

Outbreak of *Brucella melitensis* infection in Eastern Sicily: risk factors, clinical characteristics and complication rate

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SUMMARY

Brucellosis is one of the most common zoonoses in the world, especially in Southern Italy, where many cases are still recorded every year. 128 cases of brucellosis were observed in Messina (Sicily) in 2016, representing a tenfold increase in the number of cases of brucellosis expected.

The aim of this multicenter retrospective study was to analyze clinical and microbiological aspects of a brucellosis outbreak in the province of Messina in 2016, the incidence of its complications and the treatment combinations applied.

The principal transmission route was through the ingestion of unpasteurized fresh cheese. The mean latency period between the onset of the symptoms and diagnosis was 35.58±42.75 days. A late diagnosis increases the risk of developing complications. Drug-resistant strains of *B. melitensis* to Trimethoprim/Sulfamethoxazole and Ciprofloxacin were found in blood cultures of 58.4% patients.

Brucellosis is still present in Sicily. A diagnostic delay predisposes to complications requiring prolonged therapies. The finding of *Brucella melitensis* strains resistant to the most widespread treatments is worrisome and needs further investigation. Moreover, the use of alternative combination antibiotic therapy is recommended.

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INTRODUCTION

Brucellosis is a major public health problem involving both animals and humans in many countries around the world. In 2012, the CDC published a list of countries characterized by a high risk for brucellosis infection (Center for Disease Control and Prevention 2017). Surprisingly, the geographical distribution included not only historically endemic Mediterranean countries, such as Portugal, Spain, South of France, Italy, Greece, Turkey and North Africa, but also Mexico, South and Central America, Eastern Europe, Asia, the Caribbean and the Middle East, where it has been hypothesized that the prevalence of brucellosis has increased due to growing international tourism and migration (Pappas *et al.*, 2006; Greenfield *et*

al., 2002). Although, in recent years, a generally downward trend in the number of notified cases has been recorded in Italy - in 2014 only 8 cases were reported - Sicily still has a higher than average incidence each year (European Centre for Disease Control and Prevention 2017; Istituto Superiore di Sanità 2013).

Brucellosis is mainly transmitted to humans through the ingestion of unpasteurized dairy products (raw milk and fresh cheeses), although contact with infected animals, placentas or aborted fetuses are other ways of acquiring the disease (Hasanjani Roushan *et al.*, 1999).

Clinical manifestations are non-specific and include fever, night sweats, arthralgia, asthenia, anorexia, and low back pain, so that sometimes brucellosis can be mistaken for more common pathologies such as malignancies, vascular and collagen diseases, tuberculosis, malaria, rheumatic fever or leishmaniasis (Cunha *et al.*, 2012; Pappas *et al.*, 2005). Moreover, brucellosis is characterized by frequent organ-based complications - so-called *focal* brucellosis - such as spondylodiscitis, sacroiliitis, meningitis, meningoencephalitis and endocarditis, requiring management strategies different from those of the *systemic* manifes-

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tation (Aygen *et al.*, 2002; Mantur *et al.*, 2007; Colmenero *et al.*, 1996; Buzgan *et al.*, 2010; Hasanjani Roushan *et al.*, 1999; Bosilkovski *et al.*, 2007).

Brucellosis can also be classified according to the duration of symptoms and the completion of correct antimicrobial therapy into acute (<8 weeks, no antibiotic therapy), subacute (8-52 weeks, incomplete or inappropriate antimicrobial therapy) or chronic (>52 weeks) (Andriopoulos *et al.*, 2007). Subacute and chronic forms are more frequently accompanied by complications (Buzgan *et al.*, 2010).

Recommendations for treatment have not changed since 1986 when the World Health Organization suggested an optimal combination therapy including doxycycline plus either rifampin or streptomycin, but the literature reports many cases in which WHO indications have not been followed (Corbel, 2006). Moreover, the recommended treatments are not always completely effective, with a 5-15% of relapse (Ariza *et al.*, 2007). After 1986, a number of clinical studies assessed the efficacy of different regimens with two or three antibiotics with the aim of reducing the risk of recurrence and preventing the possible onset of a chronic condition (Sturniolo *et al.*, 1993; Cascio *et al.*, 2013). One of the first studies on *Brucella*'s sensitivity to the common antibiotic molecules was by Kinsara *et al.* (Kinsara *et al.*, 1999). It showed the resistance of *Brucella* to cotrimoxazole and suggested carrying out a surveillance of susceptibility of *Brucella melitensis* to antibiotic molecules in all endemic geographic areas.

