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Corynebacterium ulcerans diphtheria: an emerging zoonosis in Brazil and worldwide

ABSTRACT

The article is a literature review on the emergence of human infections caused by *Corynebacterium ulcerans* in many countries including Brazil. Articles in Medline/PubMed and SciELO databases published between 1926 and 2011 were reviewed, as well as articles and reports of the Brazilian Ministry of Health. It is presented a fast, cost-effective and easy to perform screening test for the presumptive diagnosis of *C. ulcerans* and *C. diphtheriae* infections in most Brazilian public and private laboratories. *C. ulcerans* spread in many countries and recent isolation of this pathogen in Rio de Janeiro, southeastern Brazil, is a warning to clinicians, veterinarians, and microbiologists on the occurrence of zoonotic diphtheria and *C. ulcerans* dissemination in urban and rural areas of Brazil and/or Latin America.

DESCRIPTORS: *Corynebacterium Infections,* epidemiology. Disease Reservoirs, veterinary. Zoonoses. Communicable Diseases, Emerging. Review.

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INTRODUCTION

Diphtheria is a disease with acute evolution that shows local and systemic manifestations. It remains an important cause of morbidity and mortality on different continents, even in countries with child immunization programs.[4,35,58,82,92] The classical forms of diphtheria are caused mainly by *Corynebacterium diphtheriae*, which produces diphtheric toxin (DT), and are characterized by the presence of a grayish pseudomembrane at the infection site, due to the effects from multiplication of this bacillus and the host's immune response.[40,65,114] DT is a potent exotoxin of protein nature that has the capacity to act on all tissues, with special tropism for the myocardium, nervous system, kidneys and suprarenal glands.[40,82,93] Only the samples infected by specific bacteriophages that carry the gene *tox* are toxinogenic.[33]

Protection against DT can be acquired through vaccination with diphtheric toxoid (e.g. DTP and other combinations containing the diphtheric component), but not with somatic antigens.[6,35,a] The level of immunity diminishes at the end of childhood and adolescence, depending on the vaccination calendar and the reservoir of *C. diphtheriae* in the population.[35,77] Diphtheria may affect partially immunized individuals, independent of age, race or sex. Partial decline in immunity, allied with lack of vaccine boosts and lower exposure to the

bacillus, contribute towards sporadic occurrences of epidemics.[23,34,69,77]

In today's era of vaccination, the various changes observed in the epidemiology of diphtheria[14,35,69] can be partially explained by the prevailing low levels of diphtheric antitoxin in the adolescent and adult population.[15,29] In the 1990s, in Eastern Europe, the greatest epidemic of diphtheria recorded since the start of mass vaccination occurred, accounting for 80% of the cases notified worldwide. Most of the cases and deaths occurred among adults infected by the epidemic clone of *C. diphtheriae* subsp. *gravis,* which still circulates in Russia, Latvia, Lithuania and Belarus.[89,90,112,113]

In addition to changes in age group, diphtheria cases without pseudomembranes and cases of invasive infection of a variety of origins have been observed in individuals who had previously undergone vaccination. These cases of invasive infection such as endocarditis, osteomyelitis, arthritis, pneumonia and renal abscesses have been predominantly correlated with atoxigenic samples of *C. diphtheriae* subsp. *mitis*.[17,24,48,53,66,68]

The World Health Organization (WHO) has created the Disability-Adjusted Life Year (DALY) index, in which each unit represents one year of healthy life lost through disease, hospitalization or even death (taking into account the life expectancy in each country), per 100,000 inhabitants. The 2002 data on the mortality and incidence of diphtheria correlated the following regions with greater DALY indexes: Central America [Haiti (> 50 DALY units) and the Dominican Republic], Sub-Saharan Africa [Democratic Republic of the Congo and Serra Leone] and Asia [Nepal and Myanmar] (> 15 DALY units).[b] The current epidemic that has been occurring in India presents DALY > 7 and vaccination coverage less than 80%. Only 56% of the children are protected against diphtheria (≥ 0.1 UI/ml).[4]

The countries of South and North America, Western Europe and Oceania present DALY indexes of less than one.[b] In Brazil, the national immunization program was established at the start of the 1970s.[c,d] According to data from the Brazilian Ministry of Health, vaccination coverage now reaches 99% of the child population. However, over recent decades, cases of diphtheria have still been occurring in most Brazilian states.[48,66,68,e,f,g,h]

Over the period 2008/09 alone, 89 diphtheria cases were confirmed. In 2010, in three municipalities in the state of Maranhão, where vaccination coverage reaches 56%, 27 diphtheria cases were confirmed, with two deaths, and the majority were in children with complete vaccination schemes. The possibility of underreporting of cases in Brazil and other developing countries cannot be dismissed: there may be unawareness among the population regarding the need to seek medical care and difficulties in obtaining clinical-laboratory diagnoses in cases of diphtheria, especially when patients are partially protected against the action of DT.

Starting in the middle of the 1980s, the number of diphtheria of zoonotic nature caused by *C. ulcerans* increased in different countries. In England, the number of diphtheria cases due to *C. ulcerans* exceeded the number reported with the classical etiological agent, *C. diphtheriae*.[5,76,96,107,109,113,115] Although toxigenic *C. ulcerans* has now been recognized in several industrialized countries as an emerging pathogen, its capacity to cause disease in humans, including among the inhabitants of urban centers, is still often neglected.[19,70,108]

Thus, the present review had the aim of analyzing aspects of the emergence of *C. ulcerans* as an etiological agent for zoonotic diphtheria in Brazil and other countries that carry out triple bacterial vaccination (DTP), against diphtheria, tetanus and pertussis, and other combinations containing the diphtheric component.

