

vitamin D in the oral preparations and did not include vitamin D intake from food (including milk powder) or that obtained from sun exposure. Infants in the trial did not receive any other medication to prevent influenza. At the trial initiation and subsequently, after every 2 months, serum calcium, inorganic phosphorus and 25-hydroxyvitamin D levels were measured at the department of clinical laboratory of the hospital. Suspected influenza-like cases⁹ were immediately followed-up in the pediatric infectious department, and the presence of respiratory viral antigens was assessed in pharyngeal secretions using an enzyme-linked immunosorbent assay (ELISA). If the infection was determined preliminarily to be caused by the influenza A virus by ELISA, the clinical manifestations of influenza were assessed and a polymerase chain reaction (PCR) was performed to detect the virus in throat secretions. In addition, bacterial cultures of pharyngeal secretions were performed. Infants with influenza A infection received basic treatment for symptoms and vitamins C and B were also given. However, no antiviral medication, such as ribavirin or oseltamivir, was used. In cases of bacterial infection, antibiotics were offered. In cases where wheezing or bacterial infection worsened, patients were hospitalized for further treatment.

A number of parameters and symptoms were monitored as indicators of drug efficacy. Caregivers and medical staff in the pediatric infectious department initially measured the body temperature of infants once every 4 hours, but after its return to normal for 24 hours, body temperature was measured once every 8 hours. Coughing and wheezing were assessed by medical staff daily to monitor changes; routine blood examinations for white blood cell count were performed, and C-reactive protein levels were monitored. Furthermore, pathogenic detection was conducted on days 1, 4 and 7 of treatment in pharyngeal secretions using fluorescent quantitative reverse transcription-PCR to detect influenza viral loads. Briefly, total RNA was extracted from pharyngeal secretion samples and the M gene was amplified using the following influenza A gene-specific primers: M-1/forward (F), 5'-CGACTGCAGCGTAGCGCTT-3' and M-2/reverse (R), 5'-CATCCTGTATATGAGGCCAT-3'. The expected amplified fragment length was 372 bp.¹⁰ GAPDH (452 bp) served as the reference gene and was amplified using the following primers: upstream primer/F, 5'-CCATCACCATCTTCCAGGAG-3' and downstream primer/R, 5'-CCTGCTCACACCTTCTTG-3'.

Safety was evaluated using the following indicators: (1) possible poisoning symptoms, including anorexia, vomiting, diarrhea and weight loss,¹¹ which were monitored by caregivers daily and medical staff during follow-up sessions; (2) serum calcium, inorganic phosphorus and 25-hydroxyvitamin D levels, which were analyzed immediately following observation of poisoning symptoms; and (3) heart, liver and kidney function as determined from biochemical blood examinations. A flow diagram of the trial design is shown in Figure 1.

Statistical Analysis

Data processing and statistical analyses were performed using the Statistical Package for the Social Sciences version 17.0 software package (SPSS Inc, Chicago, IL). The results of each group were compared using the Student *t* test or χ^2 test.

RESULTS

Patient Demographics

We analyzed 168 and 164 infants in the low-dose vitamin D and high-dose vitamin D groups, respectively, of 52.3% were male. The mean age of the infants was 7.8 ± 2.6 months. Five infants were premature, but all had a gestational age at birth of ≥ 32 weeks. No infants were postterm. The groups were comparable, with no

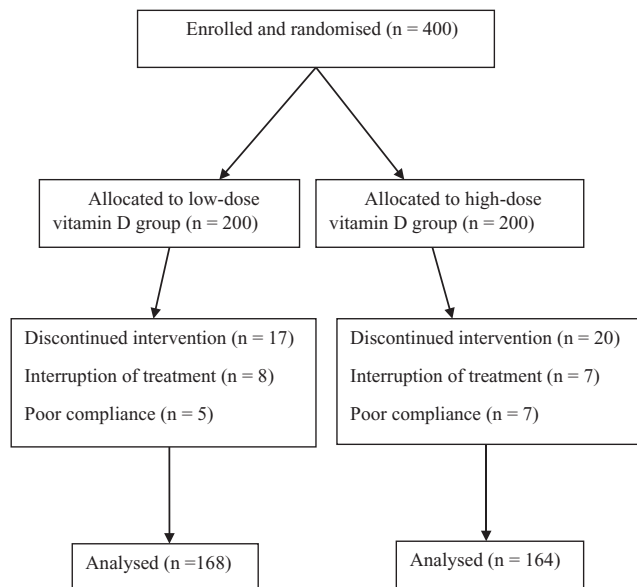


FIGURE 1. Trial design flow diagram. AEs indicates adverse events.

