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Immunogenicity, immune persistence, and safety of japanese encephalitis vaccine schedules among adults in Ningxia, China

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Live attenuated and vero-cell-inactivated Japanese Encephalitis vaccines (LJEV, IJEV) have been in common use in young children in China since 1989 and 2004, associated with large reductions in Japanese encephalitis (JE) incidence. In 2013, northern China reported JE outbreaks among adults born before JE vaccine availability, a trend that worsened in 2017–2018. We conducted an open-label, randomized, controlled trial (ChiCTR2500103235) to assess the immunogenicity, immune persistence, and safety of three JE vaccine schedules in 40–69-year-olds to provide evidence for adult targeted JE immunization efforts. Outcomes were seroconversion proportions and seropositive prevalences; adverse events were monitored. The vaccines were immunogenic with no significant difference between vaccination groups. Seropositivity remained above 80% at one year post-vaccination. No serious adverse events occurred. All three schedules had good, persistent immunogenicity and favorable safety profiles in 40–69-year-old adults, providing evidence supporting vaccinating adults in response to the emergence of adult JE in northern China.

Japanese encephalitis (JE) is a vector-borne zoonotic disease that is transmitted by *Culex* mosquitoes and can cause severe illness with high risk of long-term sequelae and a high case fatality rate, especially among children[1]. JE is seen in the Western Pacific and Southeast Asia World Health Organization (WHO) regions and is an important cause of viral encephalitis in Asia. JE has been recognized as an emerging infectious disease in several countries, notably Australia and Nepal, occurring in epidemics[2–4]. Through mosquito monitoring and JE surveillance, countries have found Genotype V JE virus circulating, which may cause new threats[5].

JE is vaccine-preventable, and JE vaccine is the most effective and economical measure to prevent JE, as has been shown by rigorous evaluations of JE vaccination of children[6,7]. There are two types of JE vaccine licensed in China - one is a live, attenuated, SA14-14-2 strain JE vaccine (LJEV), manufactured in China and prequalified by WHO since October 2013. LJEV is used in China and other Asian countries, including Nepal[8,9], South Korea[10,11], and Sri Lanka[12,13]. The other JE vaccine is an inactivated, Vero cell-derived P3 JE vaccine (IJEV) that is also manufactured and used in China and has been exported to Thailand[14,15]. In addition, several JE vaccines developed outside China, such as the inactivated Vero cell-derived SA 14-14-2 vaccine (IXIARO®)[16], and a live attenuated chimeric JE vaccine, have been used in both children and adults[17]. These vaccines provide important references for JE vaccination strategies globally.

China introduced JE vaccine into the National Immunization Program (NIP) at the end of 2007 with implementation starting in 2008. LJEV is given in a two-dose schedule at 8 months and 2 years of age. IJEV is given in four doses, with two doses at 8 months with a 7–10-day interval, followed by doses at two and six years of age. Since introduction into the national program, the incidence of JE in children under 15 years of age declined markedly, from 2.59/100,000 in 2006 to 0.083/100,000 in 2019[18–20].

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In recent decades, however, JE is becoming an increasingly important illness in adults. In some endemic areas, JE has become relatively more prominent in adults because of improved JE control in children brought about by childhood immunization programs[21,22], such as in South Korea[23,24], Japan[25], and India[26].

In China in 2006, JE outbreaks began to be reported among adults in northern provinces[27]. In 2013, adult JE outbreaks increased[18]—a trend that accelerated in 2017 and 2018 when more than ten northern provinces reported high numbers adult JE cases and outbreaks in which 80% of cases were among adults over 40 years of age[19]. The incidence of JE in adults has increased[28,29]. For example, in Ningxia province during 2008–2017, two cases were reported in 2010, and five cases were reported in 2017. In the summer of 2018, however, Ningxia had a major outbreak with 162 cases and 31 deaths, in which 90% (146/162) of the cases were among adults over 40 years of age[28,30,31].

National annual numbers of cases, ranging from 125 in 2024 to 1800 in 2018, were reported during 2018–2024, with the incidence showing a decreasing trend peaking at 0.13 per 100,000 population in 2018, and down to the lowest level of 0.0089 per 100,000 population in 2024. In 2019, we conducted a vaccination campaign among 40–70-year-old and 40–75-year-old adults in parts of Ningxia and Gansu using one dose of LJEV before the epidemic season. There have been no outbreaks in China since 2019.

The emergence of adult JE cases, following many years of routine JE vaccination of children, raises the question of using JE vaccines to protect adults. However, there is little experience with JE vaccination of adults. Our study explored the immunogenicity, immune-persistence, and safety of JE vaccination in adults. We evaluated seroconversion proportions, seropositive prevalences, and safety of three JE vaccination schedules in adults, 40–69 years of age living in a rural area: a single dose of LJEV, two doses of IJEV in a 7-day interval, and two doses of IJEV in a 28-day interval, compared with an unvaccinated control group. We report results of our study.

Results

Baseline characteristics

From May 20 to 31, 2020, 648 potential participants were screened for eligibility and were recruited; 628 (96.91%) consented to participate, 471 to the vaccination groups and 157 to the control group. Among subjects in the vaccination groups, 161 were assigned to Group LJEV, 158 to Group IJEV-IJEV 7-day interval, and 152 to Group IJEV-IJEV 28-day interval. Upon enrollment, baseline blood samples were drawn and after blood draw, participants assigned to a vaccination group received group-specific protocol vaccine. All participants completed 1-month and 1-year visits to assess safety and immune-persistence. Second and third blood samples were obtained from 620 participants; fourth blood samples were obtained from 618 participants (Fig. 1). Participant age, sex, and timing of blood draws and vaccinations are shown in Table 1. There were no significant differences among the four groups in age and sex.

Baseline antibody titers, after a 5-fold dilution, ranged from 1:5 to 1:160 among study participants, and baseline seropositive prevalence was 55.86%. By study group, SPPs for LJEV, IJEV-IJEV 7-day interval, IJEV-IJEV 28-day interval, and control were 50.00% (80/160), 54.84% (85/155), 61.84% (94/152) and 57.05% (89/156), respectively. The baseline mean GMT and 95% confidence interval (CI) was 12.20 (95%CI: 11.30–13.18), ranging from 11.59–12.85 by study group, with no statistically significant differences by group ($F = 0.29$, $P = 0.83$) .

