wjpmr, 2017,3(10), 130-134

SJIF Impact Factor: 4.103

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH
www.wjpmr.com

Research Article
ISSN 2455-3301
WJPMR

DETERMINATION OF BACTERIAL LOAD IN DOG MILK WITH ITS ASSOCIATED RISK FACTORS IN JOS, PLATEAU STATE

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Article Received on 15/09/2017 **Article Revised on 06/10/2017** **Article Accepted on 27/10/2017**

ABSTRACT

The study determines the bacterial load in canine milk with associated risk factors in Jos metropolis. Thirty milk samples were analyzed for bacterial load count and isolation of bacteria present in the milk. The result of bacterial load count in relation to breed showed that Alsatian breed has 3.54×10^{10} cfu/ml as the highest and 1.01×10^{10} cfu/ml the lowest for indigenous breed and the total mean bacterial load count is 11.49×10^{10} cfu/ml. Bacteria isolated were *Escherichia coli, Klebsiella* species, *Staphylococcus* aureus and *Bacillus* species. The associations of bacterial contaminations were significant only in relation to vaccination status and litter size. The study showed that revealed that milk could have high bacterial contamination although it is a sterile secretion lacteal secretion to nourish young animals and that it can be a source of infection that could cause neonatal death. Therefore, there is need for fast bacteriological diagnostic approach in cases neonatal mortality in dogs to provide adequate therapy for both the dam and the neonates.

KEYWORDS: Bacteria load, Contaminations, Dog, Milk, Risk factors.

INTRODUCTION

Lactation is a critical period in dog breeding and precocious weaning is usually associated with high mortality and morbidity. Dog milk not only fulfills the entire nutritional requirements for rapidly-growing puppies but also protects them against infectious diseases. The protective effect of dog milk is due to the combined action of a variety of protective factors present in colostrum and milk such as immunoglobulin, immune-competent cells, antimicrobial fatty acids, polyamines, fucoylated oligosaccharides, lysosome and lactoferrin (Rocio *et al*., 2009).

Microbiological studies focused on dog milk are scarce and available ones have been restricted to the identification of potential pathogenic bacteria in clinical perinatal infections, including lactational mastitis in bitches and septicaemia in neonatal puppies (Jung *et al*., 2002; Scafer-somi *et al*., 2003; Ververidis *et al*., 2007).

The list of bacteria which can be responsible for milk-borne disease includes *Brucella* species and *Campylobacter jejuni* (Samie *et al*., 2007); *Bacillus cereus, Shiga* toxin producing *E. coli (E. coli* 0157:h7), *Coxiella brunette, Listeria monocytogenes, Mycobacterium tuberculosis, Mycoplasma bovis, Mycoplasma avium subspecie paratuberculosis, Salmonella* species, *Yersinia enterocolitica* and certain strains of *Staphylococcus aureus* that are capable of producing highly heat stable toxins in milk (Dhanashekar *et al*., 2012). Milk is highly nutritious and can serve as an ideal medium for the growth and multiplication of various micro-organisms. Neonatal deaths in dogs have been associated with bacterial contamination of milk from bitch (Dumon (1998). There is paucity of information on the bacterial contamination of dog milk in the study area which is the major dog breeding city in Nigeria and this prompted this research work.

METHODOLOGY

Sampling Size and Technique

A total of 30 milk samples were collected from the two local government areas of the metropolis (Jos South and North). The target population of this research was lactating bitches in the study area.

Sample Collection

Drops of milk were collected aseptically into sterile universal bottles. The samples were labeled and transported in a cold flask to Microbiology Laboratory of

Central Diagnostic Division, National Veterinary Research Institute, Vom, Plateau state, Nigeria.

Media Preparation

All media were prepared and sterilized in Pyrex conical flasks with sterilized foil paper serving as flask cover.

Nutrient Agar

28g of nutrient powder was dispersed in 1 liter of sterile distilled water and allowed to dissolve for 10 minutes, then swirled to mix the solution which was afterwards autoclaved for 15 minutes at 121°C, cooled to 47°C and poured into plates (Sagar, 2015).

Blood Agar

0.56g of nutrient agar will be dissolved in 20ml of sterile water and poured into MacCartney bottles and placed into an autoclave for sterilization. The bottles will be removed and allow to cool to 47°C. 2ml of sterile defibrinated blood will be added to 18ml of the nutrient agar and will be properly mixed by swirling. The mixture will be then poured into sterile Petri dish and made to be evenly distributed over the plate surface (Acharya, 2013).

MacConkey agar preparation

26g of the powder was weighed and dissolved into 500ml of distilled water by heating slightly. It was sterilized by using autoclave for about 15min at 121^{o}c allow to cool to 48-50^{o}c and then poured into plates, allow to solidified and incubate for sterility check at 37^{o}c for 18-24hours (Acharya, 2013).

Culture Method

Bacteria load count

10 test tubes are arrange and labeled 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10}. 9ml of distilled water is added to each test tube, 1ml of the sample is added to the 1st test tube and subsequently 1ml is drawn from 1st into the 2nd and continue same manner to the last test tube. 1, 3, 5, and 7th test tube where inoculated into sterile nutrient agar using 100µl microliter pipette and spread and allow to soak before incubating at 37^{0}c for 18-24hours. The plate was counted using Stuart colony counter after incubation is completed.

