

Case Study

## Emergency response to occupational brucellosis in a pharmaceutical manufacturing enterprise

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**Abstract: Objectives:** Human brucellosis, as a zoonosis, is a serious public health problem in many developing countries, including China. To date, there has been no case report on occupational brucellosis and the emergency response in the pharmaceutical manufacturing industry. The aim of this report is to describe an emergency brucellosis outbreak in this unusual setting and the associated treatment methods from an occupational health perspective. **Methods:** The emergency response included a field epidemiological investigation, a hierarchy of control strategy, and validation of control measures. **Results:** Seven workers in the defrosting, grinding, and hydrolysis departments suffered from human brucellosis during two periods. The main symptoms were whole-body joint pain and undulant fever for several weeks, and *Brucella*-specific serum antibody was found in the patients. A hierarchy of exposure control strategy was implemented, including elimination, engineering control, administrative control, and the use of personal protection equipment. Raw material and environmental monitoring, measurement of placental temperature, *Escherichia coli* inactivation tests, and health examinations demonstrated that these measures were effective. **Conclusions:** This report describes an emergent case and the response to two consecutive outbreaks of occupational brucellosis among workers in a pharmaceutical manufacturing enterprise. The hierarchical control strategy used in this case may prevent new outbreaks, and several lessons can be learned from this event.

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**Key words:** Brucella, Brucellosis, Emergency, Infectious disease, Occupational exposure

### Introduction

Brucellosis caused by *Brucella* bacteria is one among the most prevalent zoonotic infectious diseases. Globally, it is estimated that more than 0.5 million new cases of brucellosis emerge each year<sup>1)</sup>, and this disease remains a serious public health problem in many developing countries, including China. *Brucella* belongs to the  $\alpha$ -2 subgroup of the phylum Proteobacteria, which are gram-negative, partially acid-fast, aerobic, facultative intracellular coccobacilli or short rods. *Brucella melitensis*, which commonly infects sheep and goats, is the most common species that infects humans<sup>2)</sup>. The prevalence of human brucellosis is dependent on factors such as husbandry practices, dietary habits, the methods used to process milk and dairy products, and environmental sanitation<sup>3)</sup>. Brucellosis can be transmitted from infected livestock to humans via ingestion or inhalation of the bacteria, conjunctiva, or skin abrasions<sup>4)</sup>. Human brucellosis has a number of different manifestations<sup>5)</sup>. However, the most commonly reported symptoms and signs are fever, fatigue, malaise, chills, sweats, headaches, myalgia, arthralgia, and weight loss<sup>6)</sup>.

Besides being an infectious disease, human brucellosis is also considered an occupational disease because it usually occurs during occupational activities that expose workers to *Brucella*. Populations such as abattoir workers, veterinarians, lab technicians, hunters, farmers, and livestock producers are involved in such activities<sup>7)</sup>; and occupational brucellosis is predominantly reported in the animal husbandry, agriculture, meat processing, and vaccine production industries. In these occupational fields,

exposure to *Brucella* can be prevented by maintaining good hygiene and using protective clothing/equipment<sup>4</sup>. To date, there has been no case report on occupational brucellosis in a conventional manufacturing setting such as the pharmaceutical manufacturing industry. Moreover, the emergency response practiced for this occupational infectious disease is considerably different from that used for ordinary infectious diseases. The former usually gives priority to occupational hazards in the workplace, and may adopt a targeted hierarchical strategy of exposure control. The latter focuses on infection source control, cutting off transmission routes, and protecting vulnerable people in the general population.

In this report, multiple methods, including a field epidemiological investigation, a hierarchy of control strategy against *Brucella* exposure, and an evaluation of control strategy effectiveness were used for urgent eradication of occupational brucellosis in a pharmaceutical manufacturing enterprise, and provided a scientific basis for the supervision and administration of this event by the local government.