Immunogenicity outcomes

Twenty-nine days after completion of a single dose of LJEV or two doses of IJEV, and on day 58 after enrollment in the control group, the SCPs for Group LJEV, Group IJEV-IJEV 7-day interval, Group IJEV-IJEV 28-day interval, and Group Control were 39.87%, 37.75%, 39.86% and 10.97%, respectively; SPPs were 79.75%, 80.79%, 85.81%, and 65.16%, respectively; GMTs were 25.13, 30.23, 27.92 and 15.50, respectively. Immunogenicity outcomes are shown in Table 2, Table 3 and Fig. 2.

There were no significant differences among the three vaccinated groups, and the three vaccinated groups all had higher SCPs, SPPs, and GMTs than the control group (all $P < 0.0001$).

Seroconversion proportions were determined for one dose of both vaccines. Eight days after the first dose in Group LJEV and Group IJEV-IJEV 7-day interval, SCPs were 25.64% and 23.65%, respectively; SPPs were 68.59% and 75.68%; and GMTs were 20.20 and 21.66. For Group LJEV and Group IJEV-IJEV 28-day interval, on day 29, after one dose in these two groups, respective SCPs were 39.87% and 28.57%; SPPs were 79.75% and 81.63%; and GMTs were 25.13 and 21.98 (Table 2). There was no significant difference in SCP and GMT on day 8 between one dose of LJEV and IJEV. SCPs of one dose of LJEV on day 29 was statistically significantly higher than one dose of IJEV ($P = 0.034$), as were the GMTs ($P = 0.014$).

Seropositivity and seroconversion were determined by age group. Baseline SPPs among individuals aged 40-49, 50-59, and 60-69 years old were 43.28%, 56.60%, and 67.14%, respectively. Trend test ($Z = -4.8656$, $P < 0.0001$) indicates a significant trend in SPP by age group. Corresponding GMTs were 9.40, 12.82, and 14.19. For Group LJEV, respective SCPs after 29 days were 56.86%, 38.89%, and 24.53% among individuals aged 40–49, 50–59, and 60–69 years old; SPPs were 78.43%, 81.48%, and 79.25%. For Group IJVE-IJEV 7-day interval, SCPs after 29 days were 44.90%, 37.5%, and 31.48% among individuals aged 40–49, 50–59, 60–69 years old; SPPs were 81.63%, 77.08% and 80.79%. For Group IJEV-IJEV 28-day interval, SCPs after 29 days were 46.81%, 50.00%, and 23.53% among individuals aged 40–49, 50–59, 60–69 years old; SPPs were 85.11%, 90.00% and 82.35%. For the control group, SCPs on day 58 were 16.98%, 5.77% and 10.00% among individuals aged 40–49, 50–59, 60–69 years old; SPPs were 45.28%, 75.00% and 76.00%, respectively. Age-group results are shown in Table 2, Table 3 and Fig. 2. GMTs by time are shown in Table 3.

Immune persistence was assessed at one year post vaccination or enrollment (for the control group). Seropositive proportions at one year for Group LJEV, Group IJEV-IJEV 7-day interval, Group IJEV-IJEV 28-day interval, and control group were 81.29%, 81.69%, 85.91% and 69.43%, respectively; corresponding GMTs were 25.13, 30.23, 27.92 and 15.50. Vaccinated groups were significantly higher than the unvaccinated control group ($P < 0.001$).

Seroconversion proportions by baseline serostatus (seropositive and seronegative) were assessed in a post-hoc analysis. For Group LJEV, SCP at day 8 post-vaccination was 37.66% for pre-vaccination seronegative subjects and 14.10% for pre-vaccination seropositive subjects, and at day 29 post-vaccination, respective SCPs were 60.26% and 20.25%, respectively. For Group IJEV-IJEV 7-day interval, SCP at day 8 after the first dose was 43.75% for pre-vaccination seronegative subjects and 8.64% for seropositive subjects, and at 29 days after the second dose, respective SCPs were 54.69% and 26.19%. For Group IJEV-IJEV 28-day interval, SCP at day 29 after the first dose was 51.79% for pre-vaccination seronegative and 14.29% for seropositive subjects, and 29 days after the second dose, respective SCPs were 62.50% and 26.09%. For the control group, SCPs at day 29 for pre-seronegative and seropositive subjects were 15.38% and 3.37%, respectively, while at day 58 they were 19.40% and 4.60%. Results are shown in Fig. 3. Statistically significant differences were observed in SCP between pre-vaccination seronegative and pre-vaccination seropositive subjects across all four groups (for the control group, pre-vaccination corresponds to day 0, and post-vaccination corresponds to day 29 or 58).

Safety

The vaccines used in this study were well tolerated, and no severe adverse events or deaths were reported. For groups LJEV, IJEV-IJEV 7-day interval, IJEV-IJEV 28-day interval, 2.01%, 2.80% and 0.71%, had adverse events after the first dose. After a second dose of IJEV, 2.27% and 0 had adverse events (Table 4). The most common adverse events were local injection site reactions including induration, redness, pain, and swelling.