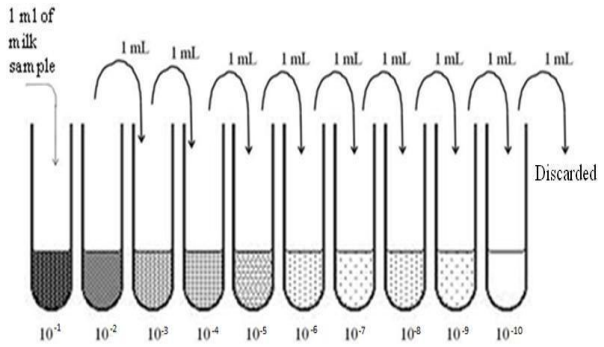

Figure 1: Serial dilutions of milk samples in 10 test tubes containing 9 ml of sterilized distill water each Subculture procedure.

Discrete colonies from the nutrient agar where sub-cultured in blood agar (BA) and MacConkey agar (MCA).colonies where picked based on macroscopic examination and streaked with a flamed wire loop on the BA and MCA and incubated at 37^{0}c for 18-24hours.

Gram Staining

Preparation of smear

Normal saline was dropped on a clean grease free slide. A colony is picked with a sterile wire loop from the subculture plate and smeared on the normal saline, the slide was heat fixed by passing it through flame thrice and allowed to air dried.

Staining procedure

The slide was flooded with crystal violet and allowed to stand for 1minute and was washed-off with tap water. The slide was also flooded with lugols iodine and allowed to stand for 1minute and was washed-off. Acetone alcohol was applied to the slide and wash-off immediately with tap water and counter staining was done with carbon fuchsin for a minute then washed-off, drained and air dried. The slide was examined microscopically using ×100 objective lens. Gram staining was done as described by Todar *et al.*, (2005).

Expression of results

The countable bacterial colonies from two consecutive plates of each sample were converted into colony forming units per millilitre (cfu/ml) using a formula given by ISO 7218:2007(E):

$N = \Sigma C /$

$V \times (n1 + 0.1\ n2) \times d$

Where; N = number of bacterial colonies counted, ΣC = sum of colonies identified on two consecutive dilution steps where at least one contained 10 colonies, V = volume of inoculum on each dish/plate (ml), n = number of dilutions and d = dilution rate corresponding to the first dilution selected (the initial suspension is a dilution). Method adopted by Kanyeka, (2014).

Statistical Analysis

Chi-square test was used to analyze the date and the results were also presented in tables.

RESULTS

Bacterial load count (cfu/ml) and isolation

A total of 24(80%) milk samples collected were positive for bacterial count while 6(20%) were negative. Out of the positive samples, 11(36.67%) were Caucasian breed with the mean bacterial load count (MBLC) of 2.07×10^{10}, 2(6.67%) were Alsatian with MBLC of 3.54×10^{10}, 5(16.67%) were bull mastiff with the MBLC of 1.17×10^{10}, Neopolitan breed were 1(3.33%) with MBLC 1.10×10^{10}, 2(6.67%) were indigenous breed with 1.01×10^{10}, 7(23.33%) were Rottweiler breed with MBLC 1.40×10^{10} and 2(6.67%) were cross breed with MBLC 1.20×10^{10} and the total mean bacterial count was 11.49×10^{10}.

Table 1: Mean bacterial load count based on breed.

Breed	Number of sample examined	Mean bacterial load count (cfu/ml)
Caucasian	11(36.67%)	2.07×10^{10}
Alsatian	2(6.67%)	3.54×10^{10}
Bull mastiff	5(16.67%)	1.17×10^{10}
Neopolitan	1(3.33%)	1.10×10^{10}
Indigenous	2(6.67%)	1.01×10^{10}
Rottweiler	7(23.33%)	1.40×10^{10}
Cross	2(6.67%)	1.20×10^{10}
Total	30(100%)	11.49×10^{10}

Escherichia coli was present in milk samples of all the breeds of dog examined while *Klebsiella* species was also present in all except Neopolitan mastiff and Indigenous breeds of dogs that were examined. *Staphylococcus aureus* was present in milk sample of Caucasian, Bull mastiff, Indigenous and Rottweiler breeds of dogs examined while *Bacillus* species was also present in Caucasian, Bull mastiff, Rottweiler breeds and Cross breed of dogs examined.

Table 2: Bacterial isolated based on breed.

Breeds	*Escherichia coli*	*Klebsiella sp*	*Staph. aureus*	*Bacillus* spp
Caucasian	+	+	+	+
Alsatian	+	+	-	-
Bull mastiff	+	+	+	+
Neopolitan	+	-	-	-
Indigenous	+	-	+	-
Rottweiler	+	+	+	+
Cross breed	+	+	-	+

(%) Contamination 7(100) 5(71.4) 4(57.1) 4(57.1) Associated Risk Factors

Table 3: Prevalence of bacterial contamination based on age of bitches.

