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# Phenotypic Alterations in T-cell Subpopulations in the Milk of Normal and Mastitic Buffaloes

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## ABSTRACT

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The T-cell subpopulation (BoCD4', BoCD8' and B0WC1' T cells) were analyzed in milk samples collected from five normal and mastitic buffaloes by flow cytometry. Milk obtained from mastitic buffaloes revealed a significant increase in $BoCD4^+$ and $BoWC1^+$ T cells as compared with that from normal buffaloes ($P<0.05$). An increase was also recorded in $BoCD8^+$ cells of mastitic buffaloes as compared with normal buffaloes, but this elevation was not significant ($P>0.05$).The mean ratio of BoCD4': BoCD8'T lymphocytes in the milk of healthy

animals was 0.9+0.03. However, this ratio was lowered in buffaloes with sub clinical mastitis, indicating the presence of a higher proportion of $BoCD8^{+}$ than of BoCD4‘ T lymphocytes in their milk. Hence, this study showed that the CD4:CD8 ratio could be used as an indicator of susceptibility to bovine mastitis.

**Keywords:** Mastitis, T cells, Phenotypic Alterations

## INTRODUCTION

Bovine mastitis is a disease complex with multifaceted aetiopathogenesis, different degrees of intensity, as well as variation in duration and residual effects and has remained one of the major constraints of growth in the dairy industry (Schalm *et al.,* 1971; Sasidhar et *al.,* 2002; Denis *et al.,* 2009; Sindhu *et al.,* 2009). This disease is characterized by inflammation of the parenchyma of the mammary gland regardless of the cause. Cellular infiltration in response to bacterial pathogen in the mammary gland microenvironment plays an important role in the pathogenesis and establishment of intramammary infections. Bacterial infections of the mammary gland are characterised by an early infiltration of neutrophils into the milk, followed by a predominance of mononuclear cells (Nickerson and Nonnecke, 1986). Although lymphocytes play an important role in immune response, the functional and phenotypic distribution of specific lymphocyte subpopulation during mammary infection is not well established. Most of the work on T-lymphocyte profile in mammary gland secretion (MGS) was conducted in cattle due to the availability of monoclonal antibodies (MAbs) against leucocyte differentiation antigens. There is paucity of research work on the immunopathogenesis of the disease in buffalo. In this study, efforts have been made to analyse T-cell subpopulation in normal and mastitic buffaloes in order to understand the immunopathogenesis of this disease.

## MATERIALS AND METHOD

A total of 354 quarter milk samples collected from 89 lactating apparently healthy Murrah buffaloes from the Buffalo Research Centre, CCS Haryana Agricultural University, Hisar, were screened for sub clinical mastitis. On the basis of the International Dairy Federation criteria, samples found positive for bacteriological examination and showing somatic cell count more than 5 lakhs per millilitre of milk were identified as subclinically infected with mastitis. The study was planned to compare the cellular immune response of normal and mastitic animals.

### Characterisation of T-cell sub-population

Populations of different T cells viz., $BoCD4^+$, $BoCD8^+$ and BoWCT were analysed using MAbs against these T-cell markers by flow cytometry as per the method of Sharma (1990).These MAbs recognise BoCD4\ $BoCD8^+$ and BoWC1 $^+$ T cells. The details of these

MAbs are as follows:

| S.**No.** | **Clone** | **MAb** | **Lymphocyte recognised** |
|---|---|---|---|
| 1 | CC 30 | BoCD4 | T-helper cells |
| 2 | CC 63 | B0CD8 | Cytotoxic T celis |
| 3 | CC 15 | BoWC1 | Gamma-delta-positive T cells |

**Leucocyte isolation from milk**

Mononuclear cells were isolated from the milk of 10 lactating (5 normal and 5 mastitic) animals. Milk was first aseptically collected into sterile flasks and then centrifuged at 3000 r.p.m. for 20 min at 4°C. The creamy layer was removed using a sterile spatula and the milk gently decanted. The cell pellet was resuspended in 20 ml PBS. Cell suspension was layered on to histopaque gradient (Sigma Chemicals Company, USA) and centrifuged at 1200 r.p.m. for 30 min at 4°C. Mononuclear cells at interface were separated using a sterile pasture pipette. The cell pellet was resuspended in 10 ml PBS and centrifuged at 1200 r.p.m. for 20 min at 4°C in refrigerated centrifuge (REMI-C-23). The supernatant was discarded and cells were finally resuspended in 5 ml PBS.