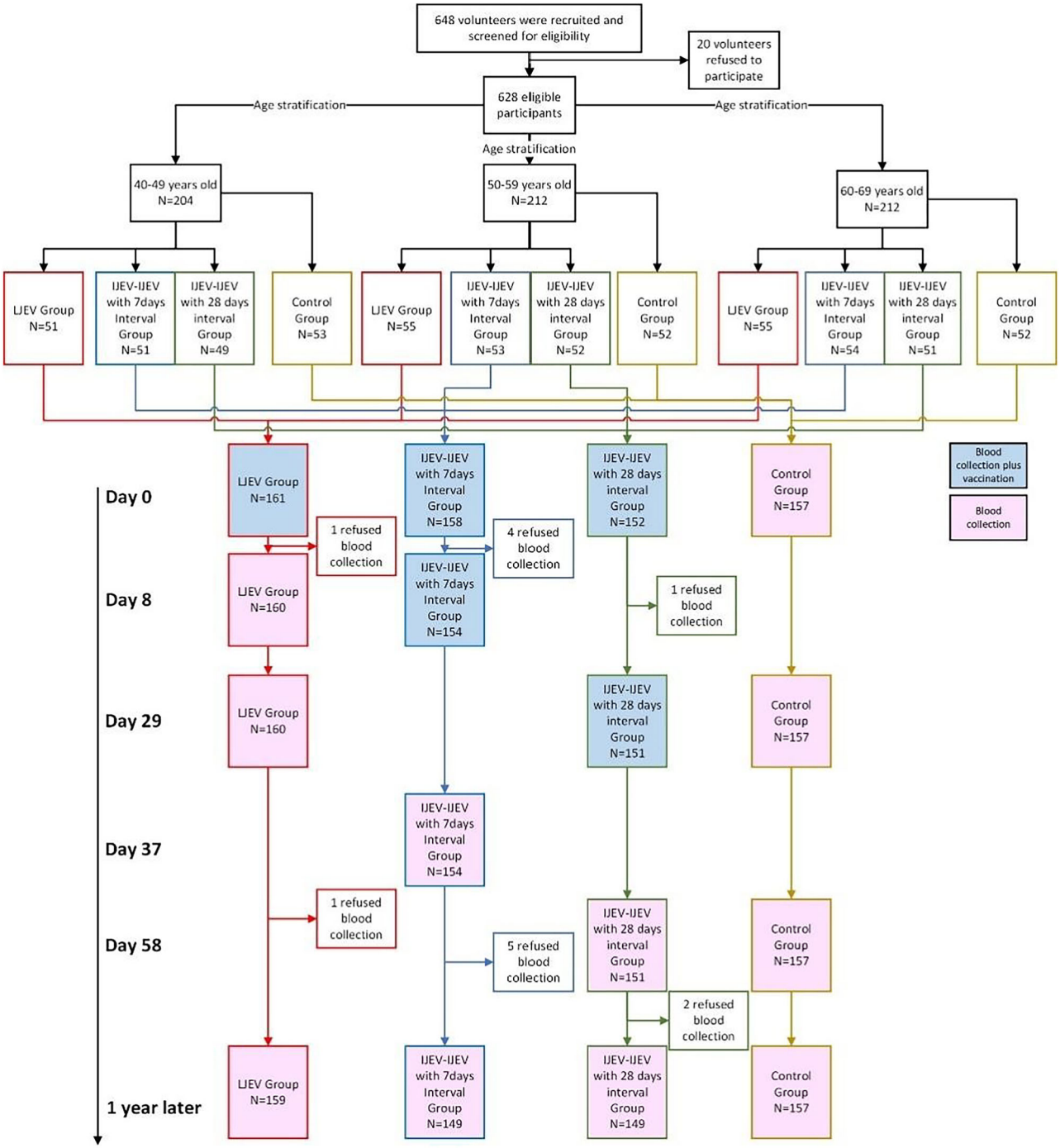

Fig. 1 | Trial profile. This figure represents the flow of participants through the clinical trial evaluating various vaccination strategies, with stratification by age group and allocation into four groups. Each group is represented by a different color frame for clarity: Group LJEV (red), Group IJEV-IJEV with 7-day interval (blue), Group IJEV-IJEV with 28-day interval (green), and Group Control (yellow). The black arrow on the left side and the color-coded lines guide the reader through the timeline and of interventions and assessments.

Discussion

Our randomized, open-label, controlled clinical trial found that in 40–69-year-old adults with a baseline JE seropositivity of 56%, seroconversion proportions 29 days after receiving one dose of LJEV or two doses of IJEV given at either a 7-day or 28-day interval were similar, with no statistically significant differences by schedule. Among baseline seronegative individuals, seroconversion proportions in the three schedules were similar, ranging from 55% to 63%. One year after vaccination, seropositive proportions were sustained above 80% in the three vaccinated groups, significantly higher than the unvaccinated control group at 65%. Both live and inactivated vaccines were well tolerated in the three schedules, and no vaccine-related serious adverse events were reported. Study results provide evidence that vaccination of adults against JE can safely raise population immunity and may therefore have a role in mitigating the contemporary emergence of adult JE cases and outbreaks.

Our study was set in Ningxia, which had an outbreak of JE primarily among adults in 2018, two years prior to our study. The baseline seropositivity in the adults in our study was 56%, indicating that much of the

Table 1 | Characteristics of participants and vaccination status

	LJEV group	IJEV-IJEV with 7 days interval group	IJEV-IJEV with 28 days interval group	Control group
N	161	158	152	157
Age (years)				
mean ± SD	55.00 ± 8.63	54.37 ± 9.33	54.82 ± 8.10	54.29 ± 9.56
Age groups (years)				
40–49 [*n* (%)]	51 (31.68)	51 (32.28)	49 (32.24)	53 (33.76)
50–59 [*n* (%)]	55 (34.16)	53 (33.54)	52 (34.21)	52 (33.12)
60–69 [*n* (%)]	55 (34.16)	54 (34.18)	51 (33.55)	52 (33.12)
Gender				
Male [*n* (%)]	56 (34.78)	56 (35.44)	68 (44.74)	49 (31.21)
Female [*n* (%)]	105 (65.22)	102 (64.56)	84 (55.26)	108 (68.79)
Interval between doses (days)				
median (min, max)	0 (0, 0)	8 (7, 8)	29 (8, 30)	0 (0, 0)
Days from the first dose to the first blood sample				
median (min, max)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Days from the first dose to the second blood sample				
median (min, max)	8 (8, 8)	8 (8, 8)	29 (8, 30)	21 (21, 21)
Days from the last dose to the third blood sample				
median (min, max)	29 (29, 29)	29 (21, 30)	29 (28, 29)	29 (29, 29)
Days from the last dose to the fourth blood sample				
median (min, max)	510 (510, 519)	502 (502, 511)	481 (480, 503)	482 (481, 492)

Table 2 | Seropositive prevalences and seroconversion proportions at different time points before and after immunization in different age groups and total participants