Given the history of this neglected zoonosis, it is easy to see the dangers accompanying a new brucellosis outbreak, like the one which occurred in 2016 in the province of Messina (Facciola *et al.*, 2018). Our study aims to show the characteristics of this outbreak - time of diagnosis, complications, antimicrobial sensitivity of the microorganisms isolated from blood cultures - and to introduce the possibility of using new therapeutic schemes available in the case of emerging resistance.

MATERIALS AND METHODS

This retrospective observational study analyzed the epidemiological characteristics of a brucellosis outbreak in the province of Messina during a period from January to

December 2016. At that time, 128 reports were sent to the Provincial Health Authority (PHA) of Messina. Fifty-six cases were admitted either to the "G. Martino" University Hospital or to the "Papardo" Hospital. The electronic and hard copy records of the hospital admissions have been examined and included, while we excluded 72 cases followed as outpatients due to lack of data.

Brucellosis was suspected in the presence of a history of ingestion of fresh cheeses (ricotta, tuma) of unknown origin accompanying suggestive clinical signs, such as fever, profuse sweating, arthralgia, hepatomegaly, splenomegaly. The diagnosis was certain when positive laboratory tests, like Wright reaction, a standard agglutination test (SAT), or an enzyme-linked immunosorbent assay (ELISA) test to dose specific IgM and IgG versus *Brucella melitensis*, or a blood culture isolate of *Brucella melitensis* was retrieved. For each included patient, we evaluated sex, age, date of onset of symptoms, clinical characteristics and complications with organ localization, laboratory data, therapeutic regimens (two drugs, rifampin and doxycycline, or three drugs, two-drug regimen plus gentamicin, according to the clinical conditions of the patient), and adverse reactions to the antimicrobials used. The data were analyzed using descriptive statistics (mean; median; standard deviation, SD; percentage).

RESULTS

128 cases were reported during the period from January to December 2016, with the highest incidence occurring in February, when 34 cases (26.5%) were notified, and a gradual decrease throughout the rest of the year (Figure 1). Table 1 summarizes the vital statistics (age, sex) of the 56 inpatients we included in the study. These general data were unavailable in the case of outpatients, therefore we had to exclude them from the analysis.

Out of the 56 cases included in the analysis, in 41 patients (73.2%) an in-depth interview made it possible to identify the source of infection, which was the distribution of unpasteurized dairy products (ricotta cheese, or other fresh cheeses) during a representation of the living nativity during the period from December 2015 to January 2016.

The average number of days between the onset of symp-

Figure 1 - Incidence of Brucellosis cases in Messina during 2015 and 2016.

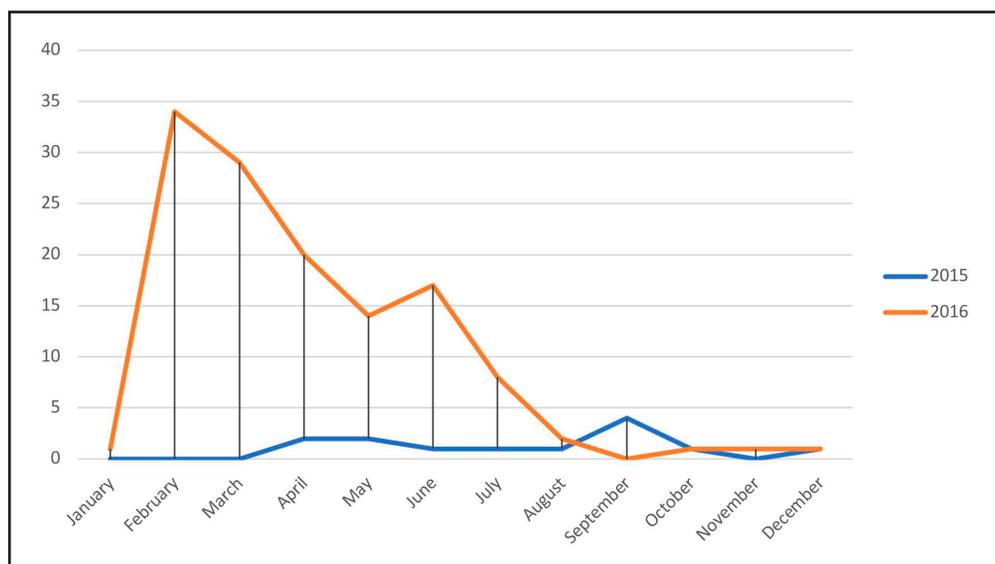


Table 1 - Descriptive analysis of the population included in our study.

