Protective Action of Camel Milk in Mice Inoculated with *Salmonella enterica*

Roberto R. De Almeida Cardoso MD[1], Marina Ponte MD[2] and Vanuza Leite[2]

[1]Department of Allergy and Immunopathology, Faculdades Integradas do Planalto Central (FACIPLAC), Brasília DF, Brazil
[2]Santa Paula Laboratory, Brasília DF, Brazil

ABSTRACT: **Background:** In some countries people believe that camel milk can protect against various aggressors, whether due to infections, diabetes, or even autism. Little has been scientifically demonstrated regarding the veracity of these beliefs.
Objectives: To study the anti-infectious action of camel milk.
Methods: Fifty mice were divided into 5 groups of 10 animals each: 3 control groups and 2 test groups. Except for one of the control groups, all groups were intraperitoneally inoculated with a strain of *Salmonella enterica*. The rations in the test groups were supplemented with camel milk or cow milk.
Results: A statistically significant survival was observed in the mice supplemented with camel milk. The death rate after Salmonella inoculation was only 40% in the study group, as compared to 100% in the control groups where the mice were not protected, and 80% in the group supplemented with cow milk and injected with Salmonella.
Conclusions: Camel milk is an excellent nutrient and because of its specific properties, particularly its anti-infectious action, should be used to replace other milks.

IMAJ 2013; 15: 5–8

KEY WORDS: camel milk, cow's milk, immunoglobulins, salmonellosis, infection

For Editorial see page 35

Milk is an important nutrient in human nourishment. In some communities, camels represent the most important source of this nutrient. Some projects, for example, the one sponsored by SNV (Netherlands Development Organization) through the Resource Mobilization Center in Kenya, have demonstrated that the rational use of this animal is highly valuable for feeding poor populations [1].

Camel milk has several beneficial characteristics, such as the absence of diabetes in populations that consume it [2] and tolerance by patients who show intolerance to lactose. Even though camel milk does contain lactose [3], it is a nutrient for individuals who are allergic to cow milk [4,5]. Only one report of an individual allergic to camel milk was found in the literature [6].

Despite these benefits, studies on camel milk have received less attention than studies on the milk of other domestic animals, and most of them were carried out by researchers with little institutional support. The present study demonstrates, in a controlled manner, the anti-infectious action of camel milk.

MATERIALS AND METHODS

We used 50 mice of the *Mus musculus* species. The mice were white, male, with age ranging from 50 to 60 days and average weight 40 g. They were divided into 5 groups of 10 animals each. All the groups were observed at the same time.

FEEDING

The commercial ration for mice was used. In the groups fed with an addition of unpasteurized cow milk or camel milk, the ration was weighed and soaked with either of the two milks for 2 hours, after which no further increase of ration weight was observed, showing that the milk absorption had reached its maximum. The two types of milk and the soaked rations were kept at -4°C until the time of utilization. Water and rations were supplied ad libidum.

INFECTING AGENT

Strain of *Salmonella enterica* subspecies enterica were supplied by PNCQ (Programa Nacional de Controle de Qualidade – National Program of Quality Control), sponsored by SBAC (Sociedade Brasileira de Análises Clínicas – Brazilian Society of Clinical Analyses).

After dilution of 5 cfu (colony-forming units) in 1 ml of 5% glucose solution, we injected 0.15 ml into the right lower abdominal quadrant of the animal that was immobilized upside down. This amount was arrived at after checking which dose would kill 50% of a similar mice population with normal rationing in 5 days of follow-up.

ANIMALS

The mice were divided into five groups, as follows:

- Control Group 1 (CG1) – non-injected mice, normal ration
- Control Group 2 (CG2) – injected mice, normal ration
- Control Group 3 (CG3) – normal ration, mice injected

with Salmonella inactivated by heat (100°C) for 20 minutes; inactivation was checked by non-growth after culture seeding for 48 hours
- Test Group 1 (TG1) – ration soaked in camel milk, mice inoculated with Salmonella
- Test Group 2 (TG2) – ration soaked in cow milk, mice inoculated with Salmonella.