Groups	Sampling time points	40–49 years old			50–59 years old			60–69 years old			Total		
		N	No. of SP[a] (%)	No. of SC[b] (%)	*N*	No. of SP[a] (%)	No. of SC[b] (%)	*N*	No. of SP[a] (%)	No. of SC[b] (%)	*N*	No. of SP[a] (%)	No. of SC[b] (%)
LJEV	Day 0	51	18(35.29)	—	55	27(49.09)	—	54	35(64.81)	—	160	80(50.00)	—
	Day 8 after Dose1	49	28(57.14)	13(26.53)	54	41(75.93)	17(31.48)	53	38(71.70)	10(18.87)	156	107(68.59)	40(25.64)
	Day 29 after Dose1	51	40(78.43)	29(56.86)	54	44(81.48)	21(38.89)	53	42(79.25)	13(24.53)	158	126(79.75)	63(39.87)
	1 year later	50	38(76.00)	—	50	42(84.00)	—	55	46(83.64)	—	155	126(81.29)	—
IJEV-IJEV (7 days)	Day 0	49	25(51.02)	—	53	24(45.28)	—	53	36(67.92)	—	155	85(54.84)	—
	Day 8 after Dose1	46	34(73.91)	11(23.91)	51	36(70.59)	14(27.45)	51	42(82.35)	10(19.61)	148	112(75.68)	35(23.65)
	Day 29 after Dose2	49	40(81.63)	22(44.90)	48	37(77.08)	18(37.50)	54	45(83.33)	17(31.48)	151	122(80.79)	57(37.75)
	1 year later	48	39(81.25)	—	49	39(79.59)	—	45	38(84.44)	—	142	116(81.69)	—
IJEV-IJEV (28 days)	Day 0	49	27(55.10)	—	52	33(63.46)	—	51	34(66.67)	—	152	94(61.84)	—
	Day 29 after Dose1	46	39(84.78)	21(45.65)	52	43(82.69)	11(21.15)	49	38(77.55)	10(20.41)	147	120(81.63)	42(28.57)
	Day 29 after Dose2	47	40(85.11)	22(46.81)	50	45(90.00)	25(50.00)	51	42(82.35)	12(23.53)	148	127(85.81)	59(39.86)
	1 year later	47	40(85.11)	—	52	47(90.38)	—	50	41(82.00)	—	149	128(85.91)	—
Control	Day 0	52	17(32.69)	—	52	36(69.23)	—	52	36(69.23)	—	156	89(57.05)	—
	Day 29	52	24(46.15)	9(17.31)	51	39(76.47)	3(5.88)	52	37(71.15)	1(1.92)	155	100(64.52)	13(8.39)
	Day 58	53	24(45.28)	9(16.98)	52	39(75.00)	3(5.77)	50	38(76.00)	5(10.00)	155	101(65.16)	17(10.97)
	1 year later	53	27(50.94)	—	52	39(75.00)	—	52	43(82.69)	—	157	109(69.43)	—
Total	Day 0	204	87(43.28)		212	120(56.60)		212	141(67.14)		628	348(55.86)	

[a]SP stand for seropositive.
[b]SC stand for seroconversion.

Table 3 | Antibody GMT at different time points before and after immunization in different age groups and all participants

Group	Sampling Time points	40–49 years old			50–59 years old			60–69 years old			Total		
		N	GMT (1/)	95% CI	*N*	GMT (1/)	95% CI	*N*	GMT (1/)	95% CI	*N*	GMT (1/)	95% CI
LJEV	Day 0	51	8.50	6.71–10.75	55	12.55	9.28–16.97	54	14.32	10.94–18.75	160	11.59	9.90–13.56
	Day 8 after Dose1	49	14.24	10.45–19.42	54	21.05	15.25–29.06	53	21.35	15.74–28.97	156	20.20	17.60–23.20
	Day 29 after Dose1	51	25.20	18.03–35.21	54	26.19	18.83–36.41	53	23.25	16.78–32.22	158	25.13	21.51–29.35
	1 year later	50	19.72	14.26–27.29	50	23.62	16.99–32.84	55	25.41	18.91–34.15	155	22.87	19.12–27.36
IJEV-IJEV (7 days)	Day 0	49	11.52	8.67–15.31	53	10.27	7.90–13.34	53	17.78	13.28–23.81	155	12.85	10.92–15.11
	Day 8 after Dose1	46	22.22	15.84–31.19	51	16.54	12.48–21.91	51	27.71	20.85–36.84	148	21.66	18.22–25.75
	Day 29 after Dose2	49	31.45	22.14–44.67	48	22.13	15.96–30.68	54	38.49	27.30–54.27	151	30.23	24.85–36.78
	1 year later	48	27.09	19.19–38.23	49	19.44	14.27–26.49	45	41.25	28.46–59.80	142	27.60	22.63–33.67
IJEV-IJEV (28 days)	Day 0	49	10.73	8.32–13.84	52	12.88	9.63–17.24	51	13.12	10.32–16.69	152	12.22	10.53–14.19
	Day 29 after Dose1	46	25.45	18.71–34.62	52	21.96	16.33–29.52	49	19.17	14.76–24.90	147	21.98	18.66–25.89
	Day 29 after Dose2	47	26.86	19.24–37.49	50	34.47	24.93–47.66	51	23.54	17.74–31.24	148	27.92	23.36–33.38
	1 year later	47	22.84	16.94–30.80	52	24.43	17.98–33.19	50	23.62	17.75–31.43	149	23.65	19.99–27.97
Control	Day 0	52	7.56	6.19–9.23	52	16.38	12.29–21.82	52	14.72	11.51–18.82	156	12.21	10.52–14.18
	Day 29	52	10.13	7.90–13.00	51	18.69	14.13–24.7	52	14.72	11.51–18.82	155	14.05	12.08–16.34
	Day 58	53	10.13	7.94–12.93	52	20.27	15.21–27.01	50	18.40	14.02–24.15	155	15.50	13.22–18.17
	1 year later	53	10.82	8.35–14.01	52	20.00	14.84–26.96	52	19.22	15.07–24.5	157	16.04	13.7–18.77
Total	Day 0	201	9.40	8.32–10.61	212	12.82	11.13–14.77	210	14.91	13.10–16.97	623	12.20	11.30–13.18