METHODOLOGICAL PROCEDURES

Studies and surveys on zoonotic diphtheria were gathered by searching in the Medline/PubMed and SciELO databases. The search terms used were: "diphtheria", "*Corynebacterium ulcerans*", "zoonotic diphtheria", "laboratorial diagnosis", "diphtheria clinical cases", "molecular epidemiology" and "infected animals", covering the period from 1926 to 2011. Studies focusing on descriptions of diphtheria cases due to *C. diphtheriae* that occurred outside of Brazil were excluded. In addition, articles available in electronic format in Portuguese and English focusing on the diphtheria situation in Brazil, laboratory procedures and serum levels of diphtheric antitoxin antibodies in adults, were analyzed.

[b] Wikipedia: The Free Encyclopedia. Diphtheria World Map - DALY - WHO2002 [cited 2010 Nov 15]. Available from: http://en.wikipedia.org/wiki/File:Diphtheria_world_map_-_DALY_-_WHO2002.svg

[c] Ministério da Saúde, Fundação Nacional de Saúde, Centro Nacional de Epidemiologia. Evolução temporal das doenças de notificação compulsória no Brasil de 1980 a 1998. *Bol Epidemiol*.1999; 3 Ed Espec:1-48.

[d] Ministério da Saúde, Fundação Nacional de Saúde. Difteria: situação atual da doença [cited 2002 Mar 23]. Available from: http://www.funasa.gov.br/guia_ epi/htm/doencas/difteria/index.htm

[e] Ministério da Saúde, Fundação Nacional de Saúde, Centro Nacional de Epidemiologia. Guia de vigilância epidemiológica. 4. ed. Brasília (DF); 1998 [cited 2010 Mar 10]. Difteria; capítulo 5.6. Available from: http://www.cro-rj.org/biosseguranca/Guia de%Vigilancia Epidemiologica.pdf

[f] World Health Organization. Diphtheria. Geneva; 2010 [cited 2010 Sep 7]. Available from: http://www.who.int/topics/diphtheria/en/index.html

[g] Santos LS, Sant'Anna LOS, Cosme LMSS, Hirata Jr RH, Mattos-Guaraldi AL. Perfis fenotípicos e genotípicos de amostras de *Corynebacterium diphtheriae* isoladas em recente surto epidêmico ocorrido no Maranhão. In: 44º Congresso Brasileiro de Patologia Clínica; 2010; Rio de Janeiro, Brasil. *J Bras Patol Clin,* 2010;46:4.

[h] Ministério da Saúde, Sistema de Informação de Agravos de Notificação – SINAN. Casos confirmados de difteria: Brasil e Grandes Regiões, 1997-2006. Brasília (DF); 2007 [cited 2010 Mar 10]. Available from: http://portal.saude.gov.br/portal/arquivos/pdf/casos_difteria.pdf

MICROBIOLOGICAL AND CLINICAL CHARACTERISTICS

The genus *Corynebacterium* belongs to the group of irregular Gram-positive rods, which grow aerobically and are immobile, non-sporulating and catalase-positive. Over the last few years, more than 60 species of corynebacteria have been described, and many of them are related to colonization and/or infection in humans and animals. Phylogenetic analysis on subunits of 16S rRNA sequences has made it possible to identify *C. ulcerans, C. diphtheriae* and *Corynebacterium pseudotuberculosis* as distinct species that are potential producers of DT.[57,91] *C. ulcerans* and *C. pseudotuberculosis* are capable of hydrolyzing urea, which presumptively differentiates them from the type species of the genus, i.e. *C. diphtheriae*.

The species *C. ulcerans*, which is gelatinase-positive and nitrate-negative and was described by Gilbert & Stewart in 1926, can produce several clinical conditions, depending on the type and quantity of toxins secreted.[8,36] Many studies have investigated pathogenicity mechanisms and DT production in samples of *C. diphtheria*,[13,47,49,69,73,101] but little has been documented about the virulence factors of *C. ulcerans*. The virulence mechanisms described for *C. pseudotuberculosis* include toxic lipids associated with the cell wall, which may mediate bacterial to attack by phagocytes.[42] Currently, a phospholipase D (PLD) exotoxin is considered to be the main virulence factor produced by *C. pseudotuberculosis* during cases of infection. PLD presents sphingomyelinase activity and may locally increase the vascular permeability, thereby contributing towards dissemination of the pathogen in the host's tissue.[3,50,72] *C. ulcerans* is also capable of producing PLD, as well as DT.[51]

Concern regarding the potential for *C. ulcerans* to emerge as a pathogen capable of carrying the DT-coding bacteriophage, and therefore capable of causing conditions of classical diphtheria, is not new. Studies have shown that some bacteriophages are selective regarding *C. ulcerans* and do not induce DT production.[45] However, it has also been observed that the bacteriophage β has the capacity to infect samples of *C. diphtheriae*, *C. pseudotuberculosis* and *C. ulcerans*, thereby constituting a risk of emergence of conditions of diphtheria caused by this species.[1,38,95,99] Variability of the capacity to produce DT, between samples of *C. ulcerans*, has already been documented.[39,116] The majority of clinical cases in humans and animals have been correlated with DT-producing strains (Tables 1 and 2).[11,18,56,62]

The following DT effects are produced by *C. ulcerans*: frequent occurrences of nose bleeding during infections; skin lesions that mimic typical cutaneous diphtheria; lesions of the tracheobronchial tree, with pseudomembrane; hemorrhage; compromised cervical lymph nodes; and cell death due to apoptosis.[8,17,63,111,115] Occurrences of necrosis, mucosal ulceration and clinical syndromes in the lower respiratory tract, such as pneumonia and granulomatous pulmonary nodules, have been attributed to production of DT and PLD.[16,44,52,97]

