

Anthrax in cattle in southern Brazil: 1978-2006¹

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ABSTRACT.- Schild A.L., Sallis E.S.V., Priebe A.P., Soares M.P., Almeida M.B., Ladeira S.R.L., Schramm R. & Riet-Correa F. 2006. **Anthrax in cattle in southern Brazil: 1978-2006.** *Pesquisa Veterinária Brasileira* 26(4):243-248. Laboratório Regional de Diagnóstico, Faculdade de Veterinária, Universidade Federal de Pelotas, Campus Universitário s/n, Pelotas, RS 96010-900, Brazil. E-mail: alschild@terra.com.br

Ten outbreaks of anthrax occurred in cattle from 1978 to 2006 in southern Brazil, in 5 municipalities on the border with Uruguay, a country where the disease is frequent. The 10 outbreaks represented 0.2% of all bovine specimens received during the period by the Regional Diagnostic Laboratory of the Federal University of Pelotas, causing 267 deaths in a risk population of 6,605 head. The disease affected young and adult cattle mainly during summer. Only one farmer reported that sheep and horses were also affected. Clinically the peracute form was more frequent, but in some outbreaks the acute form with a clinical manifestation period of 6-48 hours was also observed. The source of infection was not established; but the reduced rainfall, associated with low, flat, flooded lands used for agriculture followed by animal grazing after harvest was probably related to the disease occurrence. Annual vaccination is an efficient way to prevent the disease.

INDEX TERMS: Anthrax, *Bacillus anthracis*, infectious disease, disease of cattle.

RESUMO.- [Carbúnculo hemático em bovinos no sul do Brasil: 1978-2006.] Foi realizado um estudo retrospectivo da ocorrência de carbúnculo hemático em bovinos na área de influência do Laboratório Regional de Diagnóstico (LRD) da Faculdade de Veterinária da Universidade Federal de Pelotas. Entre 1978 e março de 2006, dez surtos da enfermidade ocorreram em cinco municípios da região sul do Rio Grande do Sul, todos localizados na fronteira com o Uruguai. A enfermidade representou 0,2% dos diagnósticos realizados no LRD no período. Ocorre em bovinos jovens e adultos, principalmente em verões quentes e secos, e com menor freqüência em outras épocas do ano. Freqüen-

temente, a doença se apresenta na forma hiperaguda, entretanto casos de carbúnculo hemático agudo podem ser observados. Não foi possível estabelecer a origem das infecções, mas precipitações pluviométricas menores que as normais, em campos planos, sujeitos a alagamentos freqüentes, utilizados para plantio e posterior uso das restas (restolhos) para a criação dos animais podem ter favorecido a ocorrência dos surtos. A prática da vacinação anual dos animais é a forma de evitar eficientemente os surtos da doença.

TERMOS DE INDEXAÇÃO: Carbúnculo hemático, *Bacillus anthracis*, doenças infecciosas, doenças de bovinos.

INTRODUCTION

Anthrax is a highly fatal infectious disease caused by *Bacillus anthracis*, a Gram-positive, aerobic, encapsulated bacillus which produces spores that in favorable conditions can persist in the environment for decades before infecting the host. It is one of the oldest known diseases affecting animals and man (Hart & Beeching 2002). More recently considerable importance has been given to *B. anthracis* due to its potential use in biologic warfare and terrorism (Öncü et al. 2003). It was considered a threat to public health in USA, after 2001, when the bacteria were liberated intentionally in that country (Bales et al. 2002).

Anthrax is endemic all over the world, but the possibility of the bacteria to grow and multiply as saprophytes in the environment

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has not been established definitively. Apparently, it is present in the normal soil flora in low numbers, but under ideal conditions, in soil rich in nitrogen and organic matter, with pH above 6 and a temperature higher than 15.5°C, associated with high rains followed by dry conditions, the number of spores increases and the herbivores become infected. It has been recognized that spores of *B. anthracis* float on water, which causes their dispersion in wet conditions, followed by concentration when dry conditions occur (Turner et al. 1999, Hart & Beeching 2002).