significant differences in terms of sex, age, feeding patterns, location of residence or contact with influenza-infected individuals (all $P > 0.05$) (Table 1). The treatment for 8 infants in the low-dose vitamin D group and 7 infants in the high-dose vitamin D group was interrupted because of secondary bacterial infection and hospitalization; however, all these infants were treated and no deaths occurred.

Clinical Characteristics

During the 4-month follow-up of all participants, there were 157 cases of influenza-like symptoms, including fever, coughing, a runny nose and wheezing. All influenza-like cases were further analyzed using an ELISA and PCR. ELISA results revealed 125 cases of influenza virus A infection and PCR revealed 121 cases; the coincidence rate of the 2 test results was 97.1%. In this trial, PCR results were considered the most accurate method for establishing influenza virus A infection. There were 78 and 43 cases (121 cases in total) in the low-dose vitamin D and high-dose vitamin D groups, respectively, and there was a significant difference between the 2 groups ($\chi^2 = 14.6324$, $P < 0.05$). Fourteen infants with influenza A virus infections (11 in the low-dose vitamin D group and 3 in the high-dose vitamin D group) had secondary bacterial infections that progressed to pneumonia and required further treatment.

The symptoms of the influenza cases in each group, including the duration of fever, coughing and wheezing, were compared. At trial completion, the median duration of a fever in the high-dose vitamin D group (95% confidence interval [CI]: 22.18–36.77) was shorter than that in the low-dose vitamin D group (95% CI: 35.20–47.53), and the difference was significant ($t = 2.4688$, $P = 0.0161$) (Fig. 2). The median duration of coughing in the high-dose vitamin D group (95% CI: 1.42–2.57) was also significantly shorter than that of the low-dose vitamin D group (95% CI: 3.43–4.38, $t = 4.8118$, $P = 0.0000$) (Fig. 3). The median duration of wheezing in the high-dose and low-dose vitamin D groups was 1.38 ± 1.02 days (95% CI: 0.99–1.82) and 2.36 ± 1.40 days (95% CI: 2.83–3.69), respectively, and there was a significant difference between the groups ($t = 3.1142$, $P = 0.0018$) (Fig. 4).

TABLE 1. Infant demographics

Variable	Low-dose Vitamin D (n = 168)	High-dose Vitamin D (n = 164)	χ^2/t test (P)
Sex			
Male	89 (53.0)	84 (51.2)	0.0425 (0.8367)
Female	79 (47.0)	80 (48.8)	
Age (mo)	7.7±2.5	8.0±2.7	0.8013 (0.4240)
Gestational age at birth (wk)			
<32	0 (0.0)	0 (0.0)	0.0012 (0.9719)
≥32 to <37	4 (2.4)	5 (3.0)	
≥37 to <42	164 (97.6)	159 (97.0)	
≥42	0 (0.0)	0 (0.0)	
Feeding pattern			
Exclusive breastfeeding	93 (55.4)	83 (50.6)	0.7996 (0.6704)
Mixed	55 (32.7)	59 (36.0)	
Artificial formula	20 (11.9)	22 (13.4)	
Location of residence			
Urban	101 (60.1)	93 (56.7)	0.2247 (0.6355)
Rural	67 (39.9)	71 (43.3)	
Contact with influenza patients			
Yes	32 (19.0)	29 (17.7)	0.7593 (0.6841)
No	44 (26.2)	50 (30.5)	
Unsure	92 (54.8)	85 (51.8)	

