

Biological and antibacterial efficacy of selected disinfectants and antiseptics commonly used in veterinary practices against *Brucella abortus*

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Abstract

The proper disinfection of contaminated animal environment plays an important role in the prevention and control of brucellosis. In this study, we evaluated in- vitro the germicidal efficacy of commonly used disinfectants including Virkon® S (Potassium peroxy-monosulfate and sodium chloride, 1%), Sumasol (chlorinated powder, 0.6%) and QACs (Quaternary ammonium compounds) and two antiseptics; including bovine iodine (iodine, 1%) and dettol 1% on *Brucella abortus* strain isolated from cows in a dairy farm and their surrounding environment under different interfering conditions; namely minimum inhibitory concentration (MIC). The results revealed that virkon S and dettol exhibited high efficacy against *Brucella abortus* at different concentrations and contact periods either in presence or in absence of organic matter. Therefore, periodical assessment of the disinfectants formulations in- vitro and also in- vivo conditions, with the target of enhancing and improving of brucellosis prevention and control in animals and humans is highly required.

Key words: Antiseptic, *Brucella abortus*, disinfectant.

Introduction

Brucellosis is endemic all over the world, and highly distributed in different countries including Latin America, the Middle East, Africa, and Asia (**Memish and Balkhy 2004**) and results in huge economic losses through reproductive failure in animals. Brucellosis has been an endemic disease in Egypt for thousands of years. Bovine brucellosis is usually caused by *Brucella abortus*. Which has seven recognized biovars, the most reported of them are biovars 1, 2, 3, 4 and 9, where biovar 1 being the most prevalent in Latin America (**Aparicio 2013**). *Brucella abortus* is usually transmitted by contact with the placenta, fetus, fetal fluids and vaginal discharges from infected animals. *Br. abortus* may also be found in the milk, urine, semen, feces and hygroma fluids (**CFSPH**

2007). They can remain viable in contaminated environment for many months, depending on factors like suitable ambient temperature, humidity, and pH (**CFSPH -2009**).

Researchers ascertained that *Brucella* could survive in manure, water, dust, manure slurry, soil, aborted fetuses, meat and dairy products for long periods (**WHO 2006**). Brucellosis is endemic in most areas of the world due to difficulty in the control and eradication of brucellosis in animal industry because of persistent shedding of bacteria in reproductive and mammary secretions (**Radostitis et al., 1994**), problems of control/eradication programs application and/or other unknown environmental factors may have influenced the spread of the disease (**Neglia et al., 2013**).

It is known that Brucellae are sensitive to di-

rect sunlight, heat treatment and disinfection (Pappas *et al.*, 2005). Disinfection is no doubt the most effective measure for prosperous brucellosis control and eradication particularly in endemic areas (Al-Majali *et al.*, 2009). Several types of chemical disinfectants including iodides, chlorhexidine and quaternary ammonium compounds are well known as active and effective against brucellosis (Wanke 2004).

Brucellosis has only been controlled and sometimes eradicated in animal reservoirs in developed world by applying strict veterinary hygienic measures, such as control tests, culling infected animals and environment sanitation (Godfroid *et al.*, 2005). So, the environmental sanitation is considered the critical points for the control of brucellosis specially disinfection and selection the disinfectant of choice. *B. abortus* is susceptible to sunlight, high temperatures and a range of chemicals, including 0.03% formalin, 1% phenol, 0.01% beta propiolactone, sodium hypochlorite, sodium hydroxide, iodine, quaternary ammonium compounds, ether and chloro form (Animal Health Australia 2005).

So, the aim of this study, is to evaluate two types of antiseptics and three types of frequently used disinfectants in vitro, in Gamasa district, for their bactericidal activity on *Br.*

abortus and evaluated under different interfering conditions of dirty environment and variable contact periods.

