

## High seroprevalence of *Toxoplasma gondii* and HIV-1 co-infection among drug users in Yunnan province, southwest China

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Dear Editor,

*Toxoplasma gondii* was discovered more than 100 years ago and has a broad-spectrum of intermediate hosts in addition to its definitive host, felids. Although it does not cause symptomatic illness in most adults, it can lead to mental retardation in congenitally infected children and serious diseases in immunocompromised patients (Hill et al., 2005). The first report of *T. gondii* infection in China was published in Chinese in 1964; subsequently, many other cases have been carried out. Currently, most publications about the seroprevalence of *T. gondii* infection in Yunnan have examined infections in pets, domestic animals, and wild animals; however, few previously published studies have assessed human *T. gondii* infection in Yunnan province.

From March to December 2009, a voluntary cross-sectional survey was carried out among all drug users that our facility could follow in communities and detoxification centers in Qujing, Lincang, Zhaotong, Baoshan, and Dehong prefectures in Yunnan province, Southwest China. After obtaining written informed consent, an anonymous questionnaire-based interview was performed and basic socio-demographic information was acquired. Additionally,

5 mL venous blood was collected in vacuous tubes with added EDTA-2K. Plasma was extracted by centrifugation and stored at  $-80^{\circ}\text{C}$  for subsequent analyses. Anti-*T. gondii* IgG antibodies were screened with diagnostic enzyme-linked immunosorbent assay (ELISA) kits (Zhuhai S.E.Z Haitai Biological Pharmaceuticals Co., Guangzhou), and *T. gondii* IgG-positive tests were confirmed using another diagnostic ELISA kit (Tangshan INNOVITA Biotechnology Co., Tangshan). Anti-HIV antibodies were assessed using diagnostic colloidal gold kits (Alere Medical Co., Japan), and the HIV-positive tests were confirmed using diagnostic ELISA kits (Beijing Wantai Biological Pharmacy Enterprise Co., Beijing). Specimens with positive results in both assays were considered to be positive for *T. gondii*/HIV. All tests were performed according to the manufacturer's instructions.

Data obtained from questionnaires and previous laboratory tests were analyzed using the software package Statistical Package for Social Sciences (version 22.0; SPSS Inc., USA). Categorical variables were compared using  $\chi^2$  tests; all tests were two tailed and differences between means with *P*-values less than 0.05 were considered to be significant.

A total of 1,414 drug users were recruited from the following five prefectures of Yunnan Province: Qujing ( $n=151$ ), Lincang ( $n=166$ ), Zhaotong ( $n=295$ ), Baoshan

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( $n=443$ ), and Dehong ( $n=359$ ). The age of these participants ranged from 13 to 68 years old, with a median age of  $31\pm 10$  years old. Among these drug users, most were males of Han ethnicity, and approximately half were farmers, unmarried, and had more than six-years of school education. Notably, 22.2% of these individuals were minorities, including Achang, Bai, Zang, Dai, Deang, Hui, Jingpo, Lisu, Man, Mian, Miao, Tujia, Yi, and Zhuang (Table 1).

Among our cohort of 1,414 drug users, 31.6% were found to be seropositive for anti-*T. gondii* IgG antibodies, and the seroprevalence of *T. gondii* varied markedly in different stratified groups (Table 1). Drug users who were living in the China-Myanmar border region (Baoshan and Dehong) had a much higher percentage of *T. gondii* infection than those living in other prefectures ( $P<0.001$ ). Elder drug users had a higher risk of *T. gondii* infection than younger ones ( $P=0.007$ ), and a similar phenomenon could be observed among illiterate versus well-educated individuals ( $P=0.015$ ). Although the sample size ( $n=62$ ) of female

drug users was small, the rate of infection with *T. gondii* among male drug users was significantly higher ( $P=0.001$ ). Moreover, *T. gondii* seroprevalence among minorities was significantly higher than in Han Chinese (49.5% vs. 26.4%,  $P<0.001$ ), and was higher among farmers than those with other jobs or who were unemployed. Conversely, there were no significant differences between drug users who were unmarried, married, cohabitated, divorced, or widowed, suggesting that sexual intercourse was not the transmission route of *T. gondii*.

Furthermore, 24.2% (342/1,414) of all participants were found to be seropositive for HIV antibodies. The percentage of *T. gondii* infection among HIV-positive drug users was significantly lower than among HIV seronegative drug users (24.6% vs. 33.9%,  $P=0.001$ ; Table 1).

Prior to 2008, the seroprevalence of *T. gondii* in China ranged from 0.4% to 20.2% in blood donors, from 17.3% to 21.8% in intravenous drug users, and from 0.5% to 25.5% in pregnant women (Zhou et al., 2011). In this present

