slaughterhouse workers (43.8%) of the Lorestan province was incredibly higher than those previously reported in similar studies in Hamadan (13.1%) [28], Kurdistan (12%) [24], Gilan (9.8%) [29], Fars (11.7%) [30], West-Azerbaijan (13%) [31], and Sistan-va-Baluchestan (7.9%) [22] Provinces. This observation is well correlated with the very high annual incidence of brucellosis (31–41 cases per 100,000 people) reported in the Lorestan province compared to the rest of Iran [32]. In the present study, having a work history of more than 15 years as a butcher or slaughterer increased the risk of brucellosis seropositivity. This could be explained by long-term occupational exposure to *Brucella* species, but also by long-term persistence of anti-*Brucella* antibodies for up to 10 years after infection [33].

Within the Lorestan province, the prevalence of both Q fever and brucellosis was higher in Khorramabad and Aligudarz counties than in

Table 1
Seroprevalence of tularemia, brucellosis and Q fever in the general population and high-risk group (butchers and slaughterhouse workers) in Lorestan province, 2017.

	Tularemia		Brucellosis		Q fever	
	General Pop.	High risk pop.	General Pop.	High risk pop.	General Pop.	High risk pop.
Negative (%)	134 (92.4)	135 (93.8)	108 (74.5)	70 (48.6)	98(67.58)	80 (55.6)
Positive (%)	7 (4.8)	4(2.78)	29 (20.0)	63 (43.8)	25 (17.2)	43 (29.9)
Borderline (%)	4 (2.8)	5 (3.5)	8 (5.5)	11 (7.6)	22 (15.2)	21 (14.6)

Table 2
Seroprevalence of tularemia, brucellosis and Q fever in the general population and high-risk group (butchers and slaughterhouse workers) in different counties of the Lorestan province, 2017.

	Tularemia No of positive result (% Pos.)			Brucellosis No of positive result (% Pos.)			Q fever No of positive result (% Pos.)		
	HRG	GP	Total	HRG	GP	Total	HRG	GP	Total
Aligudarz (N = 16)	0 (0.00)	1 (12.50)	1 (12.50)	3 (37.50)	6 (75.00)	9 (56.25)	6 (75.00)	2 (25.00)	8 (50.00)
Azna (N = 8)	0 (0.00)	1 (25.00)	1 (12.50)	1 (25.00)	1 (25.00)	2 (25.00)	1 (25.00)	0 (0.00)	1 (12.50)
Nurabad (N = 8)	0 (0.00)	0 (0.00)	0 (0.00)	1 (25.00)	0 (0.00)	1 (12.50)	0 (0.00)	0 (0.00)	0 (0.00)
Borujerd (N = 93)	3 (6.38)	1 (2.17)	4 (4.93)	21 (44.68)	5 (10.87)	26 (27.96)	11 (23.40)	5 (10.87)	16 (17.20)
Doroud (N = 25)	0 (0.00)	1 (7.69)	1 (4.00)	9 (75.00)	0 (0.00)	9 (36.0)	4 (33.33)	1 (7.69)	5 (20.00)
Khorramabad (N = 66)	1 (3.03)	3 (9.09)	4 (6.06)	17 (51.52)	15(45.45)	32 (48.48)	15(45.45)	15(45.45)	30 (45.45)
Poldokhtar (N = 23)	0 (0.00)	0 (0.00)	0 (0.00)	1 (9.09)	0 (0.00)	1 (4.35)	2 (18.18)	0 (0.00)	2 (8.70)
Rumeshkhan (N = 8)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (25.00)	1(12.5)
Kuhdasht (N = 12)	0 (0.00)	0 (0.00)	0 (0.00)	3 (50.00)	1 (16.67)	4 (33.33)	0 (0.00)	0 (0.00)	0 (0.00)
Alashtar (N = 22)	0 (0.00)	0 (0.00)	0 (0.00)	6 (50.00)	0 (0.00)	6 (27.27)	2 (16.67)	1 (10.00)	3 (13.64)
Dowreh (N = 8)	0 (0.00)	0 (0.00)	0 (0.00)	1 (33.33)	1 (20.00)	2 (25.00)	2 (66.67)	0 (0.00)	2 (25.00)

HRG: high risk group, GP: General population (slaughterhouse worker and butcher).

Table 3
Risk Factors of seropositivity for tularemia, brucellosis and Q fever among butchers and slaughterhouse workers in Lorestan province, 2017.