Materials and Methods

Samples and procedures:

Samples were collected from cows (milk samples, aborted foeti specimens) and their surrounding environment (swabs of teat cup, milker's hand and soil) in the examined farm by using stratified technique. Milk samples were collected from cows, meanwhile samples from stomach content, spleen, liver & lung were collected from aborted foeti as methods described by Alton *et al.* (1975). Representative samples were collected from environment in the examined cow's farm that includes swabs from milking machines, milker's hand and soil samples according to Jasper *et al.* (1974), Sadoma (1997) and Clegg *et al.* (1983). The collected samples were labeled with information including (farm site, number, sampling date and animal health status), then transferred carefully under aseptic condition in an ice-box to Brucellosis department, animal health research institute in El- Dokki-Giza, for further isolation and identification of different *Brucella* spp.

Table (1). Disinfectants and antiseptics used in this study.

Commercial name	Active ingredient	Recommended concentration	Application
Virkon® S	Potassium peroxymonosulfate and sodium chloride	1%	Animal house and equipment
QAC	Benzalkonium chloride	2%	Teat dipping
Sumasol	Chlorine	6%	equipment
Biocide	Iodine	1%	Skin and mucous membrane
Dettol	Synthetic phenol compound	1%	Skin of workers

Isolation and identification of *Brucella* spp:

The collected samples from animals (milk and aborted feti specimens) and environmental samples (swabs from milking machines, Milk-ers' hands and soil samples) were cultured for

isolation of *Brucella* spp. as methods described by Alton *et al.* (1975). Isolated *Brucella* were subsequently identified biochemically using the following tests; Co₂ requirement for growth, H₂S production, Agglutination in sera and the

growth in the presence of dyes as methods explained by **Alton et al. (1975)**.

In presence of organic matter To estimate the effect of interfering conditions during disinfection, the destroying effect of each disinfectant on tested bacteria was evaluated in the presence of organic matter by using soil or fecal samples which were collected from a cattle shelter and made into 20% soil or 20% fecal suspensions. Soil samples and fecal suspensions were sterilized by autoclaving at 115°C for 20 min and cooled at room temperature. The *Brucella* isolates were then added to achieve a final bacterial concentration / ml (27×10^8), and then the same procedures in absence of organic matter were repeated (**Suller and Russell 1999**). The exposure periods of bacterial strain to each used chemical were 10, 20 and 30 minutes.

Results

Results of the prevalence of *B. abortus* from animal and environmental samples are summarized in Table (2), bacteriological examination of animal samples revealed that, 4% of milk samples (4 out of 100) and 35.7% of aborted feti specimens (10 out of 28) were positive for *Brucella abortus*.

Meanwhile, environmental samples showed lower prevalence of *Br. abortus*. Only, one out of 20 (5%) Milker's hands swabs were positive for *Br. abortus*. Hence, 10% of bacteria were isolated from soil samples. On the other hand,

no *Brucella* was detected from teat cups samples.

The minimum inhibitory concentration results of tested disinfectants and antiseptics are illustrated in table (3), and showed that bactericidal effects of tested chemicals under different interfering conditions were determined. In absence of organic matter; Virkon® S, Dettol and Biocide exhibited high efficacy against *Br. abortus* at different concentrations and contact periods. Meanwhile, Suma sol was only effective after 20 and 30 minutes of contact with *Br. abortus*. On the other hand, 1 % of quaternary ammonium compounds showed higher bactericidal action on *Br. abortus* after 20 and 30 minutes of contact periods either in two conditions. When the bactericidal action of the tested chemicals was evaluated in the presence of organic matter, *Br. abortus* showed resistance to the majority of tested chemicals except for virkons which had bactericidal action on tested *Brucella abortus* after 20 and 30 minutes of contact time.

Among the two evaluated antiseptics, all used concentrations of dettol owned high powerful bactericidal action after 30 minutes of contact with *Br. abortus*.

Table (2). The distribution of *Brucella abortus* from animals and environmental samples.

Examined samples	Total No.	Positive No. (%)
Animals samples		
Milk samples	100	4 (4)
Aborted feti specimens	28	10 (35.7)
Environmental samples		
Teat cups	20	0
Milkers' hands	20	1 (5)
Soil	20	2 (10)

Table (3). Anti *Brucella abortus* activity of tested disinfectants and antiseptics under different conditions.