**Immunofluorescent staining and flow cytometry**

Approximately 1 x$10^6$ mammary mononuclear cells were incubated with lineage-specific MAbs against T- lymphocyte antigens for I h at 4°C. After incubation, cells were washed thrice with PBS containing 0.1% sodium azide and incubated with 1:80 diluted fuorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin G (Sigma Chemicals Company, USA) for 1 h at 4°C. Following incubation, cells were washed thrice with PBS containing 0.1% sodium azide and resuspended in PBS-sodium azide containing 3% BSA and 2% formaldehyde. A Becton • Dickinson FACS calibur flow cytometer and Hewlett Packard software were used for data acquisition and for analysis of MAb leucocyte staining patterns at the Division of Biochemistry, Indian Veterinary Research Institute, Izatnagar, Bareilly. The

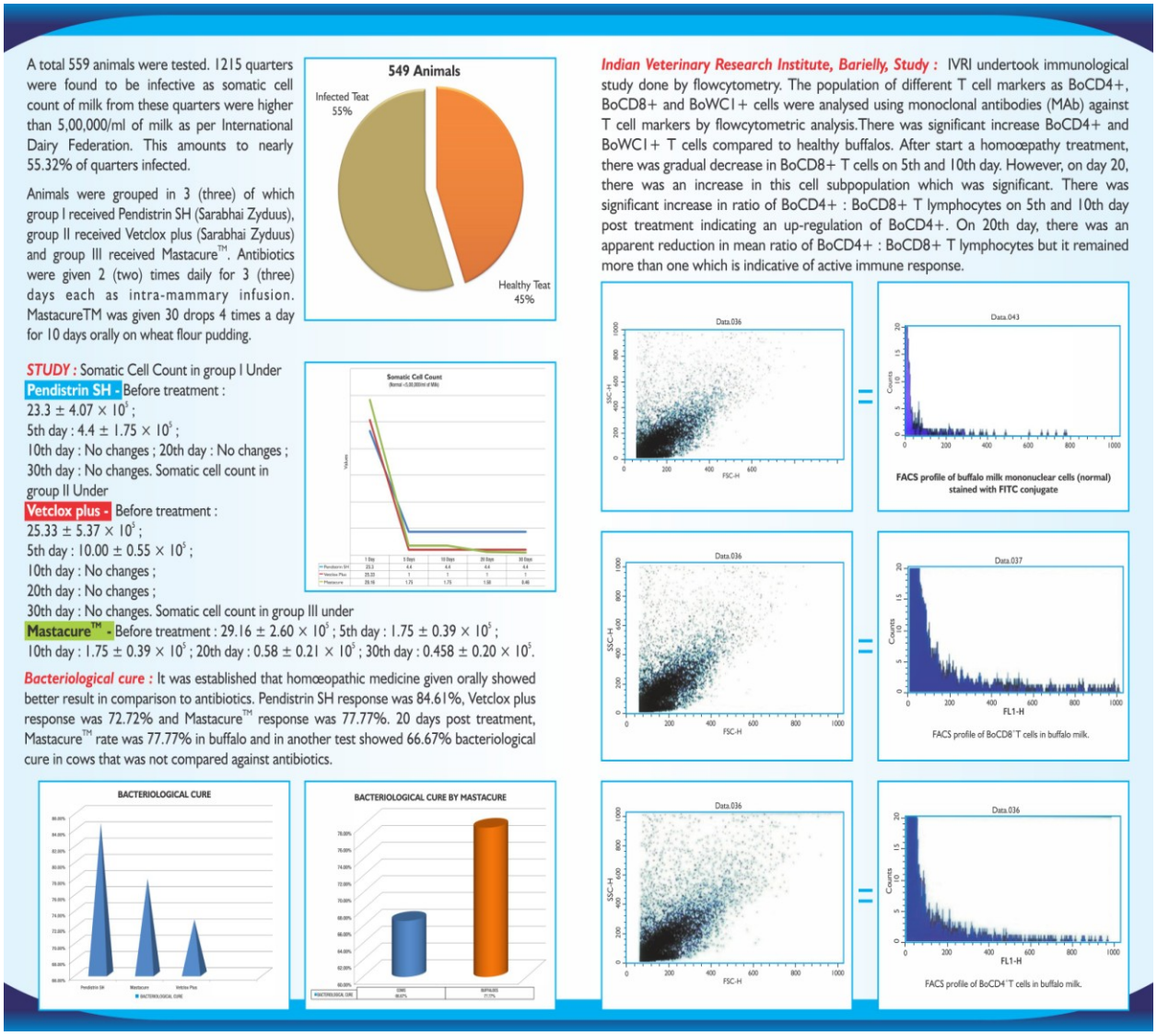
percentage of positive cells out of 10,000 was calculated by subtracting background staining with fluorescent conjugate control.