In Brazil, anthrax was reported for the first time in the forties, but there were anecdotal reports of the disease before that (Langenegger 1994). The disease was reported in Rio Grande do Sul affecting cattle, sheep, horses, goats and pigs (Saraiva 1981, Santos et al. 1988, Ferreira et al. 1991, Schild et al. 1994, Driemeier 1996), in Santa Catarina affecting pigs (Oliveira et al. 1984), in Minas Gerais affecting cattle, horses and sheep (Vaitsman 1945, Portugal et al. 1982, Langenegger 1994), in Rio de Janeiro affecting cattle (Langenegger 1994), in São Paulo affecting cattle, horses and man (Portugal et al. 1982, Langenegger, 1994), and in Pernambuco and Rio Grande do Norte, northeastern Brazil, affecting cattle, goats, and man (Braga 1942, Langenegger 1994).

The disease occurs in all South American countries and Mexico. In Uruguay, in 1965, 215 cattle died due to anthrax; later 29 outbreaks of the disease were reported in a 6 years period, affecting cattle in 81% of the cases (Leaniz-Rivara & Gil-Turnes 1972). More recently, from January 2003 to November 2005, 19 outbreaks of anthrax occurred in the Departments of Treinta e Tres, Rocha, Cerro Largo, and Durazno, being 18 in cattle, and one in horses with the death of 3 horses out of 18. Most outbreaks occurred between January and March; in a risk population of 4,103 cattle, morbidity rate was 4.38% varying from 1.25 o 13%, and fatality rate was 100%, with the exception of two outbreaks where it was of 85.7 and 83.3% respectively (Dutra 2006).

In Argentina, between 2000 and 2005, from 180 samples with suspicion of anthrax, 25 were positive. Those outbreaks started in 2003 and were markedly seasonal; 18 of them occurred from January to March (Rojas et al. 2005).

Epidemiologic conditions for the occurrence of anthrax have been studied in countries where the disease is endemic. In Australia the disease was observed on 83 farms in 1997. It was suggested that the outbreaks were associated with a long dry spell with high humidity and higher than normal soil temperatures, after a proceeding wet winter in a poorly drained area where operations were performed to renovate irrigated pasture, water channels and drainage systems. (Turner et al. 1999). In Canada, outbreaks occurred at the end of summer and start of autumn, after a long period of unusually warm and dry spring weather. The high temperatures may have facilitated the multiplication of *B. anthracis* in the environment, and due to the shortage of grass the animals were forced to graze closer to the soil and potentially became exposed to anthrax spores (Parkinson et al. 2003).

Anthrax has been controlled in endemic areas by systematic vaccination of the most vulnerable species, mainly cattle, along with restrictions in animal transportation (Turner et al. 1999).

Because the spores survive in the environment for many years, it is necessary to maintain vaccination in the absence of outbreaks (Leaniz-Rivara & Gil-Turnes 1972).

The aim of this paper is to report outbreaks of anthrax, from January 1978 to March 2006, in southern Rio Grande do Sul, Brazil.

MATERIALS AND METHODS

The records of the Regional Diagnostic Laboratory of the Federal University of Pelotas, Rio Grande do Sul, from January 1978 to March 2006, were reviewed for the occurrence of anthrax. All diagnoses were made on specimens sent by veterinarians or collected by the author(s) in visits to the affected farms. Data of the municipality and season of occurrence of the disease, age and breed of affected animals, and the use of anthrax vaccines on the farms were recorded. Outbreaks were numbered from 1 to 10 in chronologic order. Climatic data in the area of occurrence were obtained from the National Institute of Meteorology (INMET 2006).

The diagnosis was made on blood smears stained by Gram and metachromatic methylene blue, and by cultures of the agent on 5% sheep blood agar. Blood was obtained from an amputated ear or metatarsal bone, or collected with a syringe from subcutaneous exudates, jugular vein, or bloody discharges from the natural orifices. These specimens or the bacteria isolated were also inoculated into guinea pigs or mice subcutaneously or in mice by scarification of the tail.

RESULTS

Epidemiology

Ten outbreaks of anthrax were confirmed from January 1978 to March 2006, representing 0.2% of all the bovine specimens received by the Regional Diagnostic Laboratory during the period, causing 267 cattle deaths in a risk population of 6,605 head. Epidemiological data of those outbreaks are presented in Table 1. In Table 2, the meteorological data from the area where outbreaks occurred are reported.