Product	Without organic matter Contact time (minutes)			With organic matter Contact time (minutes)		
	10	20	30	10	20	30
Disinfectants, dil.						
Virkon S						
1/100	-	-	-	-	-	-
1/150	-	-	-	+	-	-
1/200	-	-	-	+	-	-
Suma sol						
5.0 %	+	-	-	+	+	+
6.0 %	+	-	-	+	+	+
7.0 %	+	-	-	+	+	+
QAC						
0.5 %	+	+	+	+	+	+
1.0 %	+	-	-	+	-	-
Antiseptics:						
Dettol						
1/100	-	-	-	-	-	-
1/150	-	-	-	-	-	-
1/200	-	-	-	+	-	-
Biocide						
0.5%	+	-	-	+	+	-
1%	-	-	-	-	-	-

+ : Growth on the inoculated plats.

- : No growth on the inoculated plats.

Discussion

The lack of hygienic carcass disposal and local husbandry methods resulting in the environmental contamination with brucellosis and are considered the main causes of disease endemicity in developing and underdeveloped countries. The transmission of this pathogen between animals usually occurs through contact with contaminated environment (**Tittarelli et al., 2005, Wilesmith 1978**). Isolation of *Br. abortus* from cow's milk constitutes a hazard to suckling calves which can also be infected by suckling colostrum or milk from infected cows. While, most of these calves rid themselves of *Brucella*, a small percentage may continue to be infected until adulthood, remaining negative to diagnostic serological tests but aborting during their first pregnancy. Such animals pose a serious threat to brucellosis control and eradication (**TerHuurne et al., 1993**).

Milk is usually the main source of brucellosis for urban populations. Ingestion of fresh milk or dairy products prepared from unheated milk

is the main source of infection for most populations, it is recommended that healthy cows should be milked first and infected cows last collected in leak-proof containers and disposed hygienically by deep burial in freshly slaked lime at sites away from water courses.

Any area in which an abortion or infected parturition has occurred should be washed down with an approved disinfectant such as hypochlorite or iodophor disinfectant at recommended working strength (**Corbel 2006**). Other authors, like **Rezaei et al. (2010)** isolated higher rates of *Br. abortus* from milk samples 21/150 (14%).

Bacteriological detection of *Brucella abortus* in different aborted feti specimens was previously cited by many authors as **Matrone et al. (2009)**.

In this study, *Br. Abortus* was mainly isolated (4% and 35.7%) from milk samples and aborted feti specimens, respectively low concentrations were found in soil samples and Milker's hands swabs, (1% and 2%) respec-

tively mean while no *Brucellae* were isolated from teat cup swabs.

The recovery rate of *Br. abortus* from aborted fetuses considered an important source of infection to other animals in the farm so should be infection to other animals and workers in the farm, other factors such as lack of hygienic measures in animal husbandry and in food handling partly account for brucellosis remaining a public health hazard (OIE, 2010).

Farm implements used for handling contaminated material should be disinfected after use by immersion in a suitable disinfectant (iodophor, phenolic soap or dilute caustic soda). Particular attention should be given to the disinfection of footwear to ensure that infection is not transferred outside the premises; the hands should be treated with an antiseptic, e.g. tincture of iodine (Corbel 2006).

The transmission cycle of microorganisms can be interrupted by disinfectants procedures needed to be readjusted on the basis of environmental temperatures, the degree of organic matter contamination provide a public health benefit (Cozad and Jones, 2003).

It is known that the disinfectant power relies on the concentration, time of exposure, temperature, and reaction conditions (Bragg *et al.*, 2014). No adequately studies on the bactericidal evaluation of disinfectants on *Br. abortus* were conducted. This work focuses on this task, in- vitro evaluation of the efficacy of commonly used disinfectants and antiseptics against *Br. abortus*.

Our findings indicated that virkon s used for disinfection of the animal house and environment was the most powerful among all tested chemicals under different interfering conditions and dettol was the strongest antiseptic can be used safely on Milker's hands. Also, we found that dilutions lower than corresponding recommended industrial concentrations could inhibit and kill *Brucella*. Pathogen concentration, and the disinfectants used, as the volatile and residual disinfectants may influence the environmental quality.