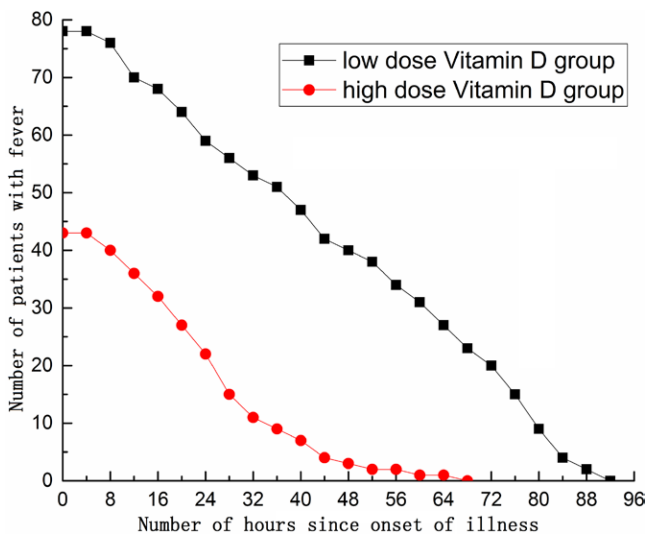


FIGURE 2. Number of patients with fever in both groups. The total number of patients that presented with a fever in both groups was 121, including 78 in the low-dose vitamin D group and 43 in the high-dose vitamin D group. The interval between each time point was 4 hours. Temperatures >37.5°C were considered to indicate a fever and those maintained at <37.5°C for >24 hours were considered normal. [full color online](#)

Viral Load Detection of Influenza Virus A

Influenza virus A nucleic acids were detected using RT-PCR in throat swabs from infants with influenza-like symptoms on days 1, 4 and 7 of follow-up in both groups; the mean viral loads were compared. No significant difference was observed between the mean throat swab viral loads of the 2 groups for the first RT-PCR detection (6.81±3.45 and 6.47±3.92, respectively; $t = 1.1251$, $P = 0.2610$). Conversely, there were significant differences between

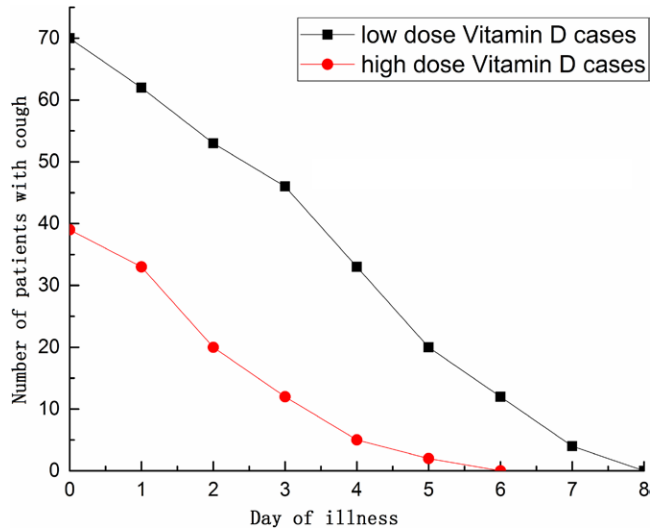


FIGURE 3. Number of patients with cough in both groups. The total number of patients that presented with a cough in both groups was 109, including 70 in the low-dose vitamin D group and 39 in the high-dose vitamin D group. The disappearance of a cough was considered a return to normal. [full color online](#)

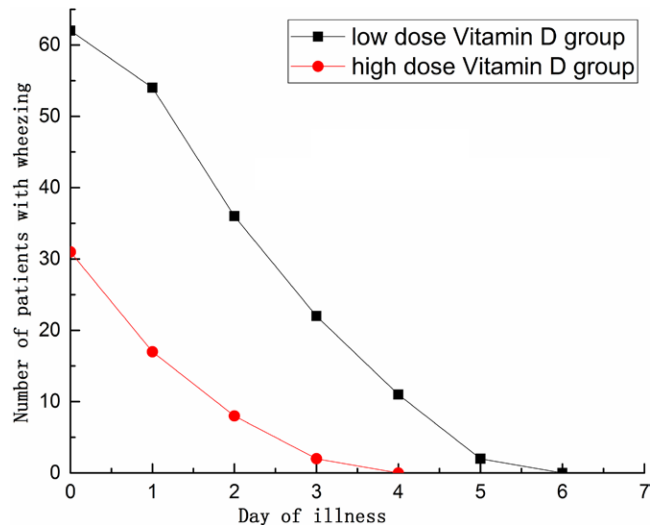


FIGURE 4. Number of patients with wheezing in both groups. The total number of patients that presented with wheezing in both groups was 93, including 62 in the low-dose vitamin D group and 31 in the high-dose vitamin D group. The disappearance of wheezing was considered a return to normal. [full color online](#)