All outbreaks occurred in cattle in the southeastern and southern region of the state, in municipalities on the border with Uruguay (Fig.1). Five outbreaks occurred between January and March, three outbreaks between May and July, and two outbreaks in October-November.

Outbreaks 2, 3, 6, 8 and 9 affected crossbreed beef cattle, in Outbreaks 4 and 5 the records had no information about breed, Outbreak 7 affected Red Angus and Holstein cattle, and Outbreaks 1 and 10 affected Hereford cattle. Morbidity rate varied between 0.53 and 15% and fatality rate was 100%, except in Outbreaks 4 and 8, in which fatality rate was 94.7 and 96.6% respectively. Farmers informed that two cattle in Outbreak 4 and one in Outbreak 8 survived after treatment with antibiotics. Outbreaks 1, 4, 5, 6 and 9 affected 1-2 years old cattle, but in two of those outbreaks (1 and 5), animals 4 years old or older were also affected. In Outbreak 7 cattle of different ages were affected. In Outbreaks 2, 3 and 8 the disease affected 4-year-old or older cattle. The veterinarian involved with Outbreak 10 reported the death of 50 adult cattle and 8 calves.

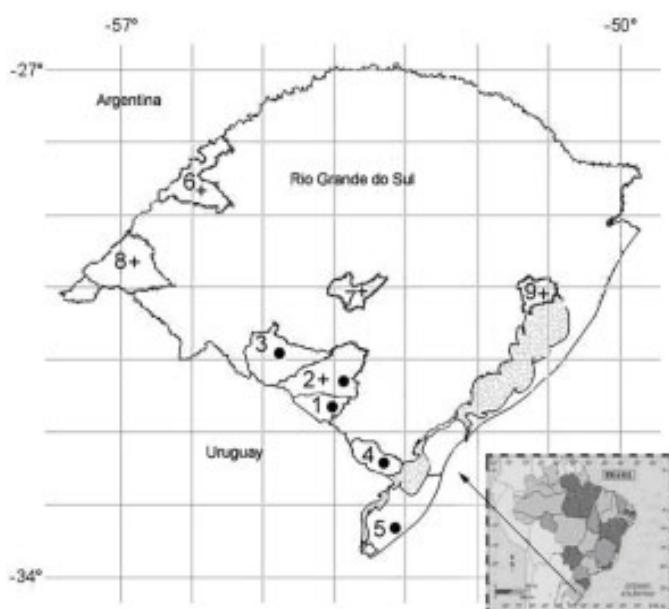
In Outbreak 2 the farmer informed that in a neighboring farm approximately 30 cattle died with the same disease. The veterinarian involved with Outbreak 8 reported that in two

Table 1. Epidemiologic data of the outbreaks of anthrax in southern Rio Grande do Sul

| Municipality/ Outbreak no. | Month/ year | Vacci- nation | Age (years) | Cattle number | | | | | |
|-------------------------------|----------------|------------------|-------------------|---------------|----------|-------|-----------|-----------|----------|
| | | | | At risk | Affected | Death | Morbidity | Mortality | Fatality |
| Jaguarão / 1 | 2-3/1988 | no | 1.5 adult | 1200 | 43 | 43 | 3.58% | 3.58% | 100% |
| Sta Vitória do Palmar / 2 | 5/1990 | no | 4 | 80 | 1 | 1 | 1.25% | 1.25% | 100% |
| Sta Vitória do Palmar / 3 | 10/1991 | na ^a | 4 | 300 | 18 | 18 | 6% | 6% | 100% |
| Bagé / 4 | 1/1993 | na | 1-2 | 300 | 38 | 36 | 12.6% | 12% | 94.7% |
| Sta Vitória do Palmar / 5 | 6-7/1996 | yes | 2-4 | 1100 | 8 | 8 | 0.72% | 0.72% | 100% |
| Bagé / 6 | 11/1999 | yes | 2 | 425 | 8 | 8 | 1.88% | 1.88% | 100% |
| Aceguá / 7 | 5/2003 | no | Different ages | 1000 | 60 | 60 | 0.6% | 0.6% | 100% |
| Sta Vitória do Palmar / 8 | 1/2006 | no | 4 | 200 | 30 | 29 | 15% | 14.5 | 96.6% |
| Sta Vitória do Palmar / 9 | 2/2006 | yes | 2 | 800 | 6 | 6 | 0.75% | 0.75% | 100% |
| Dom Pedrito / 10 | 1-3/2006 | no | 4 calves | 1200 | 58 | 58 | 4.83% | 4.83% | 100% |
| Total | | | | 6605 | 270 | 267 | 4.08% | 4.04% | 98.8% |