EPIDEMIOLOGICAL CHARACTERISTICS

Classically, the species *C. ulcerans* has been described as the etiological agent of a variety of infectious conditions in animals, especially mastitis in cattle. The first cases of infection in humans have usually been associated with consumption of unboiled or unpasteurized milk and derivatives, along with rural workers who are asymptomatic carriers of *C. ulcerans* in the nasopharynx. This shows the capacity of this pathogen to circulate between human and animal hosts and cause diphtheria of zoonotic nature.[2,7,43,46,52] In a general manner, diphtheric infections caused by *C. ulcerans* have been correlated with patients who have been partially immunized with diphtheric toxoid, negative results from investigating carriers and contacts and indeterminate infection sources.[108]

As presented in Table 1, in addition to conditions with characteristics similar to respiratory and cutaneous diphtheria, samples of *C. ulcerans* have also been correlated with other clinical conditions in humans, such as sinusitis, tonsillitis, pharyngitis, pneumonia and peritonitis. The growing number of cases of infections due to *C. ulcerans* in cats and dogs since 2006 emphasizes the importance of expanding the knowledge of the epidemiological aspects of this emerging zoonosis. Cases of infection due to *C. ulcerans* in various animals species such as monkeys, squirrels, otters, orcas, camels, lions, dogs, cats, pigs, goats and cattle have been described in the international literature (Figure 1 and Table 2).[1,17,18,55,62,96,103]

Among the 37 studies relating to infections in humans, it was possible to determine the sex of 37 individuals and the age in 32 cases. The majority of the cases (n = 24; 65%) and deaths (n = 5; 71%) were observed in females. Interestingly, most of the infections in animals due to *C. ulcerans* have also occurred in females (mastitis). Wagner et al[113] (2010) conducted a survey of cases of infections due to *C. ulcerans* and *C. diphtheriae* in England between 1986 and 2008, and also found that the cases were predominantly in women (76%), as were the deaths (75%). The mean age of the individuals with *C. ulcerans* (38 years) was higher than the mean age of those with *C. diphtheriae* (15 years). In addition, Table 1 shows that the individuals with *C. ulcerans* were older (mean of 53 years), with a large number over 60 years, which contrasts strongly with the age profile of

Table 1. Cases of infections due to *Corynebacterium ulcerans* in humans reported internationally in the literature.

Country (Year)	No. of Cases/Sex	Age/Child vaccination	Clinical condition	Examination on clinical material	Toxin Production	Outcome/Observations
England (1984)[7]	1/M	Adult/ND	Asymptomatic	Nasal swab	ND	Recovered/cow and milk samples positive for *C. ulcerans*
England (1984)[43]	1/F	Adult/ND	Pharyngitis	Oropharyngeal swab	Positive	Recovered/cows and milk samples positive for *C. ulcerans*
	1/M	Child (12 years)/yes	Asymptomatic	Oropharyngeal swab	Positive	
Denmark (1987)[83]	1/M	Child (9 years)/yes	Classical diphtheria	Oropharyngeal swab	Positive	ND
Denmark (1956-89)[75]	2/ND	ND	ND	ND	Positive	ND/Clone identical to one from Sweden
Switzerland (1990)[39]	1/M	Adult (63 years)/ND	Classical diphtheria with myocarditis	Pseudomembrane	Negative	ND
England (1990)[63]	1/F	Adult (73 years)/ND	Classical diphtheria	Pseudomembrane	Positive	Death
England (1992)[8]	1/F	Adult (44 years)/yes	Pharyngitis, dysphagia and hemorrhage	Tissue biopsy necrosed nasal tissue	Positive	Recovered
England (1994)[60]	1/F	Child (9 years)/yes	Macular pharyngitis and skin rash	Oropharyngeal swab	Positive	Recovered/visit to farm two weeks before clinical case
Germany (1994)[54]	1/F	Adult(42 years)/yes	Classical diphtheria	Fragment of pseudomembrane	Positive	Recovered/negative investigation on contacts
Denmark (1995)[16]	1/M	Adult (56 years)/ND	Coughing and several pulmonary nodules	Histopathology of nodules	Negative	Recovered
USA (1997)[11]	1/F	Adult (54 years)/ND	Classical diphtheria	Oropharyngeal swab	Positive	Recovered
England (2000)[12]	3/F	ND	01 Classical diphtheria / 02 Throat infections	Oropharyngeal swab	All positive	Death/ Recovered/ Patients undergoing serum therapy. All living in rural area. Negative
Germany (2001)[111]	1/F	Adult (71 years)/yes	Skin lesions on tibia (10 months)	Oropharyngeal and cutaneous swab	Weakly positive	Recovered
Japan (2001)[96]	1/F	Adult (52 years)/ND	Pharyngitis	Oropharyngeal swab	Positive	Recovered
Germany (2002)[115]	1/M	Adult (77 years)/ND	Necrotic sinusitis, fever, coughing and rhinorrhea	Nasal secretion	Positive	Death/Patient living in rural area. Death of cow with nasal discharge prior to clinical case
Switzerland (2002)[56]	1/F	Adult (71 years)/yes and booster in adulthood	Classical diphtheria	Oropharyngeal swab	Positive	Recovered
Germany (2003)[100]	1/M	Adult (40 years)/ND	Skin lesion	Cutaneous swab	Positive	Recovered
Netherlands (2003)[108]	1/F	Adult (58 years)/ND	Classical diphtheria	Oropharyngeal swab	Positive	Recovered
Japan (2003)[44]	1/F	Adult (52 years)/ND	Classical diphtheria	Oropharyngeal swab	Positive	Recovered/death of cat with nasal discharge prior to clinical case

