ARTIGO ORIGINAL

Q FEVER OUTBREAK IN AN EXPERIMENTAL WILDLIFE BREEDING STATION IN URUGUAY

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ABSTRACT

An outbreak of Q fever was reported during 2003-2004 in the Experimental Wildlife Breeding Station Department of Maldonado-Uruguay. A descriptive epidemiological investigation and nested case-control were conducted to identify factors associated with occurrence of Q fever (reservoir; animal's habitats and risk related to working activities) Seroepidemiological survey in humans and in the suspect animals were carried out.
117 workers were studied. Q fever was confirmed in 25 cases, (prevalence of 21.37%, 95% IC: 14.3-29.9). The outbreak may have been caused by aerosolization of *Coxiella burnetii* whilst carrying out grass mowing (OR: 10.91, 95% CI: 3.61-34.08). The field deer, *Ozotoceros bezoarticus*, was identified as the reservoir of the infective agent though the participation of other species was not ruled out.

KEY WORDS: Q fever. Outbreak. Rickettsial diseases. Reservoir.

INTRODUCTION

Q fever is a worldwide distributed zoonosis caused by *Coxiella burnetii,* domestic and wild animals, rodents and arthropods are the reservoirs. In animals the disease is usually unapparent, although abortions, early parturition and parturition of weak newborns were reported in ewes and goats (2, 4, 13, 17).

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Several factors take part in the transmission of the disease. It is generally carried out by airborne aerosols and dust particles which have been contaminated by infected animals and their subproducts. Infection by contaminated milk and by ticks has been reported but is not common (7, 10, 12, 18, 20).

A low number of infective agents may initiate the disease. Humans are susceptible to this disease, it is considered an occupational disease mainly affecting farm workers, slaughter house workers, veterinarians and laboratory personnel who have been in contact with affected animals or exposed to aerosols or dust particles from these animals. Self-limited illness is the most common form of Qfever with acute unspecific fever, severe headache, muscular and thoracic pain, and in some cases nausea and diarrhea. The patient may develop pneumonia or acute hepatitis. In chronic forms, patients may present endocarditis, hepatitis and chronic fatigue syndrome (1, 11).

As beef cattle production is one of the main economic resources of Uruguay, the slaughterhouse activity is one of the main industrial areas and it determines a high rate of exposure to possible zoonoses. The first human case of Q fever was reported in 1956 in abattoir personnel. Between 1975 and 2003, twenty three outbreaks were reported in workers related to the beef industry and in closed environment. The studies of serological prevalence of Q fever in different productive species, determined rates from 0.9 to 30% in bovines according to the animal category; 10.3% in sheep, 21.1% in swine and 5.5 to 21.7% in horses (14, 23).

Several open air Q fever outbreaks have been reported internationally. It is accepted that infective particles can be wind-borne for long distances and may survive in an adverse environment up to 6 months. In our country this is the first time that the disease is reported in humans in an open air situation and in which the source of infection is in wildlife (8, 21, 24, 25).

The objective of this study was to carry out an epidemiological investigation of this outbreak and to attempt to identify the reservoir so as to implement control and prevention measures.

METHODS

Preliminary investigation of the outbreak

On January 23rd 2004, the Eastern Region Health Area of the Ministry of Public Health reported in the Epidemiological Surveillance Network 5 cases of fever, respiratory syndrome and intense muscular pain in personnel dedicated to environmental maintenance in the Department of Maldonado which carried out their work in several tourist attraction areas.

The study started with a descriptive epidemiological survey. The data were acquired from several sources. A semi structured survey was carried out to

identify the areas where they could have contracted the disease, the attack rates were calculated, the type and area of their work, contact with animals or animal products and clinical symptoms.

From all these studies it was identified that these workers were mainly dedicated to grass mowing and collection, using power mowers and not using any personal protection equipment. The analysis of the attack rates by area led to the identification of two suspect areas with an attack ratio of 80%: a tourist castle and an experimental wildlife breeding station (EWBS). All personnel interviewed reported seeing rats, mice, dead pigeons and parrots. Due to these reports, a serological diagnosis of atypical pneumonia and Hantavirus was requested. Considering all the information provided and not having identified any affected family members or contacts a hypothesis of an occupational zoonosis was proposed.

Both suspect areas were studied and an active search for new cases was carried out. In the tourist castle, rodents were found and a domestic residue disposal area was found nearby but no dead pigeons and parrots were found. In the EWBS, the director and the veterinarian were interviewed using a semi closed form and all pertinent data on the animals was recorded, such as: species, number and origins, breeding records if any and previous health status.