the throat swab viral loads of the high-dose vitamin D and low-dose vitamin D groups at the second (1.26±0.52 and 4.48±1.37, respectively; $t = 40.8935$, $P = 0.0000$) and third (0.15±0.04 and 1.25±0.43, respectively; $t = 45.4385$, $P = 0.0000$) detections (Fig. 5).

Detection of Serum Calcium, Inorganic Phosphorus, and 25-Hydroxyvitamin D Levels

Serum calcium, inorganic phosphorus and 25-hydroxyvitamin D levels were measured 3 times for all infants: on the first

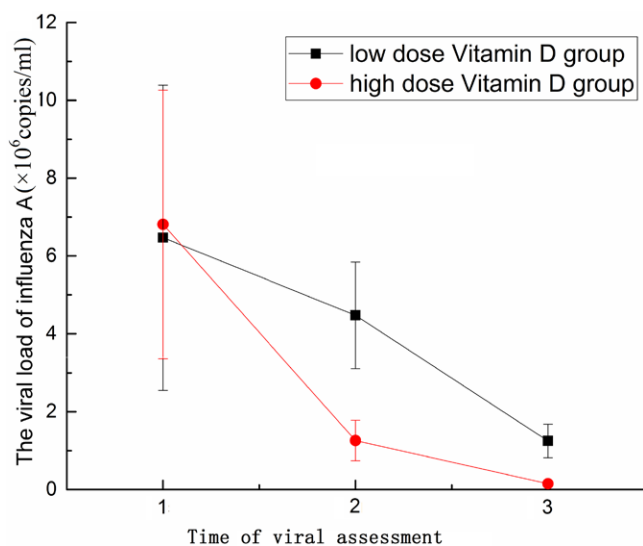


FIGURE 5. Influenza A viral loads in throat swab samples from both groups. The total number of patients that presented with a fever in both groups was 121, including 78 in the low-dose vitamin D group and 43 in the high-dose vitamin D group. * $P < 0.05$ versus the high-dose vitamin D group. The first, second and third detections involved analysing throat swab samples in triplicate using reverse transcription-PCR on days 1, 4 and 7 of follow-up. [full color online](#)

day (first detection), during the second month (second detection) and at the end of follow-up (third detection). In China, the normal levels of serum calcium, phosphorus and 25-hydroxyvitamin D levels are 2.25–2.77, 1.29–1.94 and 50–75 mmol/L, respectively.¹² The serum calcium levels in the high-dose vitamin D group were 2.49 ± 0.18 , 2.55 ± 0.19 and 2.54 ± 0.20 mmol/L in the first, second and third detections, respectively, while in the low-dose vitamin D group, they were 2.47 ± 0.19 , 2.53 ± 0.20 and 2.51 ± 0.19 mmol/L, respectively. There were no significant differences between the groups at each time point (first detection: $t = 1.0154$, $P = 0.3106$; second detection: $t = 0.9634$, $P = 0.3360$; third detection measured 3 times in the high-dose vitamin D group (1.77 ± 0.16 , 1.73 ± 0.17 and 1.75 ± 0.16 mmol/L in the first, second and third detections, respectively) and in the low-dose vitamin D group (1.74 ± 0.17 , 1.75 ± 0.18 and 1.72 ± 0.19 mmol/L in the first, second and third detections, respectively). There were no significant differences between the groups at each time point (first detection: $t = 1.7076$, $P = 0.0886$; second detection: $t = 1.0734$, $P = 0.2838$; third detection: $t = 1.6033$, $P = 0.1089$).