OBSERVATION PERIOD

Initially the animals were observed for 6 days. On the seventh day, considered day 1, the animals in Groups CG2, CG3, TG1, and TG2 were inoculated and observed for 25 days. The total duration of the experiment was 31 days.

STATISTICAL ANALYSIS

In this study, survival analysis techniques were used. This statistical method is utilized in the analysis of data for which the significant variable is the time needed for an event to occur. Here, the significant event (failure) was the death of the mouse; therefore, the answer variable is the time from the start of the experiment until the death of the mouse. This time is also known as the failure time.

Another characteristic of the set of data in survival analysis is the presence of censoring, which means the partial observation of the answer. In this case, the observations considered as "censored" are those where the mouse did not fail, i.e., the mouse had not died by the time the experiment ended.

The survival function was estimated using the non-parametric Kaplan-Meier estimator. The software used for the analyses was SAS (*R*) 9.2. For all hypothesis tests, a degree of freedom (*DF*) of 95% was considered.

To check the homogeneity of the groups, the hypothesis tests of Log-rank were used, and for survival analysis the Wilcoxon test was utilized. A hypothesis was considered null when the survival function was the same for all groups.

RESULTS

Table 1 shows that no failures (deaths) occurred for CG1 and CG3. All mice in CG2 died, and in groups TG1 and TG2, respectively, 40% and 80% of the mice died.

When the homogeneity of the survival functions (defined as the probability of survival of one individual beyond a given time, t, per treatment group) was tested, a value of $P < 0.05$ was found, which led us to reject the null hypothesis (this would mean the same survival hypothesis for all groups). Therefore, there is statistical evidence that the groups did not have the same survival function [Table 2]. The animals in groups CG1 and CG3 were statistically identical. They had the same behavior, i.e., all the mice survived [Table 1].

Comparing groups CG1 and CG2, CG1 and TG1, CG1 and TG2, CG2 and CG3, CG2 and TG1, CG3 and TG1, CG3 and TG2, TG1 and TG2, always considering the null hypothesis as the survival function being the same in these above compared groups, $P < 0.05$ was reached, which led us to reject the null hypothesis. In other words, there is statistical evidence that the groups did not have the same survival function [Table 3].

Table 1. Censoring and non-censoring numbers*

Stratum	No. of mice per group	Failures	Censorings	Censoring percentuals
CG 1	10	0	10	100,00
CG 2	10	10	0	0,00
CG 3	10	0	10	100,00
TG 1	10	4	6	60,00
TG 2	10	8	2	20,00
Total	50	22	28	56,00

*The censored mice were the animals that died. Non-censored were the mice that survived

Table 2. Homogeneity of the surviving function per group: equality over strata test

Test	Chi-square	DF	*P* > Chi-square
Log-rank	42.6634	4	< 0.0001
Wilcoxon	39.5522	4	< 0.0001

Table 3. Comparing the function of survival in the groups

Test	Chi-square	DF	*P* > chi-square
	CG1e CG2		
Log-rank Wilcoxon	21.1859 18.4629	1 1	< 0.0001 < 0.0001
	CG1 e TG1		
Log-rank Wilcoxon	4,7500 4.7500	1 1	0.0293 0.0293
	CG1 e TG2		
Log-rank Wilcoxon	12.7190 12.1019	1 1	0.0004 0.0005
	CG2 e CG3		
Log-rank Wilcoxon	21.18159 18.41629	1 1	< 0.0001 < 0.0001
	CG2 e TG1		
Log-rank Wilcoxon	11.7474 12.0819	1 1	0.0006 0.0005
	CG3 e TG1		
Log-rank Wilcoxon	4.7500 4.7500	1 1	0.0293 0.0293
	CG3 e TG2		
Log-rank Wilcoxon	12.7190 12.1019	1 1	0.0004 0.0005
	TG1 e TG2		
Log-rank Wilcoxon	4.8857 5.2898	1 1	0.0271 0.0215