### Emergency Response

On May 15, 2015, the local government ordered a pharmaceutical factory to suspend production because of a continuous occurrence of seven cases of human brucellosis in the past 3 years. The conventional emergency treatment which is usually employed for infectious diseases did not prevent repeated occurrence of the epidemic. This caused a psychological panic among workers, who no longer dared to go to work. As a result of the shutdown, these enterprises faced the risk of bankruptcy and were required to take targeted measures to prevent new occurrences of this infectious disease. At the request of the enterprise, the Zhejiang Provincial Center for Disease Control and Prevention (Zhejiang CDC) set up a group of emergency response experts to help address this problem.

The company, which produces a health care product derived from sheep placenta, is a private enterprise consisting of over 200 workers and is located in the west of Zhejiang province in China. The disease outbreaks occurred in a workshop carrying out the extraction of proteins from sheep placenta, with an annual usage of 120 tons of sheep placenta. The production process in the extraction workshop involves the following steps. (1) Placenta washing and defrosting: sheep placenta is transported from cold storage and transferred to a constant-temperature (60-70°C) cleaning pool for 1 h. (2) Placenta grinding: sheep placenta is ground in a manual meat grinder. (3) Hydrolysis: the ground placenta is hydrolyzed at 90°C using acid and proteolytic enzymes. These steps are performed using manual operations in open conditions. In addition, high-speed crushing of sheep placenta

probably produces aerosols containing harmful organisms. Following high-temperature hydrolysis, all subsequent processes take place in a “clean workshop” governed by Good Manufacturing Practice (GMP). The extraction workshop lacked both a superior general ventilation system and a local exhaust ventilation system. Workers did not wear effective personal protective equipment (PPE). Only an ozone generator was installed in the workshop. Due to an expansion in the scale of production, the source of the raw materials (sheep placenta) used in the extraction process had recently changed from the Jiangsu province of China (a low-prevalence area of brucellosis) to the Inner Mongolia autonomous region of China (a high-prevalence area of brucellosis).

From 2013 to 2015, there were two outbreaks, with four cases in September 2013 and three cases in May 2015. The workers had several symptoms, such as whole-body joint pain, undulant fever, headache, sweating, and fatigue. They were mistakenly treated for influenza in a local county hospital, but the treatment was ineffective. A serum agglutination test (SAT) based on a detection method recommended in the Chinese diagnostic criteria for brucellosis (WS269, 2007) was utilized by the local CDC, and found that the titer of serum antibody specific for *Brucella* was significantly increased in the seven patients, confirming brucellosis.

All seven patients were operators in the washing, grinding, or hydrolysis departments in the sheep placenta extraction workshop, which employed fourteen workers. The fourteen workers in the workshop had the same working conditions during the periods encompassing the first and second outbreaks. The patients included four males and three females, and were between 36-52 years of age. The clinical manifestations of the seven cases are listed in Table 1, and included whole-body joint pain (especially in those joints that experience high impact, such as the waist and knee), headache, sweating, and undulant fever (less than 39°C) for several weeks. The male patients also experienced testicular strain and pain along with insomnia, fatigue, depression, and appetite loss. Two cases had vertebral abscesses and one case had prostate hyperplasia.

In response to this emergency, the company adopted a hierarchy of exposure control strategy in the sheep placenta extraction workshop based on advice from experts. The following hierarchical measures were taken.

I. Elimination. 1) The purchased raw materials (sheep placenta) must pass quarantine inspection to ensure that the sheep placenta is free of *Brucella* bacteria. As the recipient of the sheep placenta, the pharmaceutical manufacturing enterprise must ensure that the goods they are receiving have already passed quarantine inspection. 2) Prolonged low-temperature storage: the raw materials used in placenta extraction were stored in the freezer for at least 60 days to reduce *Brucella* activity. 3) Airtight

**Table 1.** Clinical manifestations of the seven brucellosis cases that occurred in the washing, grinding, and hydrolysis processes