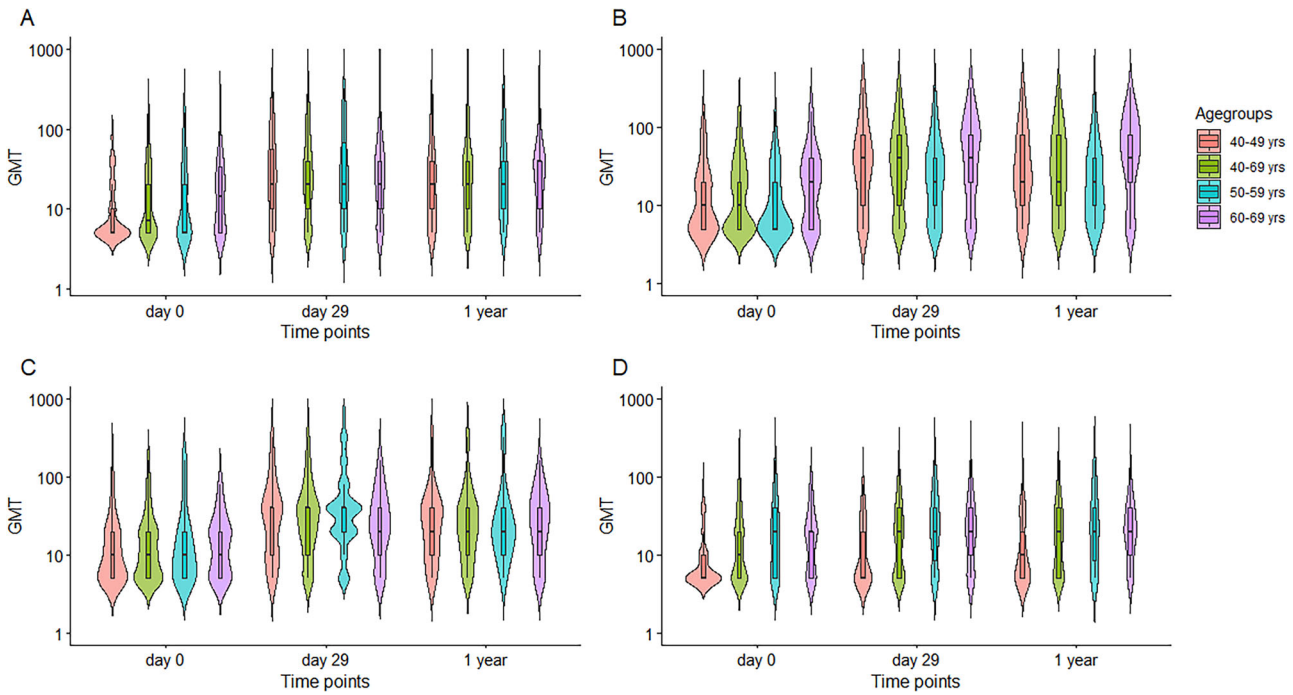

Fig. 2 | Neutralizing antibody titers against JE virus before and after vaccination in vaccination groups and the unvaccinated control group. The WHO reference is a live viral neutralizing antibody titer of 1:10 against JE virus. **A** Group LJEV, **B** Group IJEV-IJEV with a 7-day interval, **C** Group IJEV-IJEV with a 28-day interval, and **D** Unvaccinated control group.

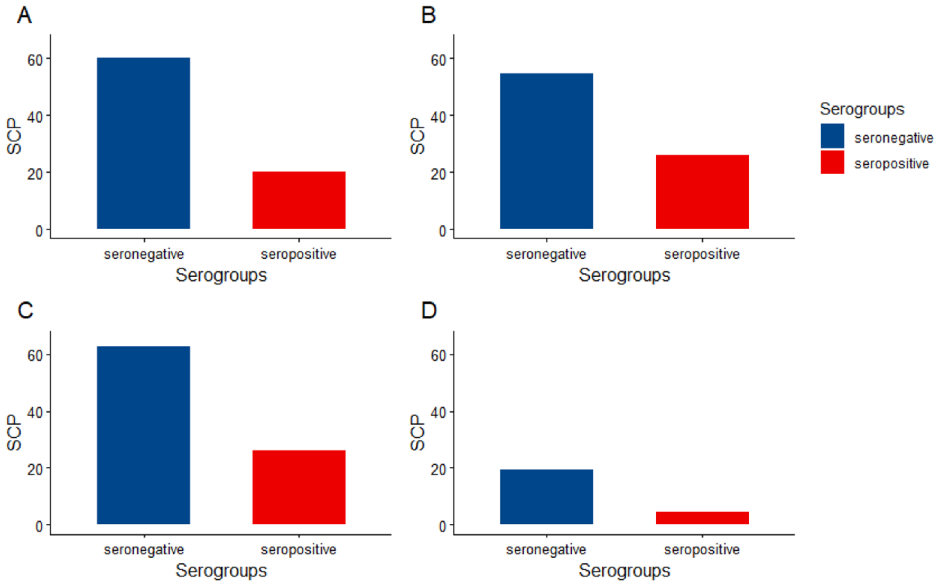

Fig. 3 | SCP levels 29 days after the full schedule in seronegative and seropositive participants. **A** Group LJEV (on day 29), **B** Group IJEV-IJEV 7-day interval (on day 37), **C** Group IJEV-IJEV 28-day interval (on day 58), and **D** control group (on day 58).

Table 4 | Adverse event after the first dose or second dose of LJEV and IJEV

Safety	The first dose No. of cases (case reporting proportion %)			The second dose No. of cases (case reporting proportion %)	
	LJEV (*n* = 149)	IJEV-IJEV 7 (*n* = 143)	IJEV-IJEV 28 (*n* = 141)	IJEV-IJEV 7 (*n* = 132)	IJEV-IJEV 28 (*n* = 139)
SAE at 30 min					
Temperature, mean (SD)	36.57 (0.22)	36.56 (0.25)	36.57 (0.26)	36.54 (0.24)	36.46 (0.24)
Any local reaction	0	0	0	2 (1.52)	0
Pain	0	0	0	2 (1.52)	0
Grade 1	0	0	0	2 (1.52)	0
Any systematic reaction	2 (1.34)	0	0	0	0
Fever	2 (1.34)	0	0	0	0
Grade 1	2 (1.34)	0	0	0	0
SAE at one month[a]					
Any local reaction	1 (0.67)	2 (1.40)	0	0	0
Pain	1 (0.67)	1 (0.70)		0	0
Grade 1	0	1 (0.70)	0	0	0
Grade 2	1 (0.67)	0	0	0	0
Redness	1 (0.67)	1 (0.70)	0	0	0
Grade 1	0	1 (0.70)	0	0	0
Grade 4	1 (0.67)	0	0	0	0
Any systematic reaction	1 (0.67)	2 (1.40)	1 (0.71)	1 (0.76)	0
Temperature	1 (0.67)	1 (0.70)	0	1 (0.76)	0
Grade 2	1 (0.67)	1 (0.70)	0	1 (0.76)	0
Other	0	1 (0.70)	1 (0.71)	0	0
Grade 1	0	0	1 (0.71)	0	0
Grade 2	0	1 (0.70)	0	0	0