**Table 1** *Toxoplasma gondii* infection characteristics among drug users in Yunnan, China<sup>a)</sup>

Variable	Total		<i>Toxoplasma</i> seropositive		$\chi^2$ value	P-value
	N	%	n	%		
Residence					113.904	<0.001
Qujing	151	10.7	19	12.6		
Lincang	166	11.7	25	15.1		
Zhaotong	295	20.9	63	21.4		
Baoshan	443	31.3	167	37.7		
Dehong	359	25.4	173	48.2		
Gender					10.542	0.001
Female	62	4.4	8	12.9		
Male	1350	95.6	439	32.5		
Age					14.169	0.007
≤25	324	22.9	103	31.8		
26–30	341	24.1	96	28.2		
31–35	370	26.2	103	27.8		
36–40	227	16.1	81	35.7		
>41	151	10.7	64	42.4		
Ethnicity					60.242	<0.001
Han	1098	77.8	290	26.4		
Minority*	313	22.2	155	49.5		
Occupation					63.097	<0.001
Jobless	513	37.8	106	20.7		
Farmer	632	46.6	268	42.4		
Others**	212	15.6	60	28.3		
Marital status					1.210	0.751
Unmarried	627	44.3	189	30.1		
Married	564	39.9	184	32.6		
Cohabitated	55	3.9	19	34.5		
Divorced or widowed	168	11.9	55	32.7		
Education					10.497	0.015
None	100	7.1	41	41.0		
1–5 years	508	36.0	176	34.6		
6–9 years	625	44.3	175	28.0		
≥10 years	178	12.6	52	29.2		
HIV serostatus					10.374	0.001
Negative	1072	75.8	363	33.9		
Positive	342	24.2	84	24.6		

a) \*, Achang, Bai, Zang, Dai, Deang, Hui, Jingpo, Lisu, Man, Mian, Miao, Tujia, Yi, Zhuang; \*\*, Cook, Electrician, Cooker, Waiter, Government staff, Businessman, Nurse, Worker, Chauffeur, Builder, Hotel manager, Miner, Carpenter, Repairman, Salesman, Doctor.

study, 31.6% of drug users were seropositive for *T. gondii*, which was much higher than the aforementioned results. As *T. gondii* could not transmit via blood or intercourse, the epidemic of *T. gondii* among drug users might suggest an increasing trend of *T. gondii* infection in China. Moreover, the seroprevalence of *T. gondii* in different prefectures of Yunnan province ranged from 12.6% to 48.2%, which might reflect an epidemic status of *T. gondii* infection in some provinces of China. Together, our findings indicate that a nationwide survey is needed to monitor the exact seroprevalence of *T. gondii* infection in China.

As such a high percentage of drug users were infected with *T. gondii*, targeted interruptions were immediately needed to prevent toxoplasmosis and new infections. Based on our present study, well-educated drug users had a lower percentage of *T. gondii* infection, implying that education was an effective measure for reducing *T. gondii* infection. Additionally, education or public health programs should focus on the elderly male minorities who are doing farm-related jobs in the China-Myanmar border region, as they were the most badly impacted by *T. gondii*.

Drug users and HIV-positive patients have been considered to be prone to *T. gondii* infection because they have suppressed or damaged immune systems; however, this opinion has been the subject of debate. Previous studies have shown that the seroprevalence of *T. gondii* was not significantly different between HIV-positive and -negative illicit drug users in Iran (Alavi et al., 2013). In this present study, the rate of *T. gondii* infection among HIV-positive drug users was lower than among HIV-negative drug users, which was likely the reason that HIV infection showed no association with *T. gondii* infection. Moreover, few sites were sampled where the rates of HIV prevalence were high and those of *T. gondii* seroprevalence were low. Together, these findings indicated that immune status showed no association with *T. gondii* infection.

Although drug users and HIV-positive patients were not found to be prone to *T. gondii* infection, they were prone to toxoplasmosis if they had been infected by *T. gondii*. Previous studies showed that latent *T. gondii* infection would modulate immune responses during HIV infection, and showed a trend for reactivation among some HIV/*T. gondii* co-infected individuals; for example, in the form of toxoplasmic encephalitis (Beran et al., 2015; Kodym et al., 2015). In this present study, 447 drug users were found to be seropositive for *T. gondii* IgG antibodies, including 84 HIV/*T. gondii* co-infected individuals. Although it was difficult to follow-up previous participants, we attempted to the best of our ability to warn them about the risk of reactivation of latent *T. gondii* infection.

In this present study, although our findings were drawn from a large sample size, there were two main limitations. First, no factors about diet culture were included in the

questionnaire. The authors knew that diet culture was a key factor in studies of *T. gondii*, and we had included this in our recent studies; unfortunately, in this present study, we could not provide this information because the primary purpose of sample collection was for HIV research, and re-interviewing these participants was impossible. These factors may not limit the main conclusions of this study, that a high prevalence of *T. gondii* infection exists in China and this demands more efforts to prevent it. Second, the findings drawn by this present study might be influenced by the partially answered questionnaires of some participants. Based on our statistical analyses, the seroprevalence of *T. gondii* showed no significant difference between the completely and partly answered questionnaires (31.9% ( $n=1351$ ) vs. 25.4% ( $n=63$ ),  $P=0.278$ ), indicating that this influence was likely negligible.

In conclusion, from our cross-sectional survey of 1,414 drug users in Yunnan Province, a much higher percentage of *T. gondii* infection was detected when compared with previously reported results from China. Further adequate studies and interruptions will be urgently needed to prevent the growing epidemic of *T. gondii*.

**Compliance and ethics** The author(s) declare that they have no conflict of interest.

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**Ethical approval** The protocol of this study was approved by the Ethics Committee of Kunming Institute of Zoology, Chinese Academy of Sciences (approval number: SWYX-2009021; approval date: January 7, 2009), and informed consent was obtained from all individual participants included in the study.

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