No	Gender	Symptom	White blood cell count ( $10^9/l$ )	Specific serum antibody <sup>a</sup>	Complications	Prognosis after antimicrobial treatment
1	Female	Fever and profuse sweating for half a month	4.60	Positive	C5C6 cervical abscess	The pain was ameliorated, but the pain in the back of the neck was still noticeable.
2	Female	Repeated pain in the hips for 6 months	5.13	Positive	L4 vertebral body lesion, accompanied by vertebral abscess	Symptoms disappear
3	Female	Repeated muscle soreness with fever for 3 months	2.6	Positive	-	Lower limb pain continues to persist
4	Male	Pain while urinating, accompanied by lumbago and fever for 1 month	6.9	Positive	-	Symptoms disappear
5	Male	Fever for 21 days	3.3	Positive	-	Symptoms disappear
6	Male	Fever and sweating accompanied with joint pain for half a month	3.9	Positive	-	Symptoms disappear
7	Male	Repeated neck pain and muscle soreness for 4 months	3.8	Positive	Degeneration of cervical vertebra	Symptoms disappear

<sup>a</sup> Specific serum antibody for *Brucella* antigen was detected through the serum agglutination test (SAT) based on the Chinese diagnostic criteria for brucellosis (WS269, 2007).

conditions and disinfection: a thermostat-controlled isolation chamber was set up to defrost sheep placenta for 1h, and water was heated to a temperature of 60-70°C and maintained for 2h to carry out moist-heat disinfection. An ultraviolet-sterilizing lamp was installed at the top of the isolation chamber, and the workshop was also equipped with a portable UV disinfection lamp. The total irradiation time was no less than 2h. 4) Automatic operation: the transport of materials was automated, reducing the potential for contact with *Brucella*.

II. Engineering controls. 1) Local exhaust ventilation: local ventilation systems were installed above the isolation chamber, meat grinder, and hydrolysis tank to remove the aerosols produced by these processes. 2) General ventilation: the ventilation rate in the workshop was maintained at more than six air changes per hour. The captured aerosols were discharged at high altitudes after alkaline treatment.

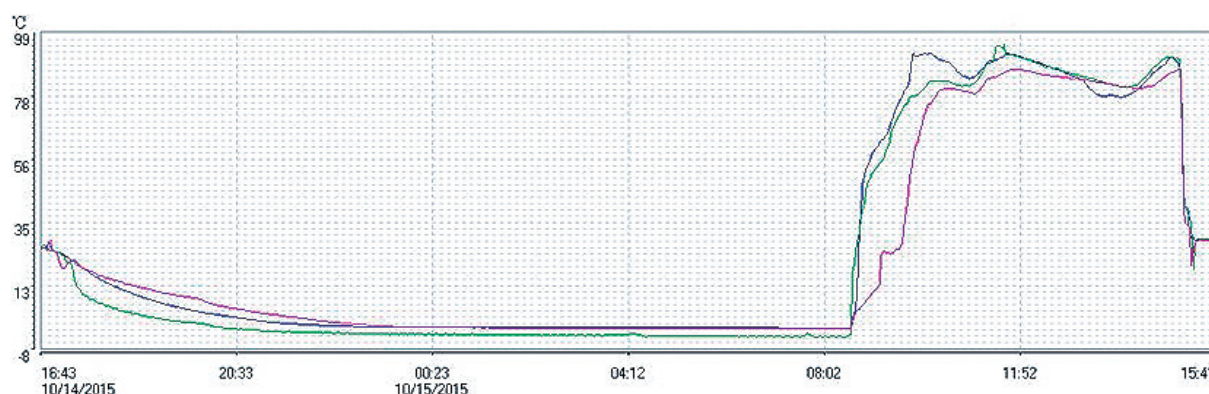
III. Administrative controls: A full-time occupational health manager was assigned in the workplace. Occupational health administrative measures including health examinations, PPE management, occupational health training, and warning and notification systems were established. The physical examination indicators for workers included regular measurement of *Brucella*-specific serum antibody titers and monitoring relevant clinical manifestations.

IV. PPE: the workers in the workshop were provided with self-priming filter dust respirators, sealed overalls, and plastic gloves.

Three months later, the control effects of these measures were evaluated through the following four approaches: testing for the presence of *Brucella* in the raw and processed materials, air, and surfaces of the workshop; measurement of placenta temperature; an *Escherichia coli* inactivation test; and detection of *Brucella*-specific serum antibodies in workers.