To be continued

Table 1 continuation

Country (Year)	No. of Cases/Sex	Age/Child vaccination	Clinical condition	Examination on clinical material	Toxin Production	Outcome/Observations
Italy (2003)[110]	1/F	Adult (75 years)/ND	Classical diphtheria	ND	Positive	Recovered
Canada (1999-2003)[17]	3/ND	ND	Skin lesion and user of renal catheter	Cutaneous swab and catheter	Positive	ND
France (2005)[62]	1/F	Adult (47 years)/yes	Classical diphtheria	Oropharyngeal swab	Positive	Recovered/dog with chronic labial lesion positive for C. ulcerans. Negative investigation on 88 contacts
Japan (2005)[96]	1/M	Adult (57 years)/ND	Mumps	Oropharyngeal swab	Positive	Recovered
	1/M	Adult (51 years)/ND	Pulmonary infection	Pulmonary tissue biopsy	Positive	
	1/F	Adult (58 years)/ND	Classical diphtheria	Oropharyngeal swab	Positive	
England (2006)[25]	1/F	Adult (elderly)/ND	Tonsillitis	Oropharyngeal swab	Positive	Death/2 dogs positive for C. ulcerans
France (2006)[1]	1/F	Adult (70 years)/during treatment	Classical diphtheria	Oropharyngeal swab	Positive	Recovered/Dog positive for C. ulcerans. Negative investigation on 56 contacts
Japan (2007)[76]	1/M	Adult (55 years)/ND	Pulmonary condition, coughing, fever and weight loss	Pulmonary tissue biopsy	Positive	Recovered
England (2007)[25]	1/M	Adult (50 years)/yes	Pharyngitis, laryngitis and abdominal pain	Oropharyngeal swab	Positive	Recovered/negative investigation on contacts
Brazil (2008)[70]	1/F	Adult (80 years)/ND	Skin lesions with pseudomembrane and pneumonia	Tracheal aspirate	Positive	death/living in metropolitan region
USA (2008)[107]	1/M	Adult (77 years)/ND	Classical diphtheria with pseudomembrane throughout respiratory tract	Oropharyngeal swab	Positive	death/living in rural area
USA (2008)[107]	1/F	Adult (66 years)/ND	Classical diphtheria	Oropharyngeal swab	Positive	Recovered/living in rural area
Germany (2009)[95]	1/F	Adult (56 years)/ Vaccination in 1960 and 1998	Classical diphtheria	Oropharyngeal swab	Positive	Recovered
England (2009)[51]	1/F	Adult (elderly)/yes	Signs of diphtheria	Oropharyngeal and nasal swab	Positive	death/living in rural area/investigation of C. ulcerans in milk samples: 2 cats and 5 dogs (2 dogs positive)
England (2010)[105]	1/M	Adult (20 years)/ND	Tonsillitis and sore throat	Oropharyngeal swab	Positive	Recovered
Japan (2010)[61]	2/ND	ND	Nd	Oropharyngeal swab	Positive	ND
Japan (2010)[59]	1/F	Adult (55 years)/ND	Peritonitis	Peritoneal fluid	Negative	Recovered

ND: Not described

Table 2. Cases of infections due to *Corynebacterium ulcerans* and *Corynebacterium diphtheriae* in animals reported internationally in the literature

Country (year)	Host (no. of strains isolated)	Production of diphtheric toxin	Clinical condition	Animal contacts (n)	death of animals (n)	Observations
C. ulcerans						
USA (1972)[71]	Monkey (1)	ND	ND	ND	ND	-
USA (1974)[32]	Monkey (1)	ND	Mastitis	ND	ND	-
Romania (1977)[81]	Monkey (1)	ND	Granulomatous Dermatitis	Negative (271 monkeys)	ND	-
England (1984)[7]	Cow (ND)	ND	Asymptomatic	Positive	No	Owner recovered from infection
England (1984)[43]	Cow (ND)	Positive	Asymptomatic	Positive	No	Owner recovered from infection
Canada (1988)[78]	Squirrel(63)	Positive	Gangrenous dermatitis	Negative (287 squirrels)	Yes (6)	The 57 surviving animals received antibiotic therapy
USA (2000)[3]	Monkey (26)	Negative	Skin lesions	Negative	ND	Microorganisms were isolated from 56% of the skin lesions after implantation and from 3% of the oropharyngeal samples
Spain (2000)[106]	Camel (1)	ND	Caseous lymphadenitis	ND	ND	-
Scotland (2002)[104]	Cat (2)	Positive	Nasal discharge	Negative	No	-
England (2002)[31]	Otter (2)	Positive	Pulmonary infection	ND	Yes (1)	Strains from the same ribotype were isolated from pulmonary tissue of both animals
England (1986-2003)[18]	Cat (7)	Positive	Nasal discharge	ND	No	The same ribotypes as isolated from 50 individuals were isolated from the cats, thus suggesting that these animals might have acted as reservoirs for the pathogen
Japan (2004)[113]	Orca (2)	Positive	Purulent pneumonia	ND	Yes	Strains isolated from blood and lung tissue of 2 orcas (male and female) kept in the same aquarium
France (2005)[62]	Dog (1)	Positive	Chronic labial lesion	ND	Euthanasia (1)	Adult recovered from condition of classical diphtheria. The pathogen was isolated from the animal's skin lesions, tonsils and nostrils
Argentina (2005)[74]	Goat (1)	ND	Cerebral abscess	Nd	Euthanasia (1)	Strains isolated from cerebral abscess and from cerebrospinal fluid
England (2006)[25]	Dog (2)	Positive	Asymptomatic	Yes (2)	No	Owner died
France (2006)[1]	Dog (1)	Positive	Asymptomatic	Yes (1)	No	Owner recovered from classical diphtheria
Japan (2008)[113]	Lion (1)	Negative	Sepsis	ND	ND	Strain isolated from blood
Japan(2009)[55]	Dog (1)	Positive	Asymptomatic	Yes (65 dogs)	No	Strain isolated from oropharynx
Germany (2009)[95]	Pig (1)	Positive	Asymptomatic	Yes (19 pigs and 1 dog)	No	Adult recovered from classical diphtheria. Strain isolated from human was identical to one from pig. Investigation of 3 human contacts was negative