		Tularemia		Brucellosis		Q fever	
	Total	No (%)	OR (95% CI)	No (%)	OR (95% CI)	No (%)	OR (95% CI)
Age(median = 38 years)							
< 38	66	1 (1.51))	1.54(0.44-5.37)	28 (42.42)	1.11(0.57-2.14)	21 (31.82)	0.82 (0.41-1.72)
38≤	78	3 (3.85)		35 (44.87)		22 (28.21	
Work history (median = 15 years)							
< 15	71	1 (1.41)	3.00(0.31-29.55)	25 (35.21)	2.00 (1.02-3.90)	20 (28.17)	1.17 (0.57-2.40)
15≤	73	3 (4.11)		38 (52.05)		23 (31.51)	
Work place							
Traditional slaughterhouse							
No	99	4 (4.04)	0.96(0.92-0.99)	42 (42.42)	1.19(0.59-2.41)	32 (32.32)	0.67(0.30-1.51)
Yes	45	0 (0.00)		21 (46.67)		11 (24.44)	
Industrial slaughterhouse							
No	88	2(2.27)	1.59(0.22-11.64)	33(37.50)	1.92(0.98-3.80)	21 (23.86)	2.06(0.99-4.27)
Yes	56	2(3.57)		30(53.57)		22(39.29)	
Butcher shop							
No	55	2(3.64)	0.61(0.08-4.45)	35(63.64)	0.26(0.13-0.53)	24(43.64)	0.35(0.17-0.73)
Yes	89	2(2.25)		28(31.82)		19(21.35)	
Contact with animals							
Cattle							
No	34	1 (2.94)	0.93(0.09-9.20)	16(47.06)	0.84(0.39-1.82)	13(38.24	0.61(0.27-1.36)
yes	110	3 (2.73)		47(42.73)		30(27.27)	
Sheep and goat							
No	38	0(0.00)	1.04(1.00-1.08)	11(28.95)	2.36(1.06-5.25)	3 (7.89)	7.07(2.04-24.50)
yes	106	4 (3.77)		52(49.06)		40(37.74)	
Camel							
No	134	4 (2.99)	0.97(0.94-1.00)	58(43.28)	1.30(0.36-4.74)	42(31.34)	0.24(0.03-1.98)
yes	10	0 (0.00)		5 (50.00)		1 (10.00)	
Cutting hand							
≤5 times	74	3 (4.05)	0.34(0.04-3.38)	30 (40.54)	1.31(0.68-2.53)	20 (27.03)	1.32(0.65-2.70)
> 5 times	70	1 (1.43)		33 (47.14)		23 (32.85)	
Ectoparasite bite							
≤5 times	119	2 (1.68)	5.09(0.68-37.98)	52 (43.70)	1.01(0.43-2.41)	33(27.73)	1.74(0.71-4.25)
> 5 times	25	2 (8.00)		11 (44.00)		10(40.00)	
Personalprotection (median = 21)							
< 21	70	0 (0.00)	1.06 (1.00-1.12)	29(41.43)	1.20 (0.62-2.32)	24 (34.29)	0.66(0.32-1.36)
< 21	74	4 (5.41)		34 (45.95)		19 (25.68)	
Job satisfaction							
No	29	2 (1.74)	4.19 (0.56-31.06)	16 (55.17)	1.78 (0.78-4.05)	11 (37.93)	1.59 (0.68-3.72)
Yes	115	2(6.90)		47 (40.87		32(27.83)	
Find themselves at risk of zoonotic disease							
No	56	1 (1.79)	0.52(0.05-5.08)	24 (42.86)	0.94(0.48-1.85)	13 (23. 21)	0.58(0.27-1.25)
Yes	88	3 (3.41)		39 (44.32)		30 (34.09)	

other counties. A previous study also reported Aligudarz County as one of the high-risk counties for these diseases in the Lorestan province [34]. In addition, a high annual incidence of human brucellosis cases was reported in the Kuhrang county of the Chaharmahal and Bakhtiari province [35], which has a common border with Aligudarz county of Lorestan province. Therefore, preventive measures such as livestock vaccination, increased public awareness about the risk associated with the consumption of unpasteurized dairy products, and personal protective measures need to be seriously strengthened in this area, especially in the high-risk population.

The seroprevalence of tularemia in butchers and slaughterhouse workers of Lorestan province was 2.8%, which is lower than that previously reported in the same occupational groups in Kurdistan (16.0%) [11] and Sistan-va-Baluchestan provinces (6.5%) [12]. The prevalence of tularemia was not significantly different between the butchers and slaughterhouse workers group, and the general population. This observation is consistent with a previous study [11], but different from other studies showing butchers and slaughterhouse workers as one of the most at-risk populations for tularemia [14]. We found that contact with sheep and goats increased the chance of tularemia seropositivity. However, a previous study in the endemic region of Georgia showed that contact with livestock were not associated with tularemia seropositivity [36]. It is to be noted that *F. tularensis* animal reservoir is primarily represented by wildlife animals, including game animals (especially lagomorphs). Butcher and slaughterhouse workers are more likely to be infected if they handle these latter animals.

In conclusion, our study demonstrates that brucellosis and Q fever are two significant occupational risks for butchers and slaughterhouse workers in the Lorestan province. Providing personal protective equipment at the workplace and educational programs facilitating diagnosis and prevention of these diseases are warranted. Sheep and goat vaccination against these two diseases is another effective way for reducing this zoonotic risk. Because we observed that contact with sheep and goats was associated with an increased risk of positive serology for all three studied zoonotic diseases, further studies are recommended to better determine the prevalence of these zoonoses in this specific livestock population of the Lorestan province. Therefore, the collaboration of the veterinary Organization to reduce the incidence of these common diseases is necessary.

Declaration of Competing Interest

The authors declare that they have no competing financial interests.

Acknowledgements

This study was approved and supported by Pasteur Institute of Iran (grant no. 941) and Lorestan University of Medical Sciences. We want to express our gratitude to the staff of Lorestan University of Medical Sciences for their support in sample collection.

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