The baseline levels of 25-hydroxyvitamin D were 42.6 ± 5.9 nmol/L and 43.4 ± 6.1 nmol/L in the high-dose vitamin D and low-dose vitamin D groups, respectively; $P = 0.2112$. In the second and third detections, the levels of 25-hydroxyvitamin D were higher in the high-dose vitamin D group (61.3 ± 9.7 and 62.8 ± 10.2 nmol/L, respectively) than in the low-dose vitamin D group (43.8 ± 6.0 and 43.1 ± 6.4 nmol/L, respectively), and the differences were significant (second detection: $t = 20.3444$, $P < 0.05$; third detection: $t = 21.0941$, $P < 0.05$) (Fig. 6).

Safety

Four infants (2 in each group) showed possible symptoms of poisoning, including vomiting and/or diarrhea. Biochemical blood examinations showed that alanine aminotransferase levels were elevated in 2 of the infants. Serum calcium, inorganic phosphorus and 25-hydroxyvitamin D levels, which were examined upon

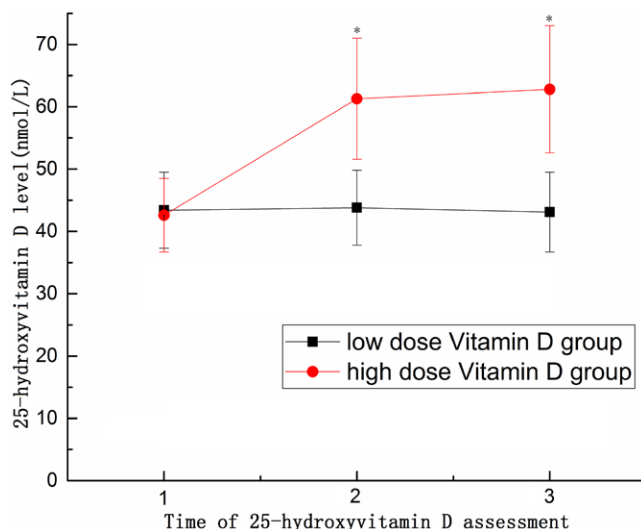


FIGURE 6. 25-hydroxyvitamin D levels in both groups. The levels of 25-hydroxyvitamin D were significantly higher in the high-dose vitamin D group ($n = 164$) than in the low-dose vitamin D group ($n = 168$) in the second and third detections. * $P < 0.05$ versus the low-dose vitamin D group. [full color online](#)

presentation of symptoms, were within the normal ranges (serum calcium level: 2.20–2.80 mmol/L; serum inorganic phosphorus level: 1.29–1.94 mmol/L; 25-hydroxyvitamin D level: 50.0–75.0 nmol/L). However, the clinical manifestations were deemed to be related to the gastrointestinal infection and not vitamin D. None of the patients exhibited circulatory, nervous, kidney or hematologic disorders during this trial.

DISCUSSION

Influenza is a viral infection that affects the general population, especially the very old and the very young.¹ In children, influenza primarily occurs in those <5 years old.^{3,13} In China, according to nationally reported data, children 0–5 years old are frequently affected; there are 352 cases per 100,000 inhabitants,¹⁴ which is similar to reports from other countries, including the United States¹⁵ and some countries in Europe.¹⁶ However, no data on infant influenza have been reported.¹⁷

A variety of prevention and antiviral treatments are currently available. The 2 general annual influenza vaccines available for children, including the inactivated influenza vaccine and live attenuated influenza vaccine, are U.S. Food and Drug Administration-approved, for children 1 year old and older, but not for infants.¹⁸ Adjuvant vaccines for children <2 years old are under investigation. These vaccines aim to boost the host's immune response to the vaccine antigen, but their efficacy is yet to be confirmed.¹⁹ Oseltamivir is currently recommended for prophylaxis and treatment of confirmed or suspected cases of influenza among high-risk groups, including children <2 years old²⁰; however, only sparse data exist with regard to infants, and the appropriate dose ranges from 1 to 3 mg/kg.^{21,22} Further research is required to determine the appropriate dose of oseltamivir and to determine its safety in infants. A systematic review and meta-analysis concluded that vitamin D supplementation was safe and protective against acute respiratory tract infections; however, this study did not analyze pathogens or focus on influenza A. Three studies were conducted on influenza A; however, they did not include infants.²³