To be continued

Table 2 continuation

Country (year)	Host (no. of strains isolated)	Production of diphtheric toxin	Clinical condition	Animal contacts (n)	death of animals (n)	Observations
England (2009)[51]	Dog (2) and Cat (1)	Positive	Asymptomatic	Yes (5 dogs and 2 cats)	No	Owner died. Investigation of milk samples was negative
Brazil (2010)[19]	Dog (1)	Negative	Asymptomatic	Yes (60 dogs)	No	Strain isolated from nasopharynx. The carrier status was eliminated by means of antibiotic therapy. Investigation of human contacts was negative
USA (2010)[103]	Dog (1)	Negative	Bronchopneumonia	No	No	Two distinct strains were isolated from the same dog over a 3-month period. Investigation of carrier contacts of the owner was negative
C. diphtheriae						
USA (2010)[41]	Cat (2)	Negative	Severe otitis	Yes (3 cats, 2 dogs, 1 horse)	No	4 strains of *C. diphtheriae* var. *belfanti* were isolated from the ears of two cats. Investigation of 10 contacts (2 owners and 8 veterinarians)was negative.
Ireland (2010)[64]	horse(1)	Positive	Skin lesions	ND	No	Strain of *C. diphtheriae* var. *Gravis* was isolated from pus. Investigation of human contacts was negative

ND: Not Described

the cases of infection due to *C. diphtheriae* in Brazil and around the world.

As presented in Table 1 and Figures 1 and 2, *C. ulcerans* has been a growing cause for concern among the public health authorities in many countries: England,[25,113] France,[1] Germany,[100] Netherlands,[108] Italy,[110] Switzerland,[39,56] Denmark,[75] Japan,[44] Canada[17] and the United States.[12,13,107] Only five cases of human infection were documented prior to the 1990s, and these were in England and Denmark. Many cases of human patients who had not presented risk factors associated with infections due to *C. ulcerans* (such as consumption of untreated milk or contact with farm animals) started to be reported from 1990 onwards. Therefore, the risk of transmission among small animals and among humans has become a cause for concern.[1,17,18,55,62,70,96,107]

In addition to cattle and goats, dogs and cats have also become responsible for transmitting *C. ulcerans* to human hosts. In two cases of diphtheria caused by *C. ulcerans* that were described in France (2005 and 2006), the source of infection was the pet dog.[1,62] Ribotyping tests were also been conducted in order to determine the genetic relationship between the microorganisms isolated from these animals and those in humans. In the United Kingdom, it was observed that toxigenic samples isolated from cats with bilateral nasal discharges belonged to the same ribotypes of *C. ulcerans* that were isolated from infected humans, thus showing these animals' capacity to act as pathogen reservoirs.[18,104] Likewise, it was observed that a sample of *C. ulcerans* isolated from humans in Italy belonged to the same ribotype found in samples isolated in England.[110]

Although *C. diphtheriae* is classically considered to be a human pathogen, cases of infection due to *C. diphtheriae* in cats (severe otitis) and a horse (purulent skin lesions) were reported in 2010,[41,64] thus suggesting that zoonotic potential was also emerging with regard to *C. diphtheriae* (Table 2).

Transmission of *C. ulcerans* between humans has still not been demonstrated, but several researchers have recommended that infected patients should be isolated.[2,7,11,43,60,70,107] In the first and only clinical case of human infection due to *C. ulcerans* notified in Brazil,[70] the patient's vaccination history and the source of infection were unknown. A few days after the patient died, one of the healthcare professionals involved in the case presented a condition of pharyngitis consequent to exposure to the pathogen. He immediately started antibiotic therapy with erythromycin, before material was collected for culturing and identification. Although the culture presented a negative result, the patient presented signs of myocarditis in clinical cardiological

Figure 1. Worldwide distribution of notified cases of infection due to *Corynebacterium ulcerans* in humans (Stars) and in animals (Triangles), 1972 to 2010.
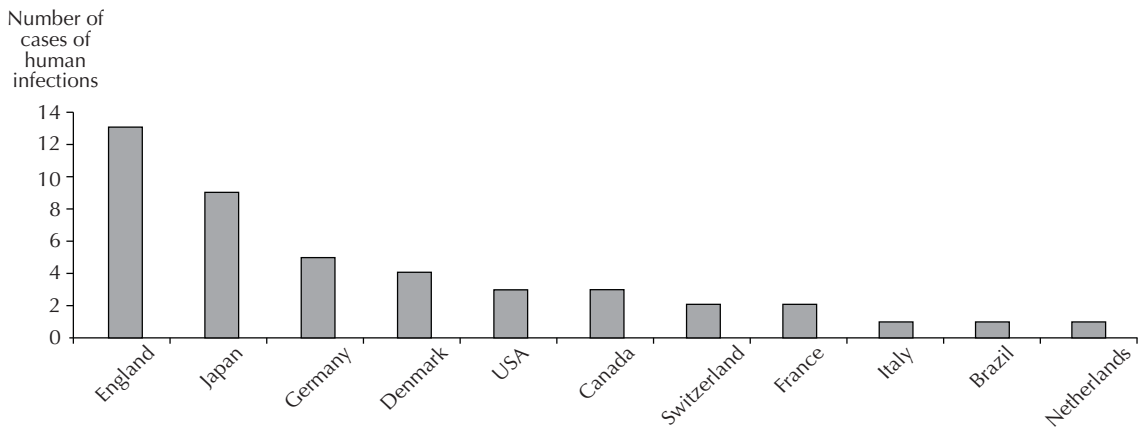

Figure 2. Distribution in different countries of cases of human infection due to *Corynebacterium ulcerans* reported in the literature available, 1984 to 2010.

examinations, including echocardiograms and repeated electrocardiograms.