Although, longer contact times under certain

conditions usually increases the bactericidal activity of disinfectants, liquid disinfectants can be less effective or inactivated under dirty conditions or at a low temperature (McDonnell and Russell 1999) and Wang *et al.* (2015).

It was clear that dirty conditions achieved by organic matter reduced the bactericidal action of all tested chemicals with slight exception of Virkon s, but longer contact times and higher concentrations could improve the germicidal effects of some of tested disinfectants on *Br. abortus*. These results indicated that the bactericidal effects of each disinfectant on *Brucella* lowered by the presence of organic matter.

Concentrations of disinfectants and prolonged contact times enhanced it, which are in harmony with that detected by Animal Health Australia (2005) and CFSPH (2007).

Our results were nearly similar to those recorded by Quinn *et al.* (1984) who studied the activity of seven disinfectants, which included representatives of phenolic, halogen, Q.A.Cs (Quaternary ammonium compounds) and aldehyde compounds, against high concentration of *Br. abortus* in the presence and in absence of organic matter. He found that these disinfectants performed well at 1% and 0.5% concentrations in the absence of organic matter. This indicates that iodine and dettol are antiseptics of choice for hand workers and veterinarians in farms while Virkon S (Potassium peroxy monosulfate and sodium chloride, 1%), Sumasol (chlorinated powder, 0.6%) and Q.A.Cs (Quaternary ammonium compounds) are disinfectants of choice for the control of brucellosis in dairy cattle farms. On the other hand, Wang *et al.* (2015) evaluated the bactericidal effects of six disinfectants (including aldehydes, halogens, quaternary ammonium compound, phenolics, and alkalines) on *Br. melitensis* and the potential factors that influence disinfection process. They revealed that the minimum bactericidal concentrations (MBCs) of these tested disinfectants were all significantly lower than the routinely used concentrations, and all the tested disinfectants were effective against *Br. melitensis*, but dirty conditions lowered this

action, which enhanced by higher concentrations and longer contact periods.

Conclusion

Among all tested chemicals, Virkon S disinfectant and dettol antiseptic posed the higher bactericidal action on *B. abortus* under different interfering dirty and contact periods conditions. In fact, these two chemicals are selected on the basis of their easy usage, low toxicity and somewhat cheap price (Alabi and Sanusi 2012). We highly recommend Virkon S for disinfection of animal houses and fields and Dettol as antiseptic for veterinarians and farm workers for proper prevention and control process of brucellosis. Also, further studies are highly required to test different disinfectants and antiseptics against *Brucella* in different conditions.

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الكفاءة البيولوجية والمضادة للبكتريا للمطهرات والمعقمات التي تستخدم عادة في الممارسات البيطرية ضد

البروسيلابورتس

*****منى محي الدين *محمود حسن عبدالحليم **محمد عبدالرحمن البابل**

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المخلص العربى

ان التطهير الجيد للأماكن الملوثة المحيطة بالحيوان يلعب دورا مهما في الوقاية والتحكم في البروسيلابورتس. وفي هذه الدراسة تم التقييم المعمل لعدد ثلاثة أنواع من المطهرات شائعة الاستخدام والتي تشمل فيركون أس (بوتاسيوم بيروكسي أحادي السلفات وكلوريد الصوديوم ١%). **وسوملسول (مسوق الكلور ٠.٠٦%)** ومركبات رباعي الأمونيوم وعدد اثنين من المعقمات التي تشمل اليود ١% والديتول على عترات البروسيلابورتس المعزولة من الأبقار الموجودة داخل مزرعة أبقار حلاية. وكذلك البيئة المحيطة بهذه الحيوانات تحت ظروف تداخلات مختلفة من حيث التركيز ومدة التعرض للمطهرات باستخدام أقل تركيزات مثبتة. وأثبتت النتائج أن فيركون أس والديتول أظهرتا كفاءة عالية ضد البروسيلابورتس في مختلف التركيزات وفترات التعرض وذلك سواء في وجود أو عدم وجود المواد العضوية. ولذلك يوصى بإجراء تقييم دورى للمطهرات والمعقمات في الظروف المعملية والحقلية وذلك بهدف سرعة تحسين طرق الوقاية والعلاج للبروسيلابورتس في الإنسان والحيوان.