## RESULTS AND DISCUSSION

T-cell subpopulation (BoCD4\ BoCD8$^{+}$ and BoWCV T cells) were analysed in milk samples collected from five normal and five subclinical mastitic buffaloes by flow cytometry.

### Proportion ofCD4$^{+}$, CD8$^{+}$andWc1$^{+}$T cells in the milk of buffaloes

The reactivity of bovine MAbs that recognised BoCD4$^{+}$, BoCD8$^{+}$ and BoWC 1$^{+}$T cells to homologous antigens on buffalo milk lymphocytes have been shown through Fig. 1 to 4 . In addition, the percentage of T- lymphocyte subpopulation in the milk of mastitic buffaloes varied during intramammary infection. Milk from mastitic buffaloes was characterised by a significant increase in BoCD4$^{+}$ and BoWC1$^{+}$ T cells



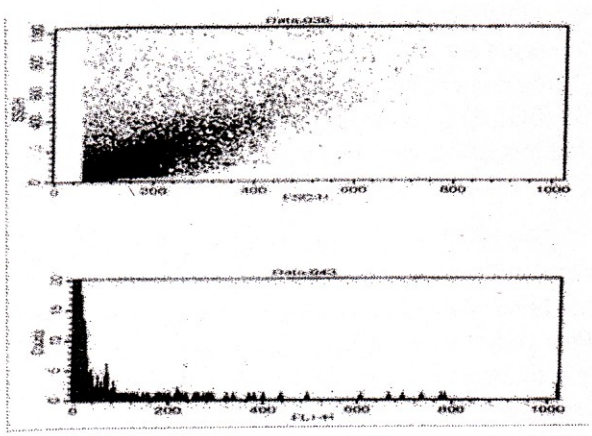


**Figure 1:** FACS profile of buffalo milk mononuclear cells (normal) stained with FITC conjugate

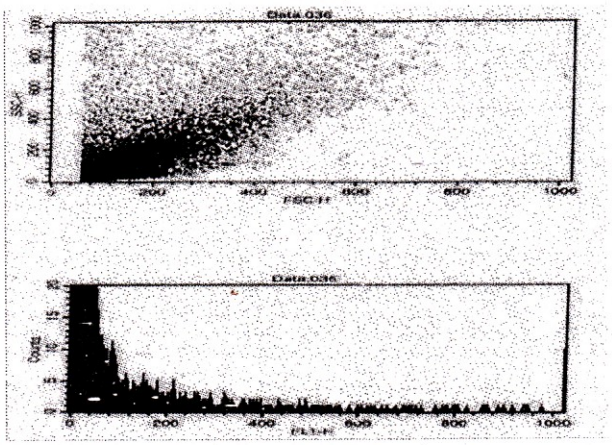


**Figure 3:** FACS profile of BoCD4⁺ T cells in buffalo milk

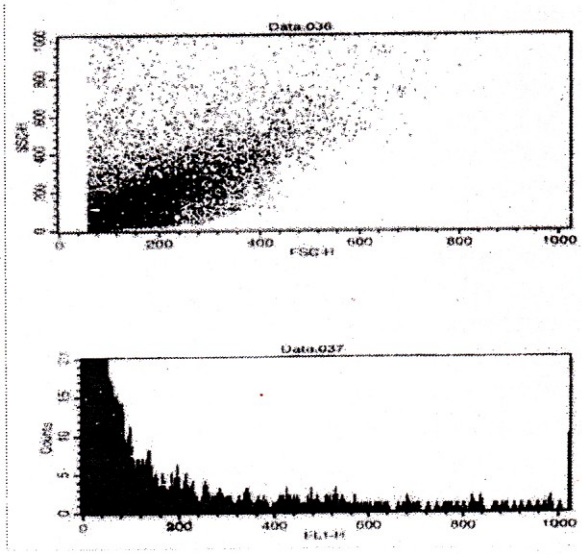


**Figure 2:** FACS profile of BoCD8⁺ T cells in buffalo milk

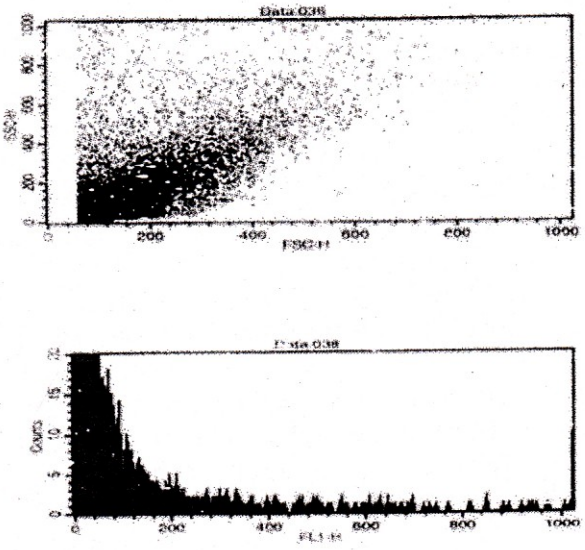


**Figure 4:** FACS profile of BoCD4⁺ T cells in buffalo milk

compared with that from healthy buffaloes *(P*<0.05). An increase was also recorded in BoCD8$^+$ cells of mastitic buffaloes as compared with normal buffaloes, but this elevation was not significant (P>0.05).

**Ratio of BoCD4$^+$:BoCD8$^+$ T lymphocytes**

The mean ratio of BoCD4*:BoCD8$^+$ T lymphocyte in the milk of healthy animals was 0.9+0.03. However, this ratio was lowered to 0.78±0.13 in buffaloes with subclinical mastitis, indicating the presence of a higher proportion of BoCD8$^+$ than of BoCD4*T lymphocytes in the

milk of mastitic buffaloes.

In this study, we have used bovine MAbs to characterize buffalo T-cell subpopulation with a view that in more closely related species there is probability that MAbs that develop against one species will recognise an epitope conserved on an orthologue in other species. The findings reported in this study supported this notion. Bovine MAbs used in this study recognised orthologue T-cell antigens in buffaloes as 7revealed by flow cytometric analysis. Davis *et al.* (2001) employed a vast panel of bovine MAbs against leucocyte differentiation molecule to characterise leucocyte antigen of water buffalo and reported that the composition of leucocyte population in water buffaloes is similar to that in cattle. The cross-reactivity of bovine MAb to the T-cell subpopulation of buffalo was exploited to analyse the T-cell subpopulation in the milk of buffaloes.