The causes contributing towards the increase in the number of cases of infection due to *C. ulcerans* in humans and animals have still not been fully elucidated. Given that similar ribotypes have been observed in human and animal infectious processes, it is possible that some samples of *C. ulcerans* may present additional virulence factors resulting from selective pressure, associated with unfavorable socioeconomic conditions, especially in overpopulated areas in which inadequate sanitary and hygiene conditions prevail. In addition, in some countries

like the United States, the number of domestic animals living inside homes and circulating in areas such as kitchens and bedrooms, is very high,[i] thus favoring the process of transmission of various agents and zoonoses to humans, possibly including *C. ulcerans*. [20]

PRESENT SITUATION IN BRAZIL AND EMERGING COUNTRIES

As illustrated in Figure 1, the number of cases notified in emerging countries is very small. In Latin America, cases in animals have been described in Argentina[74] and Brazil,[19] but in humans, only in Brazil[70] (Tables 1 and

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[i] Marquezi D. Algo novo no reino dos humanos. *Galileu.* 2007;(197):41-53.

2). There are no records in the literature of isolation of samples of *C. ulcerans* in India, China, Oceania or the entire African continent. The hypothesis that circulation of this pathogen really occurs in all continents also needs to be put forward, and that there may be underreporting of cases because of lack of availability of laboratories qualified to isolate and identify the pathogen. Other than the United Kingdom, few countries present established laboratory routines and require compulsory notification of human infection due to *C. ulcerans* and isolation of atoxigenic samples.

In August 2010, the Brazilian Ministry of Health issued a warning[j] about the diphtheria situation in this country. This document gave information about changes to the clinical-epidemiological profile of the disease, such as the absence of pseudomembranes, deviation of the age group, occurrences of zoonotic diphtheria and circulation of *C. ulcerans* and *C. diphtheriae* in Brazil. Other points that were highlighted included: adoption of the same control measures as recommended for the species *C. diphtheria*, in confirmed cases of diphtheria due to *C. ulcerans*; and notification, treatment and monitoring of all suspected cases of diphtheria, with isolation of non-toxin-producing strains, by healthcare professionals.

In Brazil, a single human case of fatal infection caused by *C. ulcerans* was described in 2008,[70] and subsequently (2010), a dog was found to be an asymptomatic carrier. Both of these cases were in the metropolitan area of the city of Rio de Janeiro (Southeastern Brazil).[19] In summary, an elderly women presented several skin lesions on her legs, covered by pseudomembranes, and she died of cardiorespiratory complications despite antibiotic therapy serum therapy. The microorganism isolated from the patient's lower respiratory tract, which produced an exotoxin presenting differences in subunit A of the molecule, was shown to be resistant to the antimicrobials penicillin G (MIC 0.19 mg/l) and clindamycin (MIC 1.5 mg/l). Variations in sensitivity profiles had previously been observed by other authors.[15,37] In the same way as found on other occasions, like in the United States,[11,107] Switzerland,[39] Germany,[54,111] England[8,63,105] and Japan,[59,61,76,96] the origin of the infection was not elucidated (Tables 1 and 2).

According to Pesavento et al[84] (2007), several diseases are reemerging. Hypotheses raised to explain these occurrences include the sanitary status of the population, migrations, adaptation by pathogens to new environmental conditions and factors, among others. According to those authors, animal shelters present many conditions that predispose towards this phenomenon: stress, immunosuppression, overpopulation, high exposure, high turnover of animals, malnutrition and indiscriminate use of antibiotics, thereby becoming a risk factor for the human and animal populations.

Such findings were the motivation for conducting a microbiological survey among apparently healthy dogs at an animal shelter. Out of a group of only 60 dogs from which nasal and ocular secretions and skin lesions were analyzed, one female dog that was an asymptomatic carrier of *C. ulcerans* was detected.[19]

Findings of *C. ulcerans* in the environment, infecting and/or colonizing humans and/or dogs[19,70] suggests that the scarcity of epidemiological data in Brazil may be related to clinicians' and microbiologists' lack of attentiveness regarding the possibility of infection and a lack of awareness of the clinical and microbiological characteristics of infectious processes caused by *C. ulcerans.* Moreover, there are no routine procedures that enable isolation and identification of this pathogen in clinical laboratories.

BACTERIOLOGICAL DIAGNOSIS: TRIAGE SCHEME

The diphtheria epidemics that have occurred over recent decades have forced a generation of clinicians, laboratory scientists and epidemiologists in different parts of the world to recall old lessons and develop new methods for microbiological diagnosis, prevention, control and treatment of diphtheria.[6,17,21,26,27-29,35] In emerging countries, in which the incidence of diphtheria remains relatively high and the vaccination coverage continues to be insufficient, laboratory support should be implemented in the light of the recurrent deficiencies in this segment.[30]

It is essential to have accurate, rapid, economical and easily performed triage, with the capacity to make a presumptive diagnosis of diphtheria cases caused both by *C. ulcerans* and by *C. diphtheriae*, in the majority of Brazilian laboratories. This would have the aim of reducing the underreporting that results from false negative laboratory results.

