

MOLECULAR ASPECTS OF BOVINE TROPICAL ADAPTATION

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INTRODUCTION

The Webster's Dictionary defines adaptation as the modification of an organism or its parts that makes it fitter for existence under conditions of its environment, or adjustment to environment conditions. Adaptation to tropical conditions may also be defined as the ability to survive and reproduce under harsh environment. On natural conditions, it means that animals must stand for heat stress, parasite challenges and diseases, very common in tropical areas. The complexity of these environment challenges allows for different genotypes with equivalent "adaptation value" emerging from very distinct mechanisms.

In bovine, the knowledge about the origins of *Bos taurus* and *Bos indicus* helps to better understand why some breeds are more adapted to tropical conditions than others. Most of all modern domesticated cattle breeds are believed to be derived from the extinct wild ox or aurochs, *Bos primigenius* (Epstein, 1971; Epstein & Mason, 1984) from which three continental races have been identified: *B. primigenius namadicus* (Asia), *B. primigenius opisthonomus* (North Africa), and *B. primigenius primigenius* (Europe). The most accepted idea is that taurine cattle were domesticated from *B. primigenius namadicus* in civilizations of Near East, 8,000 – 10,000 years ago (Payne, 1991; Epstein & Mason, 1984) and many authors have concluded that zebu breeds developed from taurines, subsequent to the original domestication event. However, a survey of mtDNA variation by Loftus et al (1994), combined with the interpretation of early Neolithic remains in Baluchistan (Meadow, 1993), strongly indicate a separate origin for the cattle populations of the Indian subcontinent. The modern (as well as the earliest) domestic cattle of this region are of humped subspecies *B. indicus*, whereas those of Europe are humpless or of *B. taurus* type.

The first bovine domesticated of Africa are believed to have been *B. taurus* (taurine) in morphology, and the *B. indicus* (zebu) breeds, which now predominate, entered the continent some few millennia later (Epstein, 1971). Differences between the indigenous African taurine breeds that survive and the European cattle include economically important traits such as heat and disease tolerance (Murray et al. 1982).

Heat stress tolerance. During their separate evolution from *Bos taurus*, zebu cattle (*Bos indicus*) have acquired genes that confer thermotolerance at the physiological and cellular levels (Hansen, 2004). Thermotolerance is also observed in some *Bos taurus* breeds that evolved in tropical climates such as Senepol and Romosinuano (Hammond et al., 1996 Magee et al., 2002). Cattle from zebu breeds have more efficient body temperature regulation in response to heat when compared to most *B. taurus* breeds of European origin (Finch, 1986; Carvalho et al., 1995; Hammond et al., 1996; Gaughan et al., 1999). The thermoregulatory efficiency of zebu may be attributed to reduced heat production as a result of lower basal metabolism. This efficiency could also be attributed to increased heat dissipation, probably related to skin differentiations such as differences in density and structure of sweat glands, vascularization, or a combination of both (Hansen, 2004). Other morphological traits like coat color (Reinsch et al., 1999) and hair length (Olson et al., 2003) are also considered to influence

body temperature maintenance under high solar irradiation and high temperatures. It is not yet known if the same genes are involved in heat tolerance in *Bos indicus* and *Bos taurus* but it is clear that in both cases tolerance is observed at the cellular level (Hansen, 2004).

The effects of heat in the organisms are related to hyperthermia and consequent impairment of tissue and organ functions through reduction on blood flow (Kregel, 2002). These effects can be observed in several tissues and systems, compromising spermatogenesis and viability of oocytes and embryos (Rivera & Hansen, 2001; Krininger et al., 2003; Roth & Hansen, 2004a).

Although there is a good amount of knowledge about the physiological aspects, the effects of heat stress at the cellular level are still being unrevealed. Some of the molecules related to heat stress are the so-called heat shock proteins (HSPs). These proteins were first described as proteins whose expression were induced by heat shock but were later associated to different stress stimuli (Kregel, 2002). HSPs are a large group of unrelated protein families that behave as molecular chaperones. Some of their functions in the cells are to stabilize unfolded proteins, unfold them, either for translocation across membranes or for degradation, to assist in their correct folding and assembly. Evidences relating HSPs to heat tolerance are not conclusive but cytoprotective functions could be attributed particularly to the HSP70 family, such as prevention or correction of abnormal protein structure and prevention of translation arrest in the injured cell. HSP70 is also related to modulation of cytokine production and antigen presentation.