Age of bitches (years)	Positive Bacterial Contamination	Negative Bacterial Contamination	Total
<1	2(6.7%)	0(0.0%)	2(6.7%)
1-2	4(13.3%)	1(3.3.7%)	5(16.7%)
3-4	9(30.0%)	3(10.0%)	12(40.0%)
5-6	7(23.3%)	1(3.3%)	8(26.7%)
>7	2(6.7%)	1(3.3%)	3(10.0%)
Total	**24(80.0%)**	**6(20.0%)**	**30(100%)**

X^2= 1.3021, df= 4, p>0.05(no significant difference)

Table 4: Prevalence of bacterial contamination based on litter size.

Litter size	Positive Bacterial count	Negative Bacterial count	Total
1-5	3(10.0%)	2(6.7%)	5(16.7%)
6-10	16(53.3%)	0(0.0%)	16(53.3%)
11-15	5(16.7%)	4(13.3.7%)	9(30.0%)
Total	**24(80.0%)**	**6(20.0%)**	**30(100%)**

X^2= 8.6111, df= 2, p<0.05(significant difference)

Table 5: Prevalence of bacterial contamination based on vaccination status.

Status	Positive Bacterial Contamination	Negative Bacterial Contamination	Total
Vaccinated	17(56.7%)	2(6.7.0%)	19(63.3%)
Not vaccinated	7(23.3%)	4(13.7%)	11(36.7%)
Total	**24(80.0%)**	**6(20.0%)**	**30(100%)**

X^2= 6.903, df= 1, p<0.05(significant difference)

Table 6: Prevalence of bacterial contamination based on breed.

Breed	Positive Bacterial Contamination	Negative Bacterial Contamination	Total
Caucasian	7(2.3%)	4(13.3%)	11(36.7%)
Alsatian	2(6.7%)	0(0.0%)	2(6.7%)
Bull mastiff	4(13.3%)	1(3.3%)	5(16.7%)
Neopolitan	1(3.3%)	0(0.0%)	1(3.3%)
Indigenous	2(6.7%)	1(3.3%)	3(3.3%)
Rottweiler	6(20.0%)	0(0.0%)	6(20.0%)
Cross breed	2(6.7%)	0(0.0%)	2(6.7%)
Total	**24(80%)**	**6(20%)**	**30(100)**

X^2= 3.7338, df= 6, p>0.05(no significant difference)

DISCUSSION

The high bacterial load recorded in this study when compared with maximum recommended level of 2.0×10^6cfu/ml for milk (EAS 67: 2007) is in agreement with Rocio *et al.* (2009) and Kanyeka, (2014) who also recorded high bacterial contaminations in their milk samples. This could be due to poor/ unhygienic management of the dogs post-natal. It could also be as a result of bacterial systemic infections or mastitis due to bacterial infections.

The bacterial species isolated (*Escherichia coli*, *Klebsiella* species, *Staphylococcus* aureus and *Bacillus* species) were partially in agreement with Sager and Remmers, (1990) and could be due to potential nature of milk to serve as pathogen carrier which can also cause serious health risk to consumers. It is the major vehicle that serves as means of transmission of milk-borne pathogen (Kanyeka, 2014). In some cases toxins or bacteria from milk affect the immune system of the neonate which might predispose the puppies to other bacterial or viral infections. According to Greene and Prescott (1998) bacterial infections are one of the major causes of neonatal deaths in many dog-breeding kennels. Based on age of bitches, this study showed that dogs of 3-4 years have highest prevalence of bacterial contamination of milk (37.5%) but it was not statistically significant. This may be because bacteria can be found in any age of the dog and the immune system of dogs at this age must have strongly developed to fight-off infections. Also based on breed Caucasian has the highest prevalence while Neopolitan has the lowest 1(4.2%) but it was not significant. This was in disagreement with Gill (2001) and may be because all breeds of dog are equally exposed to bacterial contamination if other predisposing factors are present.

The liter size was found to be a strong factor that contributes to bacterial contamination as more bacterial contaminations were recorded among those with high number of liter size. This is in partial agreement with Dzuik and Harmon, (1969); Wrathall, (1971); Svendsen and Bille, (1981) and Gill (2001) who stated that liter size was a factor in neonatal mortality in pigs and dogs and attributed it to milk infection. This could be due to stress-induced systemic infection on the dam or unhygienic environmental condition due to high number of liter.

Based on vaccination status also showed a strong association as the vaccinated bitches recorded low bacterial contamination compared to unvaccinated bitches. This could be due to improved immune status of the dam post vaccination which confers protection against some systemic infections.

CONCLUSION AND RECOMMENDATIONS

Dog milk could be highly contaminated with some bacterial organisms such as *Escherichia coli*, *Klebsiella* species, *Staphylococcus aureus* and *Bacillus* species thereby serving as vehicle for transmission of milk-borne pathogen. The associations of bacterial contaminations were significant only in relation to vaccination status and litter size. Therefore in cases neonatal mortality in dogs, bacteriological milk examination is recommended. It is also necessary to vaccinate dogs regularly as recommended by the veterinarian and also maintenance of good hygienic management in the dog kernel especially among those with high liter size.

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