		N of patients	Average time to diagnosis (days ± SD)	N of complications	N of Relapses
Total		56 (100.0%)	35.58 ± 42.75	7 (12.5%) *	3 (5.4%)
Sex	M	31 (55.4%)	28.67 ± 24.01	2 (6.5%) **	2 (6.4%) **
	F	25 (44.6%)	44.00 ± 57.55	5 (20.0%) ***	1 (4.0%) ***
Age	> 16 yrs	51 (91.1%)	37.10 ± 44.30	7 (13.7%)	3 (5.9%)
	£ 16 yrs	5 (8.9%)	22.40 ± 21.78	---	---

* 4 spondylodiscitis, 2 pericarditis
 ** Percentage relative to the male population
 *** Percentage relative to the female population
 Yrs = years; SD = standard deviation

toms and the diagnosis was 35.88 days (SD ±42.68 days; median 21.00 days); with a higher latency in women (47.68 days; SD ±56.56 days; median 30.00 days) than men (26.35 days; SD ±23.90 days; median 20.00 days). This difference resulted not significant when tested with the ANOVA method (p=0.062). Remarkably, we found that 15 patients out of 56 (26.79 %), 10 women and 5 men, were diagnosed 6 weeks after the onset of symptoms (mean 85.00 days; SD ±57.17 days; median 60.00 days; minimum 45 days, maximum 280 days). No one in our population was diagnosed after a year from the onset of the symptoms.

Children’s mean time to diagnosis was lower than the population’s average (23.00 days; SD ±20.19 days; median 17.00 days). Complications occurred in 7 adult patients (12.5%), 5 women (71.4%) and 2 men (28.6%): 5 spondylodiscitis and 2 pericarditis were diagnosed. Relapse, defined as a recrudescence of typical brucellosis symptoms associated with an increase in antibody titers due to poor adherence to treatment and inadequate duration of therapy occurred in 3 patients only (5.36%) (Ulu-Kilic *et al.*, 2013).

The most frequent clinical signs reported were: sweats (75%), fever, either during admission (64.3%), or in clinical history (35.7%), asthenia (60.7%), arthralgia (44.7%), anorexia (41.1%), hepatomegaly (33.9%), splenomegaly (30.3%), and back-pain (23.2%).

SAT was positive in 85.71% of cases: 91.7% of them had a confirmatory ELISA test positive (27.1% had positive IgM and negative IgG; 64.6% had both positive IgM and IgG; 2.1% had negative IgM and positive IgG). Alanine-transferase (ALT) higher than 50 U/L was found in 35.7% of patients (Table 2).

Blood cultures were performed in 24 patients (42.85%): half of them were positive for *Brucella melitensis*, while the other half were negative (Figure 2). *Brucella meliten-*

sis was found to be resistant to antimicrobials commonly used for brucellosis therapy in 7 cases (58.4% of the positive blood cultures). In particular, 5 *Brucella* strains were resistant to trimethoprim/sulfamethoxazole, while 2 were resistant to ciprofloxacin.

36 Patients (64.3%) were treated with a combination of two drugs, while 19 of them (33.9%) with a combination of three antimicrobials. One patient (1.8%) was treated with just one drug. Rifampin was used in 91.1% of the patients, doxycycline in 92.9% of them, gentamicin in 33.9%, trimethoprim/sulfamethoxazole in 5.4%, quinolones (either levofloxacin, ciprofloxacin or moxifloxacin) were used in 8.9% of the patients.

Adverse drug reactions, mainly gastrointestinal disorders, occurred in 10.7% of cases. In all of them the reaction was serious enough to require a suspension of the current therapy and administration of other regimens. Treatment was discontinued after 6 weeks in uncomplicated patients and 12 weeks in spondylodiscitis, according to WHO recommendations (Corbel 2006).

Table 2 - Signs, symptoms and laboratory test results at the diagnosis.