It was noted that during 1990 there was 50% mortality in native deer (*Ozotoceros bezoarticus*) due to respiratory problems and neonatal deaths. This pattern remains with sporadically outbreaks up to now. The population of deer was 100; more than 50% were female. A group of 20 goats were introduced between February and December 2003 into a small paddock near the deer. Ticks were reported but no identification was carried out.

Serology results confirmed the presence of antibodies of *Coxiella burnetii* in 5 of the notified human cases and 2 other cases found using active search. With this information all efforts were concentrated on the EWBS.

Ecosystem characterization

The EWBS is in the suburban area of the city of Pan de Azucar, Department of Maldonado. The zone is surrounded by hills; there are approximately 60 properties, mostly unproductive fields. Only 11 of these properties belong to cattle breeding farmers.

The EWBS is an especially important tourist attraction. It is located in a 12 hectare Municipal area in the foothills of Pan de Azucar hill at 500 meters above sea level. Until 1980, it was a stone quarry that was transformed later on to its present status. This previous use and its location in the foothills of Pan de Azucar hill provide a very unique micro climate. The predominant winds are from NNE and NE with weather systems originating in Brazil and SW Argentine and their passage around the hills produces high wind speeds.

EWBS premises

At the entrance there is a restaurant and children's playground. The remaining area is divided in small paddocks and enclosures dedicated to breeding and reproduction of deer and maintenance of native wild life species (such as jaguars, pumas, otters, capybaras, weasels, wild boars, ferrets, birds and reptiles). The Maze is an area with small enclosures and dense vegetation where the smaller species are kept. It is surrounded by stone quarries and areas free from domestic animals [Figure 1 (Map 1)].

1	main path
2	feline maze 1 and 2
3	maze 4 de la piedra path
4	maze 5 nocturnal rapacious birds
5	maze 3
6	deer sector
7	parador and children playground

1	vantage point
2	birdcage
3	"Campo Grande"
4	reptails
5	toilets

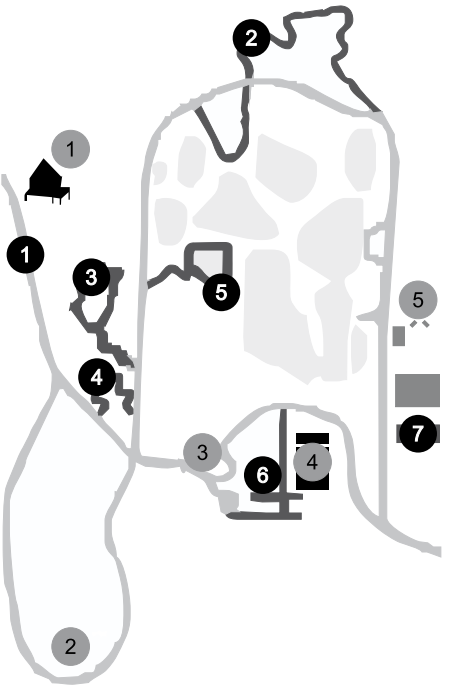

Figure 1 (Map 1). Experimental Wildlife Breeding Station premises

Human resources at the EWBS

There are 19 members in the permanent staff (one in administration and the rest in field operations), there is also a high turnover in temporary personnel. Some activities are carried out by army personnel from a nearby post, there are policemen in charge of security and 12 staff members in the restaurant. A TV station has its antenna at the top of the Pan de Azucar hill and there are two technicians from Monday to Friday. To reach the top of Pan de Azucar hill they have to cross the EWBS.

Epidemiological studies in humans

After the identification of the EWBS as the highest risk area, a nested case control study was proposed. The case group was defined as all personnel who had been occupationally related to the EWBS since January 2003 up to October 2004. (The first date is chosen to include the possibility of previous undiagnosed cases).

Case definition

Everyone that had or had had unspecific febrile syndrome with respiratory symptoms that had worked in the EWBS from January 2003 up to October 2004, with a confirmed serological diagnosis of Q fever (IgM or IgG).

Control definition

All persons who have worked in the EWBS during the same period with negative Q fever serology.