HSPs also play an important role in different apoptotic pathways; either repressing (HSP27, HSP 70 and HSP90) or inducing cell death (HSP60). Roth and Hansen (2004b) have shown that the apoptotic response observed during maturation of heat stress exposed bovine oocytes is mediated by group II caspases, a class of molecules related to DNA damage in apoptotic pathways. According to these authors, some oocytes seem to be better equipped to survive cellular stress, which could result from cumulus cells protection or differences in availability of heat shock protein 70 (HSP70). Evidences that differences in heat tolerance may be explained at the cellular level were also given by Paula-Lopes et al. (2003) comparing the effects of heat stress on cells and tissues from thermotolerant and thermosensitive breeds. Their results suggest that, besides the greater ability to regulate body temperature, Brahman and Senepol cells are also more able to survive hyperthermia resulting from heat stress.

Attempts to correlate sequence variations at HSPs to heat tolerance were not straight forward even though there are associations between variations on the gene expression level with thermotolerance (Favatier et al., 1997) and protection to several pathophysiological stresses in transgenic mice (Mestril, 2005). Several known molecules other than HSPs may be involved in thermoadaptation but identification of specific genes conferring cellular thermotolerance is still a challenge.

Resistance to parasite. An important aspect of tropical adaptation is resistance to tick and tick borne diseases. Tick resistance is a very complex trait since it is influenced by a great number of environmental and physiological factors, such as temperature, humidity, gender and age (Ashton et al., 1968). Despite that, there is a genetic component for the variation on tick resistance and estimates of heritability varies from very low to high, according to evaluation method (artificial x natural challenge), population studied and statistical method (Reviewed by Alencar, 2005).

Several studies on protein polymorphisms reported associations of specific alleles to tick count in different bovine breeds (Ashton et al., 1968; Panepucci et al., 1989; Veríssimo et al., 2004, Regitano et al., 2005). With the exception of the work done by Ashton et al. (1968), the main question about these association studies regards to distribution of allelic frequencies, since

alleles associated with resistance were considered characteristic of zebu breeds, which are more resistant to tick. Associations between BoLA class II alleles and tick resistance were reported (Stear et al., 1990; Acosta-Rodríguez et al., 2005; Martinez et al., 2005a)

In Brazil, a project is being developed at Embrapa to identify molecular markers for parasites and heat stress tolerance in *B. taurus* x *B. indicus* cross. With this purpose, a F2 population of 400 animals is being genotyped with 200 markers and also phenotyped for these traits. A preliminary analysis done with tick resistance data of 258 animals indicate a QTL ($P < 0.05$) on BTA 18, located at 61 cM from the centromere (Martinez et al., 2005b). Other preliminary results based on interval analysis points for two suggestive ($P < 0.05$) QTLs for tick resistance on BTA7 and BTA14 (Gasparin et al., *in preparation*; Miyata et al., *in preparation*). Additional animals are currently under evaluation for tick resistance and additional chromosomes are also being genotyped in order to search for QTL regions.

Aspects of tick interaction with the host immune system are also relevant for the understanding of host resistance. Tick saliva have evolved to produce several molecules that modulate host immunity (Brossard & Wikel, 2004).

Antibody response plays an important role on tick resistance since tick saliva is immunogenic and passive transfer of immunity may be obtained with sera from tick exposed animals (Roberts & Kerr, 1976). In this sense, impairment of host antibody production would be of benefit for tick survival. Kashino et al. (2005) demonstrated that resistant animals presented significantly higher levels of IgG anti-tick saliva antibodies than two different breeds of susceptible cattle. The authors concluded that this difference was actually due to a decrease in the levels of IgG1 and IgG2 saliva-specific antibodies in susceptible animals.