In Figure 3, the proposed algorithm enables isolation of Gram-positive rods that are potential DT producers, using culturing media (blood agar and tellurite chocolate agar) and biochemical tests routinely used in clinical bacteriological laboratories, i.e. deoxyribonuclease (DNAse) activity, urea hydrolysis and the reverse CAMP reaction using a beta-lysine-producing strain of *Staphylococcus aureus*. If it is possible to use Tinsdale medium, the samples of cystinase-negative Gram-positive rods can be discarded. Cystinase-positive and

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                              ┌─────────────────────┐
                              │        SWAB         │
                              └─────────┬───────────┘
                                        ↓
                              ┌─────────────────────┐
                              │     Blood agar      │
                              │         +           │
                              │ Tellurite chocolate │
                              │ agar or Tinsdale    │
                              │      medium         │
                              └─────────┬───────────┘
                                        ↓
                         ┌──────────────────────────┐      ┌──────────────┐
                         │ Sow suspected colonies of│ ───→ │    Gram      │
                         │ Gram-positive rods (GPR) │      │  staining    │
                         │     or coccobacilli      │      └──────────────┘
                         │      in blood agar       │
                         └──────────────┬───────────┘
                                        ↓
                              ┌─────────────────────┐
                              │   DNAse, urease     │
                              │     and CAMP        │
                              │  reaction tests     │
                              └─────────────────────┘
```

GPR DNAse-positive Urease-negative CAMP-negative	GPR DNAse-positive Urease-positive Reverse CAMP	GPR DNAse-negative Urease-positive Reverse CAMP	GPR DNAse-negative Urease-negative CAMP-negative
C. diphtheriae	*C. ulcerans*	*C. ulcerans*	Other corynebacteria

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                              ┌─────────────────────┐
                              │   Investigation     │
                              │  of toxigenicity    │ ───→
                              └─────────────────────┘      ┌──────────────────┐
                                                           │ Negative result  │
                                                           └──────────────────┘
                                         ┌────────────────┐      ┌──────────────────┐
                                         │  Conventional  │      │ Molecular tests  │
                                         │ biochemical    │      │                  │
                                         │    tests       │      └──────────────────┘
                                         └────────────────┘
                              ┌──────────────────────────────┐
                              │ C. ulcerans,                 │
                              │ C. pseudotuberculosis        │
                              │ or atoxigenic C. diphtheriae │
                              └──────────────────────────────┘
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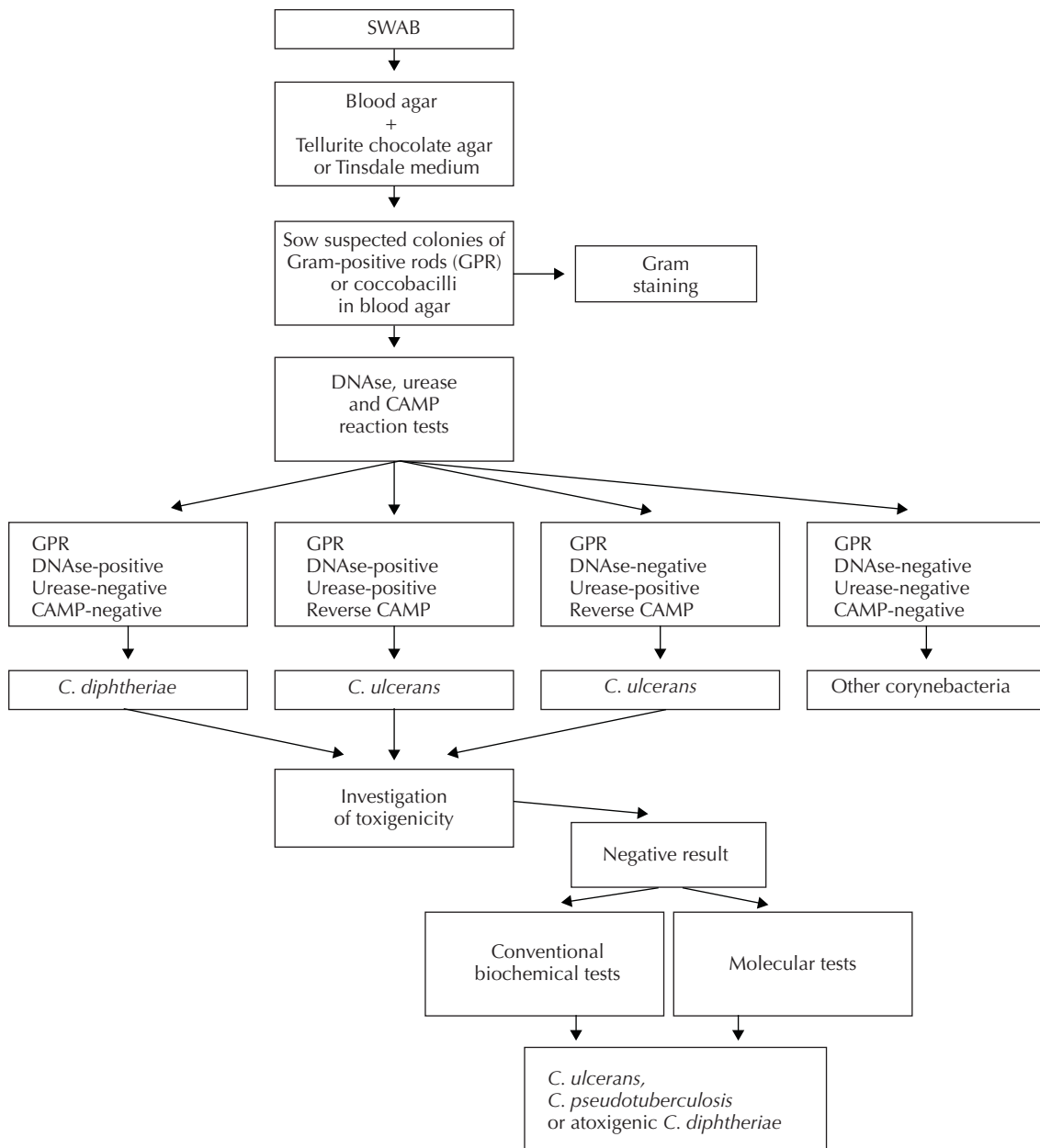
Figure 3. Scheme for triage of *Corynebacterium ulcerans*.

H$_2$S-producing samples are identified through the presence of a brown halo around the colony.[30,36]

Positive results from the DNAse test[88] suggest that *C. diphtheriae* and *C. ulcerans* are present. Unlike *C. diphtheriae*, samples of *C. ulcerans* are urease-positive and undergo the reverse CAMP reaction.[35] On rare occasions, samples of *C. pseudotuberculosis* that are potential DT producers may be isolated. These present negative results from DNAse tests and positive results in the cystinase, urease and reverse CAMP tests. Cystinase-negative and DNAse-negative samples should be considered to be "other coryneform bacteria".

In addition to conventional biochemical tests, the semi-automated API Coryne system (BioMérrieux, Lyon, France) is also capable of identifying these pathogens, including the atypical sucrose-fermenting samples of *C. ulcerans* and *C. diphtheriae* that are often isolated in Brazil.[33,67]