Table 4. Homogeneity of groups GC2 and GT2 in relation to the survival function

Test	Chi-square	DF	*P* > chi-square
Log-rank	2.4670	1	0.1163
Wilcoxon	3.0544	1	0.0805

Comparing groups CG2 and TG2, we arrived at $P > 0.05$. In this case, the null hypothesis was accepted, which is the statistical evidence that these groups had the same survival function, with a confidence interval of 95% [Table 4].

DISCUSSION

We tried to minimize the number of variables in order to obtain results that would be more reliable and easier to analyze. For this reason, as the effect of the nutrients depends on their direct action as well as on their interaction with the intestinal biota (which in turn depends on several factors, such as gender, body mass index, and age), we used homogeneous samples: same species animals, same gender, age, and weight, raised in the same environment, treated during the same period by the same technician, and fed the same samples of commercial ration. The results allowed us to conclude that the addition of camel milk, more than the addition of cow milk, to the food made the mice more resistant to the Salmonella injected.

The protection against infection conferred by consumption of camel milk was observed in experiments performed in mice infected with *Schistosoma mansoni* [7], and in humans suffering from hepatitis B. The creation of a better immunological situation probably occurred through a better adjustment of the expression Th1/Th2-type cytokines which could strengthen the cellular immune response, inhibiting the replication of the virus DNA and promoting recovery in chronic hepatitis B patients [8].

What can explain the difference in protection obtained with cow milk and with camel milk in our experiment? Cow milk and camel milk show different anti-oxidant and anti-microbial activities, higher for camel milk [9]. The protein composition and structure of the two milks – bovine and camel – are similar but not the same. Most of the whey proteins in camel milk resemble those in bovine whey proteins, except for the lack of beta-lactoglobulin in the camel milk [10]. Camel milk's lactoferrin has very high levels of bactericidal and bacteriostatic properties against Gram-positive and Gram-negative bacteria [11], more than cow and human lactoferrin. The action is similar against viruses; in this case, for example, it prevents the penetration of hepatitis C virus in leukocytes [12,13].

Other substances present in camel milk could be responsible for the protection of the mice, such as lysozyme [14], lactoperoxidase, vitamin C (present in large amounts) [15], and carbohydrates through their proven immunomodulatory action [16].

The addition of milks, specially human milk but also from other animals – such as cow, giraffe, mule, buffalo, alpaca, and camel – lowers the bacterial resistance to antibiotics, through an immunomodulatory action, but also because they act as glycodecoys for blocking bacterial lectin attachment to cell receptors [9,17].

Elements such as zinc, copper, selenium and iron are not likely to have influenced our results, since their amounts in camel milk and in cow milk are practically the same [18].

What about the small immunoglobulin G molecules in camels? These small dimeric molecules have greater capacity for tissue penetration and act more intensely than the usual tetrameric molecules with heavy and light chains [19,20]. They are easily absorbed due to their potent enzyme-inhibiting and antigen-binding capacity [21,22], quickly invade the tissues, and are capable of more effectively inhibiting the development of aggressive agents. This is the case of the rotavirus and the hepatitis C virus [23,24].

No mammal is as dependent on maternal feeding as camels. Born agammaglobulinemic, if they not fed with their mother's milk they almost certainly will die [25]. Camel milk, exclusively, will provide the baby camel with the elements necessary for its protection, and this is the milk we used in our study.

The autopsies performed on the dead mice and on the survivors allowed us to observe an intense inflammatory process in the dead animals, and the absence, or very slight reaction, in the survivors. It is worth noting that in the surviving mice fed with cow milk there was an inflammatory process, but this was of little significance. On the other hand, there were no abnormal findings in the abdominal cavity of the survivors fed with camel milk.