^a Not available.**Table 2. Mean, maximal and minimal temperatures, rainfall, and relative humidity during the period of occurrence of anthrax, and the normal values in the municipalities where the disease occurred**

| Municipality/ Outbreak no. | Month/ year | Temperature (°C) ^a | | Rainfall (mm) ^a | | Relative humidity ^a | |
|-------------------------------|-----------------------|-------------------------------|-----------------------|----------------------------|-----------------------|--------------------------------|-----------------------|
| | | (Mean) | (Normal) ^b | (Mean) | (Normal) ^b | (Mean) | (Normal) ^b |
| Jaguarão/1 | 2-3/1988 ^c | Na ^d | 27.9/19.1 | Na | 153.3 | Na | 79.9 |
| Sta Vitória do Palmar/2 | 5/1990 | Na | 20.8/11.1 | Na | 100.7 | Na | 83.6 |
| Sta Vitória do Palmar/3 | 10/1991 | 21.7/12.7 | 22.2/13.6 | 41 | 100.7 | 71.8 | 79.5 |
| Bagé/4 | 1/1993 | 27.8/20.1 | 28.2/19.1 | 177 | 119.1 | 75 | 77.4 |
| Sta Vitória do Palmar/5 | 6-7/1996 ^c | 12.52/9.61 | 17.8/8.6 | 23.68 | 105.7 | 80.49 | 84.0 |
| Bagé/6 | 11/1999 | 23.96/17.44 | 24.6/15.3 | 27.9 | 99.5 | 67.99 | 76.0 |
| Aceguá/7 | 5/2003 | 19.88/14.1 | 20.8/11.1 | 71.58 | 100.7 | 79.07 | 83.6 |
| Sta Vitória do Palmar/8 | 1/2006 | 28.90/21.50 | 28.2/19.1 | 72.10 | 119.1 | 76.58 | 77.4 |
| Sta Vitória do Palmar/9 | 2/2006 | 29.39/20.91 | 27.9/19.1 | 88.42 | 153.3 | 73.95 | 79.9 |
| Dom Pedrito/10 | 1-3/2006 ^c | 24.85/21.02 | 28.2/19.1 | 88.42 | 153.3 | 73.95 | 79.9 |

^a Data obtained from Pelotas Agro-Climatological Station, Federal University of Pelotas, Rio Grande do Sul.^b Source: INMET 1971/2000 (mean of 30 years).^c Data of the month in which the diagnosed was performed.^d Not available.

neighboring farms the disease affected non-vaccinated cattle with a morbidity of 14% and a case fatality rate of 100%, but samples from dead cattle were not sent to the laboratory.

In Outbreak 4 cattle were introduced to the farm 9 months before the outbreak, from the municipality of Dom Pedrito. In Outbreak 6 cattle had been transported recently from another farm. In Outbreak 10 cattle were born on the farm and the only transportation of cattle was between this farm and a neighboring farm belonging to the same farmer.

In Outbreaks 1 and 5 cattle were vaccinated annually against anthrax, but Outbreak 1 started during annual vaccination. In Outbreak 2 only part of the herd had been vaccinated, and only one animal from the non-vaccinated cattle died. In Outbreaks 3, 4 and 6 there was no information about vaccination. In Outbreaks

Fig.1. Map of the state of Rio Grande do Sul showing the distribution of the outbreaks of anthrax reported in this paper (●), and outbreaks reported previously (+). 1-Aceguá, 2-Bagé, 3-Dom Pedrito, 4-Jaguarão, 5-Santa Vitória do Palmar, 6-São Borja, 7-São Sepé, 8-Uruguiana, 9-Viamão.



Fig.2. Animal dead of anthrax in Outbreak 4. This animal died at 9:00-10:00 pm and was photographed at 8:00 am the next day. The carcass is very much enlarged due to bloat and gaseous distention, and dark tarry blood is exuding from the nose.