Laboratories that do not have any resources available for carrying out toxigenicity tests should send the strains for complementary analyses to the Central Public Health Laboratory of their region and/or the National Reference Laboratory and the Collaborating Center for diphtheria. Laboratories that do have resources available for conducting molecular techniques capable of

identifying *C. diphtheriae* and *C. ulcerans* and determining the presence of the *tox* gene[9,79,80,86,87] should also send the strains for epidemiological control procedures, to the same laboratories, which have this capacity.

Considering the short incubation period of this disease (between one and six days), it is recommended that material for investigating *C. ulcerans* and *C. diphtheriae* should be collected from nasal and oropharyngeal mucosa and skin lesions of people and animals that have been in contact with the case (suspected or confirmed) over the last 10 to 14 days.

ROLE OF DIPHTHERIC TOXOID IN PREVENTION

The concentration of protective antibodies in adult individuals decreases by 10% every year.[98] The importance of vaccination booster programs has been proven in Finland.[78] Data on vaccination coverage among adolescents and adults in Brazil are scarce in the literature.[15,20,22,85,102] In the city of Rio de Janeiro, only 30% of the adults are completely protected against the action of DT,[15] similar to what has been observed in other countries like Turkey.[10] These data emphasize the importance of applying reinforcement doses of diphtheric toxoid every ten years, in order to avoid decreased antibody levels in the population.[10,20]

However, the efficacy of diphtheric toxoid against the zoonotic diphtheria caused by *C. ulcerans* still remains unknown. The studies listed in Table 1 showed that the majority (approximately 75%) of the cases of zoonotic diphtheria have occurred in adult patients who had been fully or partially vaccinated with diphtheric toxoid.

There are still many issues that require better assessment, not only in relation to clinical-laboratory diagnosis, but also in relation to treating and preventing diseases caused by *C. ulcerans*. Likewise, better comprehension of the molecular epidemiology and characteristics of *C. ulcerans,* along with the toxin produced (which resembles the DT of *C. diphtheria*) is needed.

Recent studies have revealed differences in nucleotide sequences between the *tox* genes of *C. ulcerans* and *C. diphtheriae*, and between the *tox* genes of different samples of *C. ulcerans*. Some samples isolated from patients presenting conditions of extrapharyngeal infection have presented differences in DT sequences, predominantly in the domains of translocation and adherence.[9,70,80,99,109]

These facts may contribute towards situations in which individuals vaccinated with diphtheric toxoid or undergoing serum therapy do not present full protection against infections caused by *C. ulcerans*. Even if it is considered that the diphtheric toxoid may have a protective effect against diphtheria caused by *C. ulcerans* (i.e. through the presence of attenuated clinical symptoms), it should be remembered that the vaccination only presents the action of DT and probably does not impede colonization by toxigenic corynebacteria. There is agreement among researchers that, in the absence of a proven vaccine against *C. ulcerans,* diphtheric toxoid remains a reasonable alternative, especially in cases of convalescence. On the other hand, it is also believed that there is a scarcity of evidence to show that vaccination with diphtheric toxoid, even when kept up-to-date, would impede zoonotic diphtheria or other diseases caused by *C. ulcerans*.[94,107]

FINAL REMARKS

The circulation of *C. ulcerans* in many countries, added to the recent cases of infections in humans and animals described in Brazil, make it possible to raise the hypothesis that zoonotic diphtheria is occurring and *C. ulcerans* is circulating in urban and rural regions of Brazil. There is thus a need to improve the adequacy of the protocols for microbiological diagnosis of both *C. ulcerans* and *C. diphtheriae*, using alternative triage methods that could be implemented in public and private-sector laboratories. Implementation of epidemiological and laboratory-based surveillance may contribute towards increasing the number of confirmed cases of classical and zoonotic diphtheria in Brazil.

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