The results demonstrate that protection is conferred by the consumption of camel milk. This protection was also shown to be higher than that obtained with cow milk.

There is a need for more in-depth study of camels. Although the importance of camels for Bedouins has lessened over time, they still use them for transportation, labor, tent making, for food (meat, milk), for their wool, skin and hides, for shade, and for medication. They value camels as part of their heritage and as a symbol of power, endurance and patience.

We have to consider this animal as an invaluable source of milk and meat. In regions where the supply of water and food is becoming more limited every day, camels could be a complement, or even an alternative, to bovine herds, which are in fact, more vulnerable than camels.

CONCLUSIONS

Based on the tests described, it can be concluded, with a 95% confidence interval, that the groups had different survival probabilities. Groups CG2 and TG2 were the only groups that demonstrated equal survival probabilities (within a 95%

confidence interval). In other words, there is no significant difference in the survival of infected mice that were fed with commercial plus ration cow milk as compared to the infected mice fed with commercial ration without cow milk.

This study has demonstrated the superiority of camel milk as a nutrient. Group TG1, which was fed with ration and camel milk, showed a significantly higher survival than the other mice groups that were infected and did not receive camel milk as part of their food.

Acknowledgments

We thank Dr. Carlos Alberto Bottrel Coutinho for his help in preparing this report; Dromedunas, Rio Grande do Norte, Brazil, which supplied the camel's milk; the Empresa Júnior de Estatística, ESTAT Consultoria of the Universidade de Brasília for the exploratory data analysis for this research; and the FACIPLAC (Faculdades Integradas do Planalto Central, Brazil) for providing the facilities for using animals, equipment and personnel of its bioterium.

Corresponding author:
Dr. R.R. de Almeida Cardoso
Clínica de Alergia São Joaquim, Quadra 1 bloco E, salas 301/313/314, CEP 70303-900, Brasília DF, Brazil
Phone/fax: (55-61) 3321-0848
email: rronaldac@gmail.com