A survey was carried out to identify all personnel who had worked at the EWBS and a structured questionnaire was applied to identify the following:

1) Place of exposure to the agent, identification of paddocks, corrals, contact with animals or their products according to species.
2) Type of activity such as grass mowing, and collection, cleaning of corrals and presence at parturitions.
3) Risk attitudes such as drinking unpasteurized milk, animal slaughter, keeping animals at home.
4) Symptoms or previous record of Q fever.
5) Sample collection for serological diagnosis.

Epidemiological studies in animals

An in situ inspection was carried out, studying the different species and their habitat and information was collected on the animal management and breeding protocols. Samples were collected for serological studies and post mortem studies were carried out among some dead animals.

Laboratory studies.

Indirect immunofluorescence was used as diagnostic procedure for serological diagnosis in humans using Neumo-Bact ® manufactured by Vircell S.L (Granada, Spain) to detect IgM (>50) and IgG (>200). It was carried out in the National Laboratory of Public Health of the Ministry of Public Health.

For diagnosis in animals the ELISA test: Bornelli Q fever checkit for ruminants was used. Only ruminant samples were processed as there are no

commercial kits available for other species. These tests were carried out in the Veterinary Laboratories Division of the Ministry of Livestock, Agriculture and Fisheries.

As there is no high security facility (P3) in Uruguay no other diagnostic procedures were used nor isolation of *Coxiella burnetii* attempted.

Statistical analysis

The data was processed using EpiInfo (Centre for Disease Control and Prevention, Atlanta.). Through Pearson's chi-square several risk factors for Q fever were identified and the evaluation was made by Odds Ratio (OR) calculation at a significance level of 5%. 95% Confidence Interval (95% CI) was calculated. The high risk activities were analyzed according to the following definition: (1) activities that generated aerosols such as grass mowing with power mowers, raking and underbrush clearing; (2) activities in contact with animals, feeding, healing, cleansing collection of dead animals. (3) activities without apparent exposition to the infective agents such as administrative staff, park surveillance and working in the restaurant.

RESULTS

In Humans

A total of 117 persons in jobs related to the EWBS were studied: 19 permanent workers at the EWBS, 3 local government employees, 26 employed by a green areas maintenance company, 23 soldiers in maintenance activities, 32 policemen in charge of security, 12 restaurant employees, and 2 TV antenna technicians. These people worked at the EWBS from January 2003 to October 2004.

Q fever was confirmed in 25 cases, (prevalence of 21.37%, 95% IC: 14.3-29.9). The positive cases were distributed in the following way: 11 belonged to the green areas maintenance company, 6 to the EWBS, 3 local government workers, 3 soldiers and 2 TV antenna technicians. Age distribution was from 21 to 64 years of age (median 40 years), all males. The controls were 92 workers with negative serology, 98% were males and the age spread was from 20 to 62 years (median 39).

The distribution of the cases by month is shown in [Figure 2 (Graph 1)]. The highest peak of cases was in February 2004 and the active search for cases showed 3 cases in March 2003, showing that the problem started earlier. The delay in implementing the control measures determined the occurrence of new cases after the diagnosis of the outbreak.

Of the 25 confirmed cases, 17 presented clinical symptoms and positive serology and 8 cases with no clinical symptoms but positive IgG antibodies had already suffered the disease describing compatible symptoms. Fever (39 to 42°C)

was the predominant symtom, reported by all 25 cases, other symptoms were: mialgias, headaches with eye pain; in 22 cases, artrithis and sweating in 25, 24, 22 and 20 cases respectively. In 15 cases thorax X rays showed pneumonia with thoracic pain and coughs. Only one case had hepatitis.

The mean duration of the disease was 14 days (7 to 60 days), 47.06% of the affected persons had to be hospitalized for a mean of 9.7 days and 2 cases had a relapse and complications. The confirmed cases were treated with tetracycline or its derivatives during 3 weeks to shorten the disease period and prevent relapses and chronic cases (5, 9, 15, 19). All these patients were followed up serologically for 6 to 12 months. There were no fatal cases.

All positive cases had worked in the EWBS in the high risk areas and carrying out high risk jobs at least 5 days before the onset of symptoms. The survey records showed that the cases had not been exposed outside the EWBS to animals that had given birth, had abortions nor had handled afterbirth nor had taken unpasteurized milk. One of the cases had mowed grass on a farm with 2 milking cows.