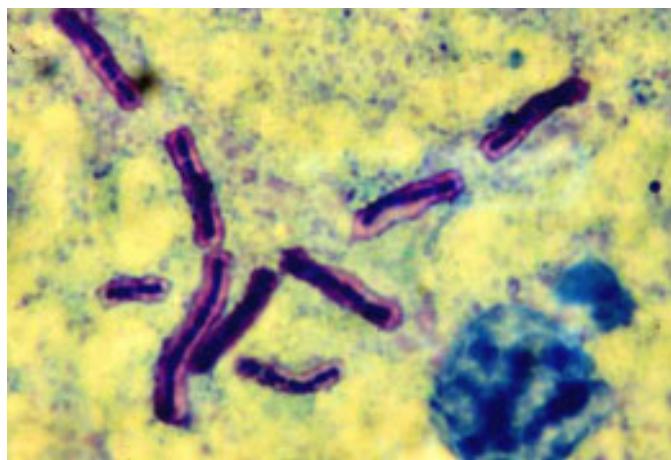


Fig.3. *Bacillus anthracis* in a blood smear from the animal shown in Fig.1, stained with polychromatic methylene and showing the typical capsule. Obj.100.

7, 8 and 10 cattle were not vaccinated against anthrax. In Outbreak 9 cattle had been vaccinated 5-7 days before the start of the outbreak.

In Outbreak 7 the farmer reported that the disease was occurring since some months ago, with sporadic deaths, and that it affected also 15 sheep out of 1,000 and 4 horses out of 30. Nearly 90% of the deaths occurred in the same paddock. On this farm, the owner also mentioned that the disease occurred more than 30 years ago, and after many years of vaccination this practice was abandoned.

In Outbreaks 1 and 10 the first cattle that died had clinical signs of tick fever and specimens sent to the laboratory were positive for *Babesia bovis*. Later, anthrax was diagnosed in cattle found dead without the observation of clinical signs. In Outbreak 4 cattle were vaccinated against tick fever 45-50 days before; anthrax was diagnosed in cattle with acute signs; they did not respond to tetracycline treatment.

Outbreak 10 occurred in a paddock where a large pond was almost dry and was the only area of the paddock with green pasture; in consequence the animals stayed there for long periods.

Clinical signs

In Outbreaks 3, 4 and 8 some cattle presented the acute form of the disease with subcutaneous edema of the neck, and other body regions, marked depression, incoordination, dyspnea, anorexia, and temperature higher than 40°C, with death within 6-48 hours. In the other outbreaks the animals were found dead, suggesting that the disease was peracute.

Pathology

Cattle found dead had dark tarry blood, which did not clot, from the natural orifices, and assumed the typical "sawhorse" posture. In one case of the acute form the farmer was bringing the affected live animal to the Diagnostic Laboratory, but the animal was dead on arrival at about 11:00 pm; on the next day, 8 hours later, anthrax was suspected because the carcass was very much enlarged due to gaseous distention of the subcutaneous tissue (Fig.2). Because *Bacillus anthracis*-like bacteria were observed in the polychromatic methylene blue stain of blood smears (Fig.3), necropsy was not performed.

Necropsies were only performed in cases of the acute form of the disease in which there was not a suspicion of anthrax, and in cases in which a previous diagnosis of tick fever was made. In those cases, gross lesions were of severe diffuse hemorrhages of the subcutaneous tissues and serous membranes. The spleen was softened and enlarged with gelatinous liquid aspect. The liver was moderately enlarged, with a yellowish or dark color.

Diagnosis

Blood smears showed large Gram-positive rods, isolated or in chains, similar to *B. anthracis*. In the polychromatic methylene blue stained slides a metachromatic typical capsule was observed (Fig.3). In blood agar, non-hemolytic, smooth colonies, 4-5mm in diameter, flat, dry, grayish, with a granular ground-glass appearance were observed at 24-48 hours of aerobic incubation at 37°C. Guinea pigs or mice inoculated with material from the specimens sent by the practitioners or with the bacteria cultured on blood agar died 16-24 hours after inoculation, presenting edema and splenomegaly at necropsy.