References

1. Musinga M, Kimenye D, Kivolonzi P. The Camel Milk Industry in Kenya, Resource Mobilization Center, 2008.
2. Agrawal RP, Budan S, Sharma P, et al. Zero prevalence of diabetes in camel milk consuming Raika community of north-west Rajasthan, India. *Diabetes Res Clin Pract* 1984; 2: 290-6.
3. Cardoso RRA, Santos RMDB, Cardoso CRA, Carvalho MO. Consumption of camel's milk by patients intolerant to lactose. A preliminary study. *Rev Alerg México* 2010; 57: 26-32.
4. Ehlayel MS, Hazeima KA, Al-Mesaifri F, Bener A. Camel milk: an alternative for cow's milk allergy in children. *Allergy Asthma Proc* 2011; 32: 255-8.
5. Shabo Y, Barzel R, Margoulis Mark, Yagil R. Camel milk for food allergies in children. *IMAJ Isr Med Assoc J* 2005; 7: 796-8.
6. Al-Hammadi S, El-Hassan T, Al-Reyami L. Anaphylaxis to camel milk in an atopic child. *Allergy* 2010; 65: 1622-9.
7. Magharaby AS, Mohamed MA, Abdel-Salam AM. Anti-schistosomal activity of colostral and mature camel milk on *Schistosoma mansoni* infected mice. *Asia Pac J Clin Nutr* 2005; 14: 432-8.
8. Saltanat H, Li H, Xu Y, Wang J, Liu F, Geng XH. The influences of camel milk on the immune response of chronic hepatitis B patients. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 2009; 25: 431-3.
9. Salami M, Moosavi-Movahedi AA, Ehsani MR, et al. Improvement of the antimicrobial and antioxidant activities of camel and bovine whey proteins by limited proteolysis. *J Agric Food Chem* 2010; 58: 3297-302.
10. Merin U, Bernstein S, Bloch-Damti A, et al. A comparative study of milk serum proteins in camel (*Camelus dromedarius*) and bovine colostrum. *Livesstock Product Sci* 2001; 67: 297-301.
11. Conesa C, Sánchez L, Rota C, et al. Isolation of lactoferrin from milk of different species: calorimetric and antimicrobial studies. *Comp Biochem Physiol B Biochem* 2008; 150: 131-9.
12. Baker EN, Baker HM. Molecular structure, binding properties and dynamics of lactoferrin. *Cell Mol Life Sci* 2005; 65: 2531-9.
13. Redwan el-RM, Tabll A. Camel lactoferrin markedly inhibits hepatite C virus genotype 4 infection of human peripheral blood leukocytes. *J Immunoassay Immunochem* 2007; 28: 267-77.
14. Duhaiman AS. Purification of camel milk lysozyme and its lytic effect on *Escherichia coli* and *Micrococcus lysodeikticus*. *Comp Biochem Physiol B* 1988; 91: 793-6.
15. Farah Z, Rettenmaier R, Atkins D. Vitamin content of camel milk. *Int J Vit Nutr Res* 1992; 62: 30-3.
16. Vos AP, M'Rabet L, Stahl B, Boehm G, Garssen J. Immune-modulatory effects and potential working mechanisms of orally applied nondigestible carbohydrates. *Crit Rev Immunol* 2007; 27: 97-140.
17. Zinger-Yosovich KD, Iluz D, Sudakevitz D, Gilboa-Garber N. Blocking of *Pseudomonas aeruginosa* and *Chromobacterium violaceum* lectins by diverse mammalian milks. *J Dairy Sci* 2010; 93: 473-82.
18. Al-Awadi FM, Srikumar TS. Trace elements and their distribution in protein fractions of camel milk in comparison to other commonly consumed milks. *J Dairy Res* 2001; 68: 463-9.
19. Hultberg A, Tremblay DM, de Haard H, et al. Lactobacilli expressing llama VHH fragments neutralize Lactococcus phages. *BMC Biotechnol* 2007; 7: 1-7.
20. Yokota T, Milenic DE, Whitlow M, Schlom J. Rapid tumor penetration of a single-chain Fv and comparison with other immunoglobulin forms. *Cancer Res* 1990; 52: 3402-8.
21. Lauwereys M, Ghahroudi MA, Desmyter A, et al. Potent enzyme inhibitors derived from dromedary heavy-chain antibodies. *EMBO J* 1998; 17: 3512-20.
22. Hamers-Casterman C, Atarhouch T, Muyldermans S, et al. Naturally occurring antibodies devoid of light chains. *Nature* 1993; 363: 446-8.
23. Martin F, Volpari C, Steinkuller C, et al. Affinity selection of a camelized VH domain antibody inhibitor of hepatitis C virus NS3 protease. *Protein Eng* 1997; 10: 607-14.
24. Pant N, Hultberg A, Zhao Y, et al. Lactobacilli expressing variable domain of llama heavy-chain antibody fragments (lactobodies) confer protection against rotavirus-induced diarrhea. *J Infect Dis* 2006; 194: 1580-8.
25. Ungar-Waron H, Elias E, Gluckman A, Trainin Z. Dromedary IgG: purification, characterization and quantitation in sera of dams and newborns. *Isr J Vet Med* 1987; 43: 198-203.

"In our world of big names, curiously, our true heroes tend to be anonymous. In this life of illusion and quasi-illusion, the person of solid virtues who can be admired for something more substantial than his well-knownness often proves to be the unsung hero: the teacher, the nurse, the mother, the honest cop, the hard worker at lonely, underpaid, unglamorous, unpublicized job"

Daniel J. Boorstin (1914-2004), American historian, professor, attorney and writer

"What we obtain too cheap we esteem too little; it is dearness only that gives everything its value"

Thomas Paine (1737-1809), English-American political activist, author, political theorist and revolutionary. As the author of two highly influential pamphlets at the start of the American Revolution, he inspired the America Patriots in 1776 to declare independence from Britain