[a]For Group IJEV-IJEV 7 days interval, adverse events after the first dose were recorded within 7 days post-vaccination.

population in rural areas may have had asymptomatic JE virus infection. This seropositivity is lower than the 82% among >40 years old in endemic parts of Guizhou province in 2017[32], lower than the 83% among 35–44 year-olds and 70% among ≥45-year-olds in Yiwu prefecture of Zhejiang province in 2015–2016[33], and lower than the 74% among ≥40-year-olds in Yanqing district of Beijing in 2017[34]. Typical epidemic JE areas in China are the rice-producing regions south of the Yellow River. JE occurs in northern China, but northern China has been considered a low-endemic area. Unlike populations in endemic regions, where most people have experienced asymptomatic infection, there is a significant number of susceptible individuals among adults in low-endemic areas.

China started universal childhood immunization against JE in 2007, and subsequently, nationwide incidence of JE decreased markedly. However, there is large number of susceptible individuals who were born before introduction of JE vaccines and have neither been vaccinated nor naturally infected. In recent years, due to changes in climatic and meteorological factors[35,36], some previously low-endemic areas in northern China, have seen a rise in JE cases, resulting in a disease pattern predominantly affecting adults.

In our study, the relatively high baseline seroprevalence will lead to lower seroconversion proportions in the per protocol analysis since most seropositive subjects would not increase NAb titers four-fold, as they are already immune. However, when analysis of seroconversion is restricted to baseline seronegative subjects, seroconversion proportions were much higher, approximately 60% compared to an overall (baseline seropositive and seronegative) seroconversion of about 30%. The same phenomenon was found in a JE vaccine immunogenicity study conducted in India, in which the SCRs in low-endemic, moderate-endemic, and high-endemic participants aged 15–65 years old were 86%, 60%, and 7%, respectively[37]. Pre-existing immunity impact on antibody response is seen with other vaccines, such as influenza vaccine[38].

The seroconversion proportion among baseline seronegative individuals who received one dose of LJEV was 60%, which is consistent with another study done in Ningxia that showed an SCP from LJEV among 41–70-year-olds of 54%[39]. The finding is also consistent with a study in South India that found that ten out of 16 (63%) participants seroconverted to LJEV[40]. A study conducted in India found a seroconversion rate after one dose of LJEV given to 90 initially seronegative 15–65-year-olds to be 85.5%[37]. This rate is significantly higher than found in our study, likely because the median age in that study was 33 years. Our study found a

seroconversion rate of 63% in initially seronegative individuals aged 40–69 years who received two doses of inactivated JE vaccine in a 28-day interval. This finding is consistent with a study conducted in Germany and Austria[16] that showed a SCP from 2 doses of Vero cell SA14-14-2 inactivated JE vaccine (IXIARO) with 28 days interval to be 65% among 65-74-year-old participants. Although IXIARO is different from our study IJEV, the consistency is reassuring.

An observation in our study was that baseline seropositivity increased with increasing age. At baseline, among 40–49-, 50–59-, and 60–69-year-olds seropositivity rates were 43%, 57% and 67%, respectively. This observation implies that Zhongwei prefecture has ongoing JE virus transmission. The increase in seropositivity that we saw in the unvaccinated control group between day 0 and one year also likely reflects ongoing infection. Although no official outbreak was reported in our study area in 2020, in 2018, 2019, and 2020, there were 162, 3, and 2 cases reported from Ningxia, respectively, indicating ongoing local transmission. The high short-term SCP (15.38% at day 29) reflects high seasonal transmission or background exposure, consistent with findings from another study done in Ningxia[39]. Since baseline seropositivity increased with age, the decreasing seroconversion rate with older age subjects cannot indicate that there is a true difference in seroconversion in older individuals, and these age subgroup results are descriptive and not intended to support statistical comparisons.

We found that after more than one year of follow-up, seropositivity was well kept, as the three vaccinated groups all sustained SPP over 81%. Thus, immuno-persistence for one dose of LJEV and two doses IJEV given in an interval of either seven or 28 days can last at least one year.

The safety and reactogenicity profile for a single dose of LJEV was similar to that of two doses of IJEV. Both vaccines have been in use for decades in China among children and are considered safe. Passive post marketing surveillance of Japanese encephalitis vaccination in China among children between 2008 and 2022 has found no safety concerns. As expected, the active adverse event monitoring we used in our study found a higher prevalence of adverse events than were identified in passive post marketing surveillance.

Another vaccination schedule consideration is the potential influence of the interval between doses. A hypothesis is that longer intervals may increase GMTs. From our study we could see that the GMTs in groups IJEV-IJEV with 7 days and 28 days interval after 29 days with full vaccination were 30.23 and 27.92, respectively. That the GMTs were similar is consistent with a study done in Germany, Austria, and Switzerland[41]. A commentary by Rodriges and Plotkin[42] discussed what can be learned from other vaccines about the influence of interval on vaccination response. For example, they describe that in an elderly population, varicella zoster virus (VZV) vaccines given with longer intervals between doses may not enhance antibody levels but are non-inferior to short intervals. Therefore, under different circumstances, it may be important to consider the potential benefits of short-term versus long-term protection in an elderly population.