DISCUSSION

The number of anthrax outbreaks from 1978 to 2006 represents 0.2% of all bovine specimens received by the Regional Diagnostic Laboratory during the period, causing 259 deaths in a risk population of 6,605 head, suggesting that it is of low economical importance in the region. Nevertheless, the endemic occurrence in the southern and southeastern region of Rio Grande do Sul suggests that cattle in these regions should be vaccinated annually. The lack of vaccination may have been responsible for the occurrence of primary cases of the disease after exposure to a primary source, such as soil from an old anthrax grave. Such a source could result in wide dissemination of the agent in the environment. Previous reports of the disease in the region

suggest that annual vaccination of cattle was the reason for the low frequency of anthrax (Schild et al. 1994). This situation appeared to be confirmed regarding Outbreak 7 where the farmer mentioned that the disease occurred more than 30 years ago, and after many years of vaccination this practice was abandoned.

The three outbreaks during 2006 represent apparently an increase in the number of occurrence. The lack of routine vaccination could be the reason for the increase in the number of outbreaks in this period. On the other hand an increase in the number of outbreaks was reported also from Argentina and Uruguay in the last 3 years, between 2003 and 2005 (Rojas et al. 2005, Dutra 2006), suggesting that other epidemiologic conditions may be responsible for such increase.

Outbreaks of anthrax are frequent in tropical and subtropical countries with high annual rainfalls. They are common after major changes in weather, such as heavy rains after a long period of drought, or a dry summer after heavy rains, always in temperatures above 15°C (Radostits et al. 1999, Turner et al. 1999, Parkinson et al. 2003). Observing the climate data (Table 2), it can be suggested that dry conditions can be associated with most outbreaks. The fact that 6 out of 10 outbreaks occurred in summer (December-March) suggests that some conditions during this season are favorable to the occurrence of anthrax, probably the higher temperatures at this time of the year, and/or the occurrence of draughts, which are more common during summer.

In an outbreak in Canada, grazing at short distance from the soil during forage shortage was suggested as a probable source of infection for cattle (Parkinson et al. 2003). This explanation seems questionable in the outbreaks reported in Rio Grande do Sul, because sheep which are numerous in the southern and southeastern region of the state and which always graze near the soil were not affected. In this area there are about 2,800,000 cattle and 1,000,000 sheep. Despite the large population of sheep in the region, anthrax was not diagnosed in this species during the period, and only one farmer mentioned the death of some of them. The reason for this difference in the occurrence of the disease between sheep and cattle is unknown.

The region where the disease occurs is characterized by low and plain lands, subject to flooding and used to cultivate rice, soybean or other grains, and for animal grazing, alternatively. Periodical plowing and frequent floods could be responsible for the presence of spores in areas where the disease occurred before. The use of previously plowed areas for animal grazing after harvest with the appearance of spores, which were previously underground, has been suggested as an explanation of the occurrence of the disease (Parkinson et al. 2003).

In Outbreak 10 the occurrence of the disease was probably associated with the concentration of animals grazing on an almost dry pond area, which was the only part of the paddock with green pasture. It is suggested that in flooded areas the spores of *Bacillus anthracis* float and disperse, and later, under dry conditions, they concentrate causing outbreaks (Turner et al. 1999).

Most outbreaks affected non-vaccinated animals of different ages suggesting that routine vaccination is very important in prevention of the disease. In Outbreak 9, cattle started to die 5-

7 days after vaccination. Probably in this outbreak the animals were incubating the disease at the time of vaccination. In outbreaks of anthrax it has been observed that mortality decreases markedly nearly 8 days after vaccination (Forshaw et al. 1996, Turner et al. 1999).

In Outbreak 4 there was the possibility of *B. anthracis* being inoculated with the blood used for the immunization against tick fever 40-50 days before. An outbreak of anthrax was recorded following the injection of infected blood for immunization against anaplasmosis (Radostits et al. 1999).

The occurrence of tick fever on two farms in the same period that anthrax occurred made the diagnosis of the disease difficult. This fact and also the occurrence of the acute form of the disease, with clinical signs not suggestive of anthrax, in the absence of necropsies, increase the difficulty of diagnosis.

Diagnosis can be reliably made with the polychromatic methylene blue staining of blood or exudates smears. Necropsies are not recommended because spores are formed in the presence of air with consequent danger of infecting both humans and animals and contaminating the environment, possibly for years. If the smears are positive for *B. anthracis*-like bacteria with the characteristic capsule the carcass should be incinerated immediately after the collection of materials for culture and/or animal inoculation.

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