The findings of this study revealed a higher proportion of $BoCD8^+$ T cells in milk compared with $BoCD4^+$ cells and BoWCr T cells. Studies in cattle have also revealed a higher proportion of $BoCD8^+$ T cells in MGSs as compared with peripheral blood (Park *et al.,* 1992;Taylor *et al.,* 1994; Shafer-Weaver *et al.,* 1996).The mean ratio of BoCD4:BoCD8 T lymphocyte recorded in this study was 0.9. Park *et al.* (1992) also recorded a similar ratio in MGS. However, their findings revealed that the CD4:CD8 ratio varied during the lactation cycle. The ratio was highest at the early lactation, i.e., 1.81 and lowest at early non-lactation (1.18). MGS of buffaloes also contain gamma-delta- positive cells (BoWC1" T cells), but their population was low compared with $BoCD4^+$ T cells and $BoCD8^+$ T cells. A similar low level of gamma-delta-positive T cells has also been reported in MGS of cattle (Park *et al.,* 1992). Results of this study demonstrated a significant increase in the proportion of $BoCD4^+$ and BoWC1* (gamma-delta-positive) T cells in the milk of mastitic buffaloes as compared with healthy buffaloes. An increase was also recorded in the proportions of $BoCD8^+$ T cells, but it was not statistically significant *{*$P>0.05$). Previous studies conducted in mastitic cows also revealed an increase in the proportion of $BoCD4^+$, BOWC1 T cells and BoCD8*T cells in MGS (Taylor *et al.,* 1997; Soltys and Quinn, 1999; Rivas *et al.,* 2000). Soltys and Quinn (1999) observed that an increase in gamma-delta-positive T cells was mainly due to a proportional increase in $BoCD4^+$ T cells only in streptococcal mastitis. However, an increase in gamma-delta-positive T-cell population in staphylococcal mastitis was attributed to upregulation or'$BoCD4^+$T cells and $BoCD8^+$T cells.

These workers suggested that this difference in the responding T-cell subset might be due to the nature of toxins released by staphylococcal and streptococcal bacteria during the acute stage of infection. Studies by Ferens *et al.* (1988) demonstrated that staphylococcal enterotoxin C1 (SEC 1) preferentially activates $CD4^{+}$T cells and that this activation was influenced by the proportion of gamma-delta-positive T cells in culture.

The significance of upregulation of gamma-delta- positive T-cell subset in the milk of mastitic buffalo has not been defined thoroughly. However, Mackay and Hein (1991) reported that these cells may also play a role in infectious disease and therefore provide an important line of defence against bacterial infections. Studies conducted by Richie *et al.* (1982) and Shafer-Weaver *et al.* (1996) showed that relative to the blood, ruminants express greater levels of gamma-delta- positive T lymphocytes in mammary secretions and mammary parenchyma. Hence, their functions are primarily associated with protection of the epithelial surface. Data by De Libero (1997) suggest that these cells play an important role in the initial host response to infectious agents. According to D'Souza *et al.* (1997), gamma-delta-positive T cells do not directly protect against infection, but instead play a role in modulating local cellular traffic by promoting the influx of lymphocytes and monocytes and by limiting the access of inflammatory cells that do not contribute to protection but can cause tissue damage. A group of several other authors also suggested that gamma- delta-positive T cells may have a protective or anti-inflammatory function (Mukasa *et al.,* 1995, 1997; Saunders *et al.,* 1998). On the contrary, studies by Spinozzi *et al.* (1998) and Zuany-Amorim *et al.* (1998) reported that gamma-delta-positive T cells have been shown to play a role in contributing to inflammatory response.

Thus, it is clear that gamma-delta-positive T cells do play a role in modulating inflammatory response; however, it is currently not known whether the primary role of increased levels of gamma-delta-positive T cells is to regulate the magnitude of the immune response or to directly contribute to host tissue protection. Further studies are required to investigate these issues.

## REFERENCES

Davis WC, Khalid AM, Hamilton MJ, Ahn JS, Park YH and Cantor GH. (2001). The use of cross reactive monoclonal antibodies to characterize the immune system of the water buffalo *(Bubalus bubalis). J. Vet. Sci., 2:*103-109.

Denis M, Wedlock DN, Lacy-Hulbert SJ, Hillerton, JE and Bundle BM. (2009). Vaccines

against bovine mastitis in New Zealand context: what is the best way forward? *New Zealand Vet. J.,* 57:132-140.

-De Libero G. (1997). Sentinel function of broadly reactive human gamma-delta T cells. *Immunol. Today.,* 18:22- 26.

D'Souza CD, Copper AM, Frank AA, Mazzaccaro RJ, Bloom BR and Orme IM (1997). An anti inflammatory role for gamma-delta T lymphocytes in acquired immunity to *Mycobacterium tuberculosis. J. Immunol.,* 158: 1217-1221.