Both live and inactivated JE vaccines—whether manufactured in China or outside China—showed seroconversion rates of approximately 60% in seronegative elderly individuals, significantly lower than the 70–100% observed 28 days after primary vaccination (one dose of live or two doses of inactivated vaccines) in children[7,43–45]. This age-related decline in immunogenicity aligns with trends seen with other vaccines. However, as has been seen with recombinant zoster vaccine providing superior and longer duration protection against shingles in the elderly than zoster vaccine live[46], protection of elderly from JE may be able to be enhanced with new-platform vaccines.

To prevent JE outbreaks in northern China, we conducted a JE vaccination campaign using one dose of LJEV in parts of Ningxia (excluding Zhongwei prefecture) and Gansu province among 40–70-year-olds and 40–75-year-olds, respectively, in rural areas in 2019 before the epidemic season; there have been no JE outbreaks since 2019 in China. However, immunogenicity and persistence from JE vaccination of adults needs additional study.

Our study has program implications. Since JE vaccination of adults is safe and effectively raises population immunity against JE virus, immunization programs can consider vaccination of adults to partly mitigate emergence of adult JE cases and outbreaks. Since seroconversion 29 days after a single dose of LJEV is higher than after one dose IJEV, one dose of LJEV could be prioritized during emergency vaccination of adults in an outbreak setting. If there are concerns about coincidental adverse events, two doses of IJEV with a 7-day interval can be considered since the number of coincidental events increases as a direct function of time. Since there were no significant differences in seroconversion after 29 days following two doses of IJEV given at seven- or 28-day intervals, with both schedules leading to seropositive proportions over 80% and sustained for a year, adults receiving two doses of IJEV can choose an interval of 7 to 28 days in non-emergency situations.

Strengths of our study include random assignment to vaccination schedule through age-stratified sequential allocation, use of a concurrent control group, and high retention rates of study participants. The control group provided evidence of ongoing infection in the study area, which is essential to properly control for natural infections in the study area during the study period. Laboratory testing conformed to WHO standards. Duration of seropositivity was assessed a year after vaccination.

Our study has limitations. The study population focus was on 40–69 years old who mainly lived in rural areas; other populations will have different infection risk factors. Our trial was conducted in a JE-endemic area with relatively high seropositivity at baseline. Therefore, results may differ if seroconversion is studied in low or non-endemic populations. However, our post-hoc analysis restricted to baseline seronegative individuals provides evidence of good seroconversion in an infection-naïve population. The observation period was limited to one year; a longer duration study is needed to assess longer-term immune persistence in adults. The sample size in this study was too small to detect rare serious adverse events, particularly in older adults (≥60 years), who may be at higher risk for such events with LJEV. If these vaccines are used in emergency settings, especially among older individuals, active surveillance for serious adverse events should be conducted.

In conclusion, we found that adults aged 40-69 years seroconverted equally well to one dose of LJEV or two doses of IJEV given at 7- or 28-day intervals. Seroconversion proportions were high among baseline negative individuals, which is the population most in need of protection with vaccination. No safety concerns were identified with the vaccines or schedules. One dose of LJEV provided a higher seroconversion proportion at day 29 compared a single dose of IJEV, inducing immunity more rapidly. Therefore, during emergency JE vaccination of adults, LJEV could be considered for response in an outbreak and mass vaccination immediately before the epidemic season in an endemic area. Use of LJEV reduces the number of doses and visits needed to protect. For routine vaccination of adults with two doses of IJEV, there is flexibility in the inter-dose interval between 7 and 28 days.

Methods

Setting

The setting was Zhongwei prefecture in Ningxia Hui Autonomous Region, China, selected due to its location in a high-risk area with a low reported incidence of JE. Zhongwei city is located at the junction of Ningxia, Inner Mongolia Autonomous Region, and Gansu Province, with an area of 17,391 square kilometers. By the end of 2023, the resident population of Zhongwei was 1,080,600. No JE cases were reported in Zhongwei prefecture from 2013 to 2016, while one case was reported in 2017 and five cases were reported in 2018.

Study design and participants

The design was an open-label, randomized, controlled phase 4 trial in which age-stratified subjects were enrolled and allocated sequentially into one of three vaccination groups, followed by enrollment of age-stratified subjects meeting the same inclusion/exclusion criteria into a control group, to

compare immunogenicity outcomes and assess background infections during the study period. The vaccination groups were: one dose of LJEV (LJEV group), two doses of IJEV given in a 7-day interval (IJEV-IJEV 7-day interval group), and two doses of IJEV given in a 28-day interval (IJEV-IJEV 28-day interval group); the control group remained unvaccinated.

Since JE vaccines were not included in the National Immunization Program until 2007, few adults over 40 years old have been vaccinated against JE. The age strata for the study were 40–49 years, 50–59 years, and 60–69 years of age. Study exclusion criteria were history of JE vaccination, history of JE disease, allergy to a vaccine component, and any condition that may interfere with immune response. Participants unable to be followed up were also excluded.

This study was approved by the Institutional Ethics Committee (IEC) of the Ningxia Center for Disease Control and Prevention (NCDC 2020-03) and registered at www.chictr.org.cn (ChiCTR2500103235). The study was conducted in accordance with the Declaration of Helsinki. All participants completed consent forms prior to enrollment.

Randomization and masking

Consenting participants were allocated 1:1:1 into Group LJEV, Group IJEV-IJEV 7-day interval, and Group IJEV-IJEV 28-day interval stratified by the three age groups (stratified randomized). Consented control group participants were enrolled in same-age-group strata. For the vaccinated groups, participants were first stratified by age group. Within each age stratum, participants were then assigned to groups LJEV, IJEV-IJEV 7-day interval, and IJEV-IJEV 28-day interval in sequential order of arrival, using a rotating allocation. For the control group, enrollment was conducted separately one week later, with participants also stratified by age group to ensure comparability.

Vaccinated groups enrollment was from 20–23 May 2020; considering that natural infections may occur during the epidemic season and that the observation period of this trial was to be more than a year in duration, we included an identically recruited control group one week after enrollment of the vaccination groups. Therefore, control group enrollment was from 29–31 May 2020. Enrolling clinicians recorded participant demographic information, including birthdate and sex.

For vaccinated groups, the order of their arrival at the study site determined study group assignment. Local investigators did not know the next study group for assignment prior to enrollment of a participant. Only after study group was assigned could the local investigator know to which group the subject was assigned. After assignment, both vaccinators and participants were aware of group assignment based on the formulation and route of immunization. Laboratory technicians were unaware of study group assignments.