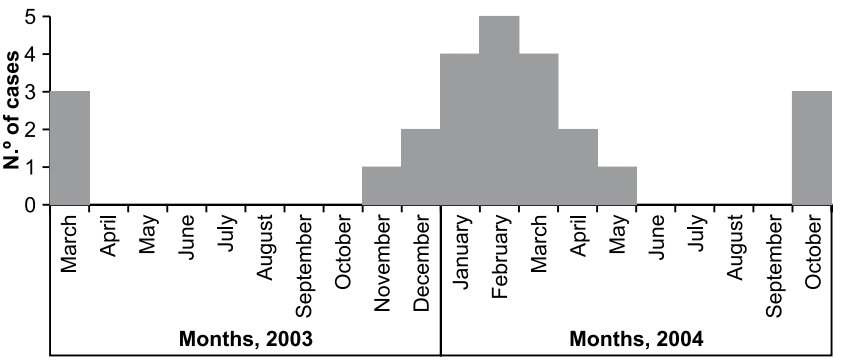

Figure 2 (Graph 1). Q fever cases by month

Risk Areas

72% of the personnel interviewed identified the "campo grande" (big paddock) and the deer enclosures as the high risk areas and they said that the majority of the people who worked there afterwards fell sick. They also indicated the strong presence of animal affected with breathing difficulty and stillbirths. The evaluation of the cases in relation with the areas where they worked allowed to identify as high risk areas the deer enclosures, with an attack rate of 82.35% (14/17), the "campo grande" enclosure with an attack rate of 81.81% (9/11) and the internal paths leading up to the "Pan de Azucar" with an attack rate of 5.41% (2/37). Chi-square showed a statistically significance $p<0.05$. No cases were recorded in other areas such as The Maze, birdhouse, restaurant and administration (Table 1).

Table 1. Distribution of the cases and controls according to the place of exposure, attack rate, OR and 95% CI.

Place of exposure to the agent	Exposed to risk		Not exposed to risk		Attack rates (%)	OR (95% IC)
	Cases	Controls	Cases	Controls		
Deer enclosures	14	3	11	89	82.35 (55.80-95.33)*	37.76 (8.22-199.90)*
"Campo grande"	9	2	16	90	81.81 (47.75-96.79)*	25.31 (4.43-188.72)*
Internal paths leading up to the "Pan de Azucar" hill	2	35	23	57	5.41 (0.94-19.53)*	0.14 (0.02-0.68)*

*IC: Confidence interval
Source: Epidemiological Surveillance-Ministry of Public Health- Uruguay

Risk activities and procedures

The study of the tasks carried out by the affected Q fever personnel indicated that 68% (17/25) were exposed to aerosol generating tasks such as grass mowing, raking and underbrush clearing; 20% (5/25) were in close contact with animals such as feeding, clearing the enclosures, parturitions, healing and cleansing the deer (it should be noted that the veterinarians and their assistants were serologically negative); 12% (3/25) were only exposed to the environment as they used the paths up the hill whilst grass mowing was being carried out. A significant statistical association was found between being affected by Q Fever and carrying out aerosol generating activities or being exposed to them, $p<0.05$. No significant statistical association was found between close contact with animals and being affected with Q fever. OR and CI indicated the aerosol generating activities as the highest risk (OR: 10.91, CI 3.61-34.08) and the sole exposition to the environment was not a risk factor. (OR: 0.08, CI 0.02-0.31) (Table2). All the Workers did not have the necessary protection to develop their tasks (masks, goggles, gloves). Important structural problems were identified (the following facilities for workers were not appropriate: toilets, dressing rooms, animal quarantine and infirmary). Moreover, there was no procedures manual.

Table 2 Distribution of the cases and controls according to the risk activities, attack rate, OR and 95%CI.

Risk activities	Exposed to risk		Not exposed to risk		Attack rates (%)	OR (95% IC)
	Cases	Controls	Cases	Controls		
Grass mowing, raking and underbrush clearing	17	15	8	77	53.13 (35.03-70.49)*	10,91 (3,61-34,08)*
Sole exposition to the environment	3	58	22	34	4.92 (1.28-14.60)*	0,08 (0,02-0,31)*

*IC: Confidence Interval
Source: Epidemiological Surveillance-Ministry of Public Health- Uruguay

In animals

The serological surveys and the remote and present anamnesis on the health status of the animals identified the deer, goats and other animals lodged in the "campo grande" enclosure (capybara and ostriches) as suspect. Stillbirths and early deaths were observed in deer and capybara and some deaths in deer with respiratory problems. Parturition in the deer started in October and carried on up to April. No confirmation of the cause of death was carried out (no post mortems nor laboratory tests) and several deficiencies were found in the enclosures and the management of the different species which were mingled with other local wildlife such as rats, field mice, possums and birds. The breeding and feeding conditions do not resemble the original environment of the different species.