To determine whether *Brucella* survived in the sheep placenta and workshop environment, biological sampling and plate cultures were performed for 3 consecutive days. The methods for sampling and culturing were based on the Chinese diagnostic criteria for brucellosis (WS269, 2007). The samples were collected from the raw materials in cold storage; from sheep placenta in the processes of washing, defrosting, grinding, and hydrolysis; from the thermostat handle, cargo conveyor belt, and meat grinder surfaces; and from the air at the height of the worker's breathing zone in the washing, defrosting, grinding, and hydrolysis areas. Three samples were randomly collected from each batch of raw materials in cold storage, and total of nine samples were collected. No *Brucella* colonies were observed in any plate culture sample, indicating *Brucella* was not present in the raw materials, air, or surfaces of the manufacturing areas.

To verify that the temperature at the center of the sheep's placenta was sufficient for sterilization following these technical modifications, temperature probes were placed in the center of three different sheep placentas and used to record the temperature in real-time for three consecutive days. Hence, there were nine central placenta



**Fig. 1.** The temperature in the center of sheep placentas in the defrosting and moist-heat disinfection processes, as recorded by three temperature probes for three consecutive days.

temperature samples. This method was based on the standard testing protocol followed in China (Safety and Sanitation Requirements for Disinfection of Tableware and Cabinets, GB17988-2008). Fig. 1 shows that temperatures of  $>70^{\circ}\text{C}$  were maintained for 2 h at the center point, providing the optimal hot and humid conditions to kill *Brucella*. In addition, *E. coli*, which has a thermal sensitivity similar to *Brucella*, was employed in an inactivation test to verify that these hot and humid conditions would be able to kill *Brucella* species. This method is based on the disinfection standard protocol employed in China [i.e., the Disinfection Technical Specification (2002 edition)]. The culture results showed that there was no growth of *E. coli* in sheep placentas, indicating that maintaining a temperature of  $>70^{\circ}\text{C}$  for 2 h in the middle of the defrosting process was sufficient to kill *Brucella* species.

Occupational health examinations of the workers in the placenta extraction workshop showed that none exhibited the clinical manifestations of brucellosis, including undulant fever and muscle or joint pain. Additionally, the *Brucella*-specific serum antibody test was negative in all workers. No new brucellosis cases occurred in this enterprise during this six-month trial.

## Discussion

The severity of the brucellosis epidemic in China is similar to that in other developing countries in the Middle East and Africa<sup>8-10</sup>. There is a close relationship between the incidence of human brucellosis and the severity of the disease outbreak in animals<sup>1,2</sup>. At present, there is a tendency for human brucellosis to spread across China, and this epidemic is spreading from pastoral or semi-pastoral environments to agricultural processing areas and even to cities<sup>11</sup>. Zhejiang province, where the outbreak emergency occurred, is not a high-prevalence area for brucellosis, which may be imported from other areas<sup>12</sup>.

Based on the two related standards in China, e.g., the Diagnostic Criteria for Brucellosis (WS269) and the Di-

agnostic Criteria for Occupational Infectious Disease (GBZ227), there are three methods for diagnosing human brucellosis: epidemiological data, clinical data, and specific laboratory examination. The seven patients were diagnosed with occupational brucellosis based on occupational activities involving potential exposure to *Brucella* carried in sheep placentas from a region with a high prevalence of brucellosis (the Inner Mongolia autonomous region of China), typical clinical manifestations (such as weeks of undulant fever and muscle and joint pain), and a significant increase in the titers of *Brucella*-specific serum antibody. The specific serum antibody detected with the SAT test can be an alternative brucellosis diagnostic method in the absence of blood cultures for etiological tests<sup>4</sup>. Moreover, after anti-*Brucella* treatment, the patients' clinical symptoms disappeared or were improved. Four of the seven brucellosis cases occurred in September, which is not generally epidemic season for human brucellosis in China (the majority of cases are reported in February-July)<sup>11</sup>. The reason for this different onset period might be that these cases of occupational brucellosis were associated with sheep placentas contaminated by the *Brucella* bacteria. Several months likely elapsed between the transportation of raw material from the source area in northern China to the destination plant in eastern China, as well as a period of storage in the factory freezer before the defrosting process.