Procedures and vaccines

The two study vaccines were LJEV (0.5 mL/dose, lot number: 201912A148-1) and IJEV (0.5 mL/dose, lot number: 201905B16), manufactured by Chengdu Institute of Biological Products Co., Ltd and Chengda Biotechnology, respectively. Both vaccines are stored at 2–8 °C.

Blood draws were approximately 3.0 mL each and were obtained four times from each study participant. For Group LJEV, blood draws were on day 0, day 8, day 29, and one year postvaccination. For Group IJEV-IJEV (7-day interval), blood draws were on day 0, day 8 (immediately before the second dose), day 37, and one year post-vaccination. For Group IJEV-IJEV 28-day interval, blood draws were on day 0, day 29 (immediately before the second dose), day 58 and one year post-vaccination. For control group participants, blood draws were on day 0, day 29, day 58 and 1 year post-enrollment.

Antibody test assay

The National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention measured antibody titers by using a standardized 50% plaque reduction neutralization test ($PRNT_{50}$) to measure NAb titers against JE Virus, in accordance with the WHO-recommended method[47]. The laboratory is a JE regional reference laboratory for WHO. The accepted immunological surrogate of protection is a serum neutralizing antibody titer of at least 1:10, as determined by $PRNT_{50}$, and referred to as seroprotection[1]. Seropositive was defined as a neutralization antibody titer of 10 or higher; seronegative was <10 titer. Seroconversion is defined as $PRNT_{50}$ titer <10 at baseline and ≥10 post vaccination, or a 4-fold rise from a baseline titer of ≥10. Dispensed BHK-21 cells were seeded in six-well plates and cultured until a monolayer was formed. Serum specimens were inactivated at 56 °C for 30 min and diluted 2-fold from 1:5 to 1:10, continuing up to 1:240. Each dilution was mixed with an equal volume of virus suspension at a titer of 200 plaque-forming units (PFU)/100 μL. After incubation for 1 hour at 37 °C, the mixture was used to inoculate BHK-21 cells in six-well plates. In addition, the virus was added (100, 50, and 10 PFU) to the cells in six-well plates as a reference. All specimens and references were incubated at 37 °C for 1 h. The liquid in each well was discarded, and the cells were overlaid with methylcellulose-MEM containing 2% FBS and 1% PS, followed by incubation at 37 °C for 5 days. The methylcellulose-MEM in each well was discarded, and the cells were stained with crystal violet. Next, the number of plaques in each well was determined. NAb titers were determined as the maximum dilution level that resulted in a 50% reduction in plaque number compared to the reference.

After administration of each dose of vaccine, adverse events were recorded by the study staff at the trial site for 30 min, and at participants' homes on day 30 through active surveillance visits. Diary cards were provided to the participants to record any adverse events within 30 days of vaccine administration. Participants with any suspected vaccine-related or serious adverse events could contact study physician.

Outcomes

The primary outcome was the seroconversion proportion (SCP) of JE virus-neutralizing antibodies before and after vaccination. Secondary outcomes were geometric mean titer (GMT) and SCP on days 8 and 29 after a single dose of LJEV or IJEV to allow a comparison of early immune responses after only one dose of each vaccine; GMT and seropositive prevalence (SPP) 29 days and 1 year after completing the vaccination schedule; and incidence of adverse events within 30 min and 1 month after each dose.

We conducted post-hoc determinations of seroconversion proportions separately for subjects who were seronegative prior to vaccination and subjects who were seropositive prior to vaccination. Primary, secondary, and additional outcomes were assessed among adults completing the study, in a per-protocol analysis.

Seroconversion was defined as either a four-fold rise in NAb titer or transitioning from seronegative to seropositive (NAb titer >10); seroconversion proportion was defined as the number of seroconverting subjects in a group divided by the number of subjects in the group; seropositive proportion was the number of seropositives in a group divided by the number of subjects in the group.

Statistical analysis

The sample size for the study was determined based on a cross-sectional study design sample size calculation. We report baseline characteristics using descriptive statistics and summarize continuous variables using mean ± standard deviation (SD) and categorical variables using *n* (%). SCP and SPP are reported as percents. Seropositivity proportions were compared using Pearson Chi-square (χ^2) tests across all four groups and between any two groups. GMTs with 95% CIs were used to describe the antibody titers and were compared by analysis of variance; paired t-tests were used to compare GMTs at different time points after vaccination among the four groups.

Adverse events by number of days after vaccination are shown as *n* (%). Solicited systemic adverse events included irritation or depression, fever, vomiting, anorexia, diarrhea, lethargy, and acute anaphylaxis. Local adverse events included induration, pain, swelling, redness, local rash, and pruritus. Adverse events due to other factors considered to be causally related to the vaccine were judged for causality by the investigators.

SAS software (SAS Institute Inc., Cary, USA) (version 9.4) was used in the data analysis. Statistical significance was set at $P < 0.05$. Figures were performed using R (version 4.3.2).

Data availability

De-identified data will be made available upon approval by researchers, with relevant agreements and approvals. Requests should be made to the corresponding author.

Code availability

The underlying SAS code used for this study are not publicly available but may be made available to qualified researchers upon reasonable request to the corresponding author.

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Author contributions

D.W. and Y.Z. supervised the data collection and verified the results; Q.K.Y., S.H.F., and H.Y.W. finished the laboratory test. D.W., Y.X.L., H.Y.W., and Y.H.H. conceptualized the study and interpreted the results; Z.Y., L.X., Z.L.P., and S.Y.R. coordinated study participant recruitment and study implementation; Z.L.W., L.Y.X., Z.H., A.Z.J., and Y.Z.D. contributed to study supervision; Z.Q., L.M.S., Y.T.T., W.Y.Q., Q.X.Y., and W.J.H. contributed to data analysis; W.D., F.S.H., W.H.Y., L.Y.X., and Lance Rodewald drafted the original version of the manuscript. All authors critically reviewed and approved the final version; All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Competing interests

The authors declare no competing interests.

Consent to participate

Written informed consent was obtained from all individuals who participated in the study.

Additional information

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