Serological tests were carried on 22 deer and 4 goats. As the deer are very sensitive to high stress conditions and difficult to catch the sample was drawn from what was available at that time: 4 adults (2 male, 2 female), 7 yearling (4 males, 3 females), 7 young calves and 4 other with no information. 22% were positive, (5/22), 2 adult females and 3 yearlings. All goats were negative.

DISCUSSION

The environmental and epidemiological data suggest that the Q fever outbreak in the EWBS was associated with the inhalation of contaminated aerosols mainly generated by grass mowing with power mowers in personnel working without adequate personal protection. Airborne spread by aerosols has been often indicated as the most common and possibly the most efficient mode of transmission of the disease. (3,6,26). The use of power mowers generates large clouds of dust thereby increasing the probabilities of inhaling large numbers of *Coxiella burnetii* spores a situation which is further aggravated when the mower operators are not using any sort of personal protection such as face masks or goggles. This lack of personal protection, the lack of Standard Operating Procedures on biosafety measures added to the general lack of safe conditions in the buildings and surrounding areas probably has contributed in great measure to the transmission of the disease. The correction of these deficiencies will surely contribute to the control and prevention of new outbreaks.

The probable source of infection were the deer as some tested positive and this species is the most numerous, with the highest number of births and many animals with symptoms compatible with those that have been reported in other ruminants.(2, 13, 22).

The peak incidence in humans coincides with the possible increase of shedding of the agent into the environment during the parturition period, as has also been reported. (2, 13, 16).

The fact that the veterinarians and their assistants were not affected indicates that close contact was not statistically important as a mode of infection; this could be related to the fact that these technicians were not involved in grass mowing operations and took better personal hygiene. Nevertheless it should be stressed that due to the high occupational exposure of the veterinarians and their assistants they should be trained in biosafety measures and provided with the adequate equipment.

Due to the lack of adequate diagnostic kits it was not possible to test other species and this does not rule out the possibility of other species being infected. The dense vegetation and the availability of food stimulate the invasion of other local species (12, 13, 27).

It was not possible to process environmental samples due to the lack of adequate facilities so no information is available on the degree of environmental contamination. The enclosures are in the lowest part of the EWBS therefore all rainfall drains onto them, this fact and the distribution of the predominant winds in the area suggest the possibility this disease spreading to neighboring farms as has been reported. (24,25) This will be presented in a further report.

CONCLUSIONS

Is the first time that Q fever is reported in humans in an open air situation and in which the source of infection is in wildlife.

Q fever is an important occupational disease, inhaled particles are a significant source of the disease and this must be taken into account when personnel are involved in aerosol generating tasks without the adequate protection.

Biosafety should be considered as top priority, Standard Operating Procedures should be prepared for each activity and all the personnel assigned to high risk activities should be trained in the use of adequate protection equipment which should be provided.

The use of power mowers in areas potentially contaminated with excretions and secretions of infected animals must be taken into account in further research.

The field deer (*Ozotocerus bezoarticus)* can be considered as the reservoir without ruling out other species.

RESUMEN

Brote de fiebre Q en una estación experimental de Fauna Autóctona en Uruguay

Se reporta un brote de fiebre Q en una Estación Experimental de Fauna Autóctona (EEFA) en el departamento de Maldonado-Uruguay ocurrido durante el 2003-2004. Se realizó un estudio epidemiológico descriptivo y posterior caso control anidado con el fin de identificar factores asociados a la ocurrencia de fiebre Q (contacto con

animales, lugares, hábitos y actividades de riesgos). El diagnóstico en humanos y animales fue realizado por serología. Se confirmaron 25 casos en humanos de 117 trabajadores estudiados (prevalencia: 21.37%, 95% IC:-14.3-29.9%). Los resultados de la investigación epidemiológica sugieren que el brote epidémico de fiebre Q en la EEFA se asoció a la inhalación de aerosoles contaminados generados fundamentalmente por el corte de pasto con máquinas bordeadoras (OR: 10.91, 95% CI: 3.61-34.08). El venado de campo *Ozotoceros bezoarticus* fue identificado como reservorio de la infección, no obstante la participación de otras especies no se pudo excluir.

PALABRAS CLAVE: Fiebre Q. Brote. Rickettsiosis. Reservorio.

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