	N of patients	Percentages (%)
Fever (BH >38°C)	36	64,3
Sweating	42	75,0
Fatigue	34	60,7
Arthralgia	25	44,7
Anorexia	23	41,1
Hepatomegaly	19	33,9
Splenomegaly	17	30,3
Low back pain	13	23,2
SAT >1:80	48	85,7
Hypertransaminasemia (UI/l)	20	35,7
Positive blood culture	11	19,7

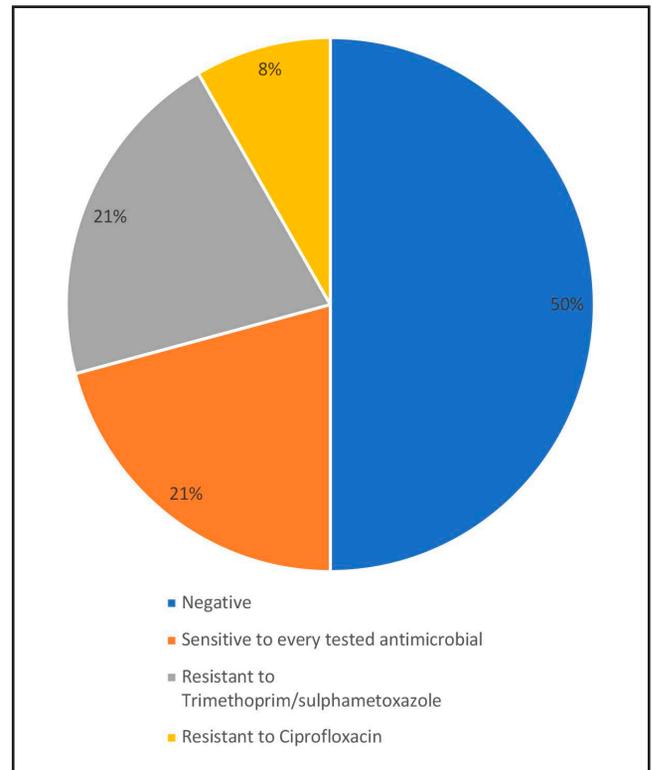


Figure 2 - Blood culture results.

DISCUSSION

Brucellosis is one of the most frequent zoonoses in the world. However, the real global incidence is not well-known due to the variation in surveillance systems used among different countries. It is believed to exceed 1/10,000 cases in some populations (Pappas *et al.*, 2006). Italy is considered an endemic region, and Sicily has one of the highest incidences (Istituto Superiore di Sanità, 2013).

In 2016 we highlighted an increase in the incidence of brucellosis in the province of Messina, just after Christmas holidays. In fact, 41 patients acquired the infection during a living representation of the nativity when dairy products from uncontrolled animals were distributed to participants. This small epidemic was luckily circumscribed to our province. Brucellosis is a subtle disease which can be easily confused with other more common infectious diseases until it has established its complications and the patient needs hospitalization.

For the purposes of this study we decided to include in the analysis only inpatients, for whom clinical data were available, rather than to describe the entire population. With regard to the population included in the study, women had a higher average time to diagnosis than men, supposedly because they have a higher pain tolerance than men, leading them to a delayed referral to a general practitioner or specialist (Woodrow *et al.*, 1972).

The analysis of data collected from medical records of inpatients showed a greater average diagnosis time in the adult population compared to pediatric cases. This is in contrast with what Dean *et al.* claimed in a systematic review and meta-analysis on the clinical manifestations of brucellosis. According to this review, the diagnosis of brucellosis in children is difficult and delayed due to non-specific initial symptoms and inadequate family anamnesis (Dean *et al.*, 2012; Shahzad *et al.*, 2016; Alnemri *et al.*, 2017; Mongkolrattanothai *et al.*, 2017; Çiftdoğan *et al.*, 2017).

Unsurprisingly, and according to the literature, neither focal brucellosis nor complications occurred in children. Brucellosis is generally considered benign when contracted during childhood, while there is a higher risk of complications and organ damage if the disease is contracted in adulthood (Ruegger *et al.*, 2017; Zheng *et al.*, 2018; Rossi *et al.*, 2018; Delmastro *et al.*, 1989; Resorlu *et al.*, 2016; Buzgan *et al.*, 2010; Petik, 2016; Ceccarelli *et al.*, 2018).

Due to the small number of patients in our sample who developed complications, we could not define an association between the latency time and the onset of complications. Half the patients who developed complications had an acute form of the disease (<8 weeks after the onset of the symptoms), while the other half had a subacute form (8-52 weeks since the onset of the symptoms).

It is important to emphasize that among the population included in our study, no patient was diagnosed in a chronic condition (>52 weeks after the onset of the symptoms). As a matter of fact, the endemic existence of the disease in Sicily makes brucellosis one of the differential diagnoses most frequently expressed when a patient comes to the ER complaining of fever and arthralgia, reporting a history of possible exposure.

The prevalence of complications in our population was similar to that reported by Doganay *et al.* which was 27.7% (Doganay *et al.*, 2003). However, they did not report the latency time between the onset of symptoms and the diagnosis, so it was not possible to compare the results.

