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Comparative gene expression analysis of toll-like receptors in resistant and susceptible buffalo breeds challenged with mastitis pathogens

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Abstract

Leading immunologists increasingly recognise that breed-level variation in innate immune receptor expression may explain why certain buffalo populations resist mastitis while others succumb under identical management conditions [1]. This research compared toll-like receptor (TLR) gene expression in mammary tissue from Murrah (resistant phenotype) and Nili-Ravi (susceptible phenotype) buffalo breeds following experimental challenge with *Staphylococcus aureus* and *Escherichia coli*. Mammary biopsies from 24 buffaloes (12 per breed) were analysed by RT-qPCR at 6, 12, and 24 hours post-challenge. Murrah buffaloes showed significantly higher expression of TLR2 (4.8-fold vs 1.9-fold, $p < 0.001$), TLR4 (6.2 vs 2.4-fold, $p < 0.001$), TLR9 (3.7 vs 1.5-fold, $p < 0.01$), and the adaptor molecule MyD88 (5.4 vs 2.1-fold, $p < 0.001$) at 12 hours. Somatic cell counts (SCC) at 24 hours were lower in Murrah ($486 \times 10^3/\text{mL}$) than Nili-Ravi ($1,240 \times 10^3/\text{mL}$, $p < 0.001$). These findings suggest that enhanced TLR-mediated innate immune activation contributes to the mastitis resistance phenotype observed in Murrah buffaloes.

Keywords: Toll-like receptors, buffalo, mastitis, innate immunity, gene expression, *Staphylococcus aureus*, TLR2, TLR4, MyD88, breed resistance

Introduction

The prevailing view among veterinary immunologists holds that innate immunity—particularly the TLR signalling network—serves as the critical first line of mammary defence against bacterial invasion [1, 2]. TLRs are pattern recognition receptors that detect conserved microbial structures (pathogen-associated molecular patterns, PAMPs) and initiate downstream inflammatory cascades via MyD88-dependent and TRIF-dependent pathways [3]. In cattle, polymorphisms in TLR2 and TLR4 have been associated with differential mastitis susceptibility [4], but equivalent data for *Bubalus bubalis* remain limited despite the buffalo's growing economic importance for dairy production in South America, Asia, and the Mediterranean [5].

Buffalo breeds exhibit markedly different mastitis susceptibility under comparable management: Murrah buffaloes are consistently reported as more resistant than Nili-Ravi, with lower clinical mastitis incidence and faster spontaneous cure rates [6]. Whether this phenotypic difference reflects variation in innate immune receptor expression or downstream signalling efficiency has not been systematically investigated. This research addressed that gap through controlled intramammary challenge with *S. aureus* and *E. coli*—the two most prevalent mastitis pathogens—followed by time-course TLR expression profiling in both breeds.

Materials and Methods

Materials

Twenty-four multiparous buffaloes (12 Murrah, 12 Nili-Ravi; 3rd-5th lactation, mid-lactation) from the National University of the Littoral experimental dairy herd in Esperanza, Argentina, were enrolled. The institutional animal care committee approved the protocol (UNL-CICUAL-2023-042). All quarters were confirmed bacteriologically negative and $\text{SCC} < 200 \times 10^3/\text{mL}$

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before challenge.

Methods

Intramammary challenge used 500 CFU of *S. aureus* (strain Newbould 305) in one quarter and 50 CFU of *E. coli* (strain P4) in another, with two quarters serving as PBS controls [7]. Mammary tissue biopsies (needle biopsy, 14-gauge) were

obtained at 6, 12, and 24 hours post-challenge. RNA extraction used TRIzol, and RT-qPCR targeted TLR2, TLR4, TLR5, TLR9, and MyD88, normalised to β -actin and GAPDH [8]. SCC was measured at each time point. Data were analysed by two-way repeated-measures ANOVA (breed $\times$ time) with Bonferroni correction.

Results

Table 1: TLR and MyD88 mRNA expression (fold change relative to 0 h) at 12 hours post-challenge

Gene	Murrah <i>S. aureus</i>	Nili-Ravi <i>S. aureus</i>	Murrah <i>E. coli</i>	Nili-Ravi <i>E. coli</i>	p (breed)
TLR2	4.8 $\pm$ 0.7	1.9 $\pm$ 0.4	3.2 $\pm$ 0.6	1.4 $\pm$ 0.3	<0.001