Mackay CR and Hein WR (1991). Marked variation in gamma delta ?T cells numbers and distribution throughout the life of sheep. *Curr. Topics Microbiol. Immunol.,* 173:107-110.

Mukasa A, Lahn M, Pflum EK; Born W and O'Brien RL.

(1997) . Evidence that the same gamma delta ?T cells respond during infection-induced and autoimmune inflammation. *J. Immunol.,* 159:5787-5794.

Mukasa A, Hiromatsu K, Matsuzaki G, O'Brein R, Born W and Nomoto K. (1995). Bacterial infection of the testes leading to autoaggressive immunity triggers apparently opposed responses of alpha beta ?and gamma delta T cells. *J. Immunol.,* 155:2047-2056.

Nickerson, SC and Nonnecke BJ. (1986) Tuberculin elicited cellular immune response in the lactating bovine mammary gland vaccinated intramammarily with *Mycobacterium bovis. Vet. Immunol. Immunopathol.,* 13:39-50.

ParkYH, Fox LK, Hamilton MJ and Davis WC. (1992). Bovine mononuclear leukocyte subpopulations in peripheral blood and mammary gland secretions during lactation. *J. Dairy Sci.,* 75: 998-1006.

Richie ER, Bass R, Meistrich ML and Demmison DK. (1982). Distribution of T-lymphocyte subsets in human colostrum. *J. Immunol.,* 129:1116.

Rivas AL, Quimby FW, Coksaygan O, Qlmstead L and Lein DH. (2000). Longitudinal evaluation of CD4' and CD8* peripheral blood and mammary gland lymphocytes in cows experimentally inoculated with *Staphylococcus aureus. Can. J. Vet. Res.,* 64:232-237.

Sasidhar PVK, Reddy YR and Rao BS. (2002). Economics of mastitis. *Indian J. Anim. Sci.,* 72:439-440.

Saunders BM, Frank AA, Cooper AM and Orme IM. (1998). Role of gamma delta T cells in immunopathoiogy of pulmonary *Mycobacterium viuam* infection in mice. *Infect.lmmun.,* 66:5508-5514.

Schalm OW, Carrol EJ and Jain NC (1971). In' *Bovine mastitis.* 1971. Ed. Lea and Febiger, Philadelphia.

Shafer-Weaver K, Pighetti G M and Sordillo LM. (1996). Diminished mammary gland lymphocyte functions parallels shifts in trafficking patterns during the postpartum period. *Proc. Soc. Exp. Biol. Med.,* 212: 271-280.

Sharma R. (1990). Immuno-pathogenesis of bovine respiratory syncitial virus in experimentally infected labs. Thesis, University of Liverpool, UK.

Sindhu N, Sharma A, Nehra V and Jain VK. (2009) Occurrence of subclinical mastitis in cows and buffaloes at an organised farm. *Haryana Vet.,* 48:85- 87.

Soltys J and Quinn MT. (1999). Selective recruitment of T cell subsets to the udder during Staphylococcal and Streptococcal mastitis: analysis of lymphocyte subsets and adhesion molecule expression. *Infect. Immun.,* 67: 6293-6302.

Spinozzi F, Agea E, Bistoni O, Forenza N and Bertotto A.

(1998) . Gamma delta T ?cells, allergen recognition and airway inflammation. *Immunol. Today,* 19:22-26.

Taylor BC, Dellinger JD, Cullor JS and Stott JL. (1994). Bovine milk lymphocytes display the phenotype of memory T-cells and are predominantly CD8+. *Cell. Immunol.,* 156 (1): 245-253.

Taylor BC , Keefe RG, Dellinger JD , Nakamura Y , Cullor JS and Stott JL. (1997).T cell populations and cytokine expression in milk derived from normal and bacteria- infected bovine mammary glands. *Cell Immunol.,* 82: 68-76.

Zuany-Amorim C, Ruffie C, Haile S, Vargaftig BB, Pereira P and Pretolani M. (1998). Requirement for gamma delta T cells in allergic airways inflammation. *Science,* 280: 1265-1267.

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5. List of published reviews in Journals and international proceedings/books

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8. Contributions to the advancement of Veterinary Medicine

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