The most frequent complication in our study was spondylodiscitis, with a 7.14% prevalence, in accordance with a study by Ulu-Kilic *et al.* reporting a variable incidence of spinal brucellosis from 2% to 54% (Ulu-Kilic *et al.*, 2013). They also reported that the spinal column is often affected in subacute and chronic forms more than in acute forms. Therefore, time to diagnosis seems crucial in the management of patients with brucellosis: the later the diagnosis, the greater the likelihood of developing long-term complications.

Cardiovascular complications during brucellosis are rare (<1%). Myocarditis and pericarditis are thought to occur rarely in absence of endocarditis (Kaya *et al.*, 2013; Gatselis *et al.*, 2011). We found isolated pericarditis in the absence of endocarditis or myocarditis in 2 women diagnosed with ETT evaluation and completely resolved without relapse with antibiotic therapy (Ceccarelli *et al.*, 2018).

Although serological tests (SAT and ELISA) play an important role, and are sufficient for diagnosis in endemic regions, the diagnostic procedure must be completed with the execution blood culture and antimicrobial sensitivity. On the other hand, *Brucella spp* is difficult and slow to grow, and its culture requires suspecting the disease before blood sampling. In fact, blood culture samples are said to be negative after 5 days of incubation, when an automatic method is employed, while *Brucella spp* often needs more time to grow (Miller *et al.*, 2018). Therefore, it is possible that the high rate of negative blood culture is also caused by lack of communication between the clinician and the microbiologist. Thus, it is important for the microbiologist to have some clinical data on the patient, to decide the right amount of time the sample has to be incubated.

The therapeutic scheme we chose was based on the review published by Yousefi-Nooraie *et al.* in the Cochrane Database of Systematic Review, which considers treatment failure and adverse effects of the most widespread regimens with doxycycline, rifampin, gentamicin, streptomycin and quinolones. The review concluded that the use of a three-drug regimen could potentially reduce the risk of complications and relapses (Yousefi-Nooraie *et al.*, 2014).

Considering this conclusion, we chose to include a treatment scheme with one week of doxycycline, rifampin and gentamicin plus five weeks of doxycycline and rifampin in our routine.

The finding of emerging resistant *Brucella* strains in Italy represents essential and innovative information. This is especially important because of the antimicrobials involved: trimethoprim/sulfamethoxazole and ciprofloxacin. cotrimoxazole resistance had already been highlighted in a study carried out by Kinsara *et al.* (Kinsara *et al.*, 1999). However, other studies did not confirm this resistance, but only show an altered sensitivity to both rifampin and cotrimoxazole (Asadi *et al.*, 2017; Marianelli *et al.*, 2007). A possible explanation for these differences lies not only in geographical differences, but probably in *Brucella* culture procedures.

Trimethoprim/sulfamethoxazole is one of the main antibiotics used in WHO recommended brucellosis treatment regimes. Isolation of resistant *Brucella* strains highlights new problems for managing antibiotic therapy. It may actually mean we could have to give up the use of an easily administered low cost oral antibiotic, which can be prescribed to children and pregnant women.

Both dual therapy (doxycycline and rifampicin) and triple therapy (doxycycline - rifampicin - gentamicin) have been performed. According to Bosilkovski *et al.*, the rationale behind the use of the combination of three drugs is based on the possibility of reducing the risk of brucellosis recurrence (Bosilkovski *et al.*, 2012). Patients treated with this therapeutic regimen have not developed recurrence, but the data presented are only partial, as some patients are still in follow-up at our centers.

Our study highlighted a still troubled Sicilian reality. The ability to buy non-pasteurized products today reflects poor health surveillance on farms.

Time to diagnosis is crucial in choosing the best therapy regimen and avoiding complications that lengthen the hospital stay, increasing the expenses for the national healthcare system.

The presence of resistant *Brucella* strains suggests that obtaining blood samples to test the sensitivity of *Brucella* spp. before starting specific antibiotic therapy is essential. It may also lead to a difficult choice of a treatment scheme, especially in children. Trimethoprim/sulfamethoxazole is the drug of choice for the treatment of brucellosis in children under the age of 8 years and a future scenario where *Brucella* becomes more frequently resistant to it will complicate the management of an already subtle disease. Setting up therapeutic regimens consisting in multiple antibiotics could reduce the risk of disease recurrence.

In conclusion, further studies are required to find new therapeutic approaches to brucellosis. Our study highlights the need for careful and continuous clinical and veterinary surveillance of this ancient, albeit current, zoonosis.

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