In this event, because of less advanced production technology and weak occupational disease prevention measures, workers were at a high risk of *Brucella* exposure via inhalation and skin contact due to several processes such as the transportation of placentas from cold storage, washing and defrosting, grinding, and hydrolysis. The seven brucellosis cases occurred in two consecutive outbreaks because those patients were only treated from the perspective of controlling the infectious disease, rather than maintaining occupational health. The occupational health control measures taken to prevent *Brucella* exposure were critical for the effectiveness of this emergency

response.

Following the advice of the emergency response expert group, targeted measures against *Brucella* exposure were taken based on the biological characteristics of *Brucella* bacteria<sup>13</sup> and hierarchical principles of occupational exposure control<sup>14</sup>. The hierarchy of exposure control usually consists of elimination, substitution, engineering control, administrative control, and PPE. In this emergency response, the elimination step involved purchasing *Brucella*-free raw materials, disinfection and inactivation, and automating transportation of materials. The negative plate cultures for *Brucella* in all samples of raw materials indicated that the enterprise had successfully implemented the policy that all of the sheep placenta purchased should pass quarantine inspection, ensuring that the raw manufacturing materials were free of *Brucella*.

Disinfection and inactivation measures in this report included a prolonged low-temperature storage period (60 days), moist-heat disinfection (a temperature of  $>70^{\circ}\text{C}$  for 2 h), and ultraviolet disinfection ( $\geq 2$  h). Based on the biological characteristics of this bacteria, ultraviolet irradiation for 5-20 min can kill live *Brucella* bacteria; incubation at  $60^{\circ}\text{C}$  (15-30 min) or  $70^{\circ}\text{C}$  (7-19 min) under hot and humid conditions can inactivate the *Brucella* bacteria; and storage at freezing temperatures for 47 days can decrease the activity of the bacteria. The disinfection and inactivation measures adopted during the emergency response were stricter than these basic measures, ensuring the inactivation of any *Brucella* potentially present in the sheep placentas. The measurement of placental temperatures and the *E. Coli* inactivation test demonstrated that these inactivation measures were effective.

Moreover, the automation of the transportation process greatly reduced the probability of exposure to *Brucella*. Engineering control measures included local exhaust ventilation installed at several critical positions and improved general ventilation with air changes occurring six times per hour throughout the workshop, which reduced the probability of aerosol exposure. The negative results of air and surface monitoring for *Brucella* demonstrated the success of these measures. Occupational health administrative measures including health examinations, occupational health training, and warning and notification systems were also established. PPE was also significantly improved. Finally, there were no new outbreaks and no increases in *Brucella*-specific serum antibodies in the workers at this enterprise during the trial run of these new procedures, indicating that the hierarchical strategy of exposure control was effective. One drawback of this emergency response was that it could not be determined which pathogenic *Brucella* species was responsible for the outbreak. The samples from the original raw materials contaminated with *Brucella* bacteria were not available because all of the raw materials had been disposed of when the outbreak was treated as a typical infectious disease

epidemic. Hence, etiological tests such as PCR were not performed.

## Conclusions

In summary, this was an emergency response to two consecutive outbreaks of occupational brucellosis occurring in an unusual setting (a pharmaceutical manufacturing enterprise), and the hierarchical control strategy reported in this study may prevent the appearance of new outbreaks. The following lessons can be learned from this event. First, for professional personnel to deal with this type of public health emergency it is important to realize that occupational brucellosis is both an infectious disease and an occupational disease, and the treatment of the latter should be approached from an occupational health perspective. Second, it is critical to pay attention to protection from the occupational biological hazards related to processing raw materials in the workplace. Third, an integrated strategy should be employed to minimize exposure to biohazards, including purchasing *Brucella*-free raw materials, disinfection, technical renovations, engineering controls, occupational health examinations, environmental monitoring, and the use of PPE.

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*Conflicts of interest:* None declared.

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