Ticks also modulate interleukins secretion and activity. Quantitative analysis of five interleukin mRNAs revealed depression of *IL-2* mRNA levels on lymph nodes of Nelore calves exposed to artificial challenge with *Boophilus microplus*, when compared to control (Nakata et al., *in preparation*). An interleukin-2 binding protein that complexes with this cytokin in the fluid phase was described in the saliva of *I. scapularis* (Gillespie et al. 2001). The observation of different mechanisms focused on the impairment of *IL2* functions in two species of tick suggests that this interleukin plays an important role in the host response to these parasites.

Tick modulation of host immune response is also crucial in tick-borne disease transmission (Brossard & Wikel, 2004). It is well documented that less economical losses are expected from these pathogens in *Bos indicus* cattle. The question whether this fact results from resistance to hemoparasite invasion, impairment of parasite development or is a consequence of lower challenge due to low tick infestation is still unclear.

Babesia are protozoan hemoparasites of cattle that share some biological aspects with *Plasmodium*. Since it was demonstrated that *Plasmodium vivax* depends on the recognition of the Duffy blood group antigen for cell invasion, this surface protein was considered as a candidate molecule to be related to resistance to *Babesia*. Supporting this idea, a high frequency (92.93%) of the null Duffy phenotype $FY^{(a,-)}$ was observed by Nakamoto *et al* (1998) in *Bos indicus* breeds whereas *Bos taurus* breeds had much lower frequency (36,25%).

Conversely, results from *in vitro* studies showed that at least in the case of *Babesia divergens* the main receptors to enter the red blood cells are glycoporphins, with no evidence implicating Duffy antigen as a receptor for this protozoan (Lobo, 2005). Recent results (Oliveira et al., 2005) do not support differences in *Babesia bovis* and *Babesia bigemina* infection rates between *Bos indicus* and cross bred *Bos taurus* X *Bos indicus*, analyzed by PCR and nPCR.

Albeit this is a very preliminary data, it suggests that zebu exposed to *Babesia* do not undergo severe symptoms of the disease but do become healthy carriers.

Nematode challenge is responsible for great economical losses, not only in tropical areas but also in temperate climate. Besides the enormous economical losses, drug control faces problems with emerging drug resistance and environment impacts. Genetic variation for resistance to gastrointestinal parasites does exist in most ruminant species (Charon, 2004).

Attempts to identify genes and QTLs related to host resistance to helminthes are in progress and promising results are already available. In a study of *Ostertagia ostertagi* conducted by Gasbarre and collaborators, QTLs for fecal egg count and immune response in Angus cattle were located at BTA 3, 5 and 6 (Sonstegard & Van Tassel, 2004). The gene encoding interferon gamma (*IFNG*) is one of the candidate genes that are located on BTA5. Associations between fecal egg count and this gene or chromosomal region were also described for sheep (Paterson et al. 2001, Coltman et al., 2001). Even though research may benefit from the investigation of some obvious candidate genes, discovery of QTLs for resistance to gastrointestinal parasites have to be conducted for each specific parasite-host interaction since different immune mechanisms may control resistance in each case. This implies on production of reference families with adequate number of animals and generations as well as pedigree control (Charon, 2004).

CONCLUDING REMARKS

There is a reasonable variation among cattle breeds for traits related to adaptation to the tropical environment. Cattle of *Bos indicus* breeds are much more resistant to tropical environment challenges, although some genetic characteristics of zebu cattle limit their usefulness as beef and dairy animals (Hansen, 2004). In dairy cattle systems, utilization of zebu crossbreds in hot climates becomes more beneficial compared to purebred European breeds as the overall level of feed resources and other inputs decline (Madalena et al., 1990; McGlothen et al., 1995).

Incorporating zebu genes for thermotolerance into European breeds, while avoiding undesirable genes, would also be of benefit. An example of this strategy using conventional breeding approaches comes from the *B. taurus* Senepol and Carora breeds in which criollo genotypes have been incorporated.

Given the complexity of the traits related to adaptation to tropical environments, the discovery of genes controlling these traits is a very difficult task. Most of this difficulty is related to a good phenotypic measure of traits such as body temperature and tick counts, since several environment and physiological factors affect them. Even though searching for these genes is a complex task, there are promising results emerging in the literature. Application of marker assisted selection in this case is one of the most favorable situations, since phenotypic evaluation in conventional breeding schemes would be very time consuming and some times conflicting with other traits' evaluations.

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