Vitamin D has received attention because influenza is more common in winter months when diminished sun exposure results in low levels of vitamin D, although the mechanism underlying influenza seasonality has not been clearly established.²⁴ Vitamin D has several immunomodulatory functions, including upregulation of antiviral peptides that are part of human innate immunity and can inactivate the influenza virus.^{5,7} There is a paucity of well-designed clinical studies supporting the use of vitamin D3 to prevent influenza in children. However, no previous studies focused on infants; the participants were aged from 6 months to 5 years (600 IU of vitamin D daily for 6 months) or 6 to 15 years (1200 IU of vitamin D daily for 4 months).^{5,7} Furthermore, the previous studies had limitations, including no assessment of the baseline vitamin D levels, and the vitamin D doses in 1 trial may have been too low to have benefits.²⁵ Despite the lack of evidence, a few reports have suggested that vitamin D is currently being used for influenza prevention; the frequency and extent of its use is unknown.^{26,27} In a previous study, infants in China received 400 IU vitamin D3 daily to prevent rickets.¹² This concentration perhaps cannot have a preventive effect against influenza, although there is no valid data. The present study evaluated the use of vitamin D3 drops for the prevention of seasonal influenza A among infants. The participants were divided into low-dose vitamin D (400 IU) and high-dose vitamin D (1200 IU) groups.

During the course of the trial, 78 of 168 (46.4%) infants in the low-dose vitamin D group and 43 of 164 (26.2%) infants in the high-dose vitamin D group presented with influenza A infections, $\chi^2 = 14.6324$, $P = 0.0001$. Numerous factors may have led to the different infection rates, including sex; age; social distancing measures^{28,29}; types of physical barrier protection (including hand hygiene, environmental cleaning and face masks)^{30,31}; and serum calcium, inorganic phosphorus, and 25-hydroxyvitamin D levels. However, we were unable to accurately evaluate social distancing measures and types of physical barrier protections, although feeding patterns, location of residence and possible contact with influenza patients were evaluated. The majority of indicators, with the exception of 25-hydroxyvitamin D levels, were significantly different between the 2 groups, which might explain why the incidence of influenza A was significantly lower in the high-dose vitamin D group than in the low-dose vitamin D group, although the mechanism is anagoric and was not analyzed in the trial.

The parameters evaluated in the infants with influenza A infections included the duration of fever, coughing and wheezing, as well as viral loads of influenza A. When monitoring the reduction in the number of days with fever, the high-dose vitamin D group exhibited a faster reduction in temperature compared with the low-dose vitamin D group. Furthermore, the high-dose vitamin D group showed a more rapid improvement in the time required to resolve cough and wheezing compared with the low-dose vitamin D group. Notably, the viral loads of influenza A in infants in the high-dose vitamin D group decreased more rapidly compared with those of infants in the low-dose vitamin D. These results suggested that high-dose vitamin D treatment exerted an antiviral effect to promote recovery.

The incidences of adverse events and severe adverse events were not statistically different between the 2 groups. Only 4 infants presented with potential poisoning symptoms, including vomiting and/or diarrhea. Biochemical blood examinations showed that alanine aminotransferase levels were elevated in 2 infants; this was considered to be due to the gastrointestinal infection and not vitamin D, thus indicating that high doses of vitamin D (1200 IU) are safe in infants. Following the end of the trial, infants were followed-up for an additional 2 months, and no sequelae were observed in the patients.

ACKNOWLEDGMENTS

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LITERATURE ABSTRACT

Edited by: Robert J. Leggiadro, MD

Trichinellosis Outbreak Linked to Consumption of Privately Raised Raw Boar Meat—California, 2017

Heaton D, Huang S, Shiau R, et al. *Morbidity and Mortality Weekly Report* 2018; 67:247–9.

On January 15, 2017, a hospital physician notified the Alameda County Public Health Department (ACPHD) in California of a patient with a suspected diagnosis of trichinellosis, a roundworm disease transmitted by the consumption of raw or undercooked meat containing *Trichinella* spp. larvae. A family member of the initial patient reported that at least 3 other friends and family members had been evaluated at area hospitals for fever, myalgia, abdominal pain, diarrhea and vomiting. The patients had attended a celebration on December 28, 2016, at which several pork dishes were served, including larb, a traditional Laotian raw pork dish, leading the hospital physician to suspect a diagnosis of trichinellosis. The event hosts reported that the meat had come from a domesticated wild boar raised and slaughtered on their private family farm in northern California. ACPHD conducted a case investigation that included identification of additional cases, testing of leftover raw meat, and a retrospective cohort study to identify risk factors for infection.

Exposure to *Trichinella* was defined as consumption of pork in which *Trichinella* spiralis larvae were identified. Thirty-six potentially exposed persons were identified, including 29 who attended the event and 7 who consumed food taken home from the event by attendees. Among the potentially exposed persons, 20 (56%) were interviewed, 16 for whom professional language interpreters were used. Clinical and exposure information from all 20 persons who were interviewed was collected using a structured questionnaire administered by telephone 28–92 days after the December 28 event. In consultation with the California Department of Public Health and the Centers for Disease Control and Prevention, ACPHD recommended serologic testing for *Trichinella* for all persons with a suspected diagnosis of trichinellosis using a commercial laboratory (Gold Standard Diagnostics, testing performed as Focus Diagnostics, Inc., San Juan Capistrano, California) enzyme-linked immunosorbent assay to detect immunoglobulin G directed against a *Trichinella* excretory-secretory antigen.

Ten confirmed and 2 probable cases of trichinellosis were identified; 11 occurred in men. Eleven patients self-identified as Asian, and 1 identified as Asian and white. Median age was 58 years (range, 39–71 years). Onset dates ranged from December 28, 2016, to January 23, 2017. Nine patients were hospitalized, 2 of whom were admitted to the intensive care unit; 9

had sepsis. Seven had acute kidney injury, and 2 had gastrointestinal bleeding, 1 case of which was attributed to nonsteroidal anti-inflammatory drug use. Eight patients had elevated creatine phosphokinase levels indicating skeletal muscle damage, and 7 had elevated peak lactic acid levels, which is an indicator of sepsis. Six had elevated peak troponin levels indicating damage to the myocardium. Ten cases were confirmed by a positive *Trichinella* serologic test; 2 patients were not tested.

The 3 pork-containing dishes reported to have been served at the event included pork stew, grilled pork and raw larb. Attendees were interviewed about preparation and consumption of the 3 pork dishes served at or taken home from the event, as well as consumption of any other pork-containing dishes served at the event and other sources of wild boar or bear meat. Attack rates and relative risks were calculated. Leftover raw pork from the implicated meal was obtained from the event host.

Larvae in an unstained touch preparation from the raw pork were verified as *Trichinella* spp. from a photomicroscopic image; samples were sent to Centers for Disease Control and Prevention's Division of Parasitic Diseases and Malaria diagnostic laboratory and identified as *Trichinella spiralis*. Consumption of larb was significantly associated with trichinellosis, with an attack rate of 100% and a relative risk of 3.33 (95% confidence interval = 1.29–8.59). No other meat dishes were associated with an increased relative risk.

The farm owner stated that there are several pigs being raised on the farm, and the swine are only given commercial feed and never cooked or uncooked meat, offal or garbage. The farmer denied any rodent infestation issues on the farm but did state that small animals had occasionally gotten into the outdoor, fenced pen and been eaten by the pigs, indicating that small mammals infected with *Trichinella* could have entered the pen and been consumed by the swine. The host was educated about reducing the risk for trichinellosis when consuming pigs from his farm by freezing raw meat for 30 days and cooking meat to a minimum internal temperature of 160 degrees F (71.1 degrees C) to kill *Trichinella* larvae.

Comment: Whereas trichinellosis is rare in the United States, it remains a public health threat, especially among populations that consume raw or undercooked wild game meat or pork from noncommercial sources. Recent outbreaks of trichinellosis have been associated with wild boar, bear, walrus and unspecified pork. This outbreak investigation indicates that high-risk meat preparation and consumption practices might be part of valued cultural traditions. Public health, agriculture and wildlife authorities should strengthen efforts to provide culturally competent education about trichinellosis prevention to private farmers, hunters and communities whose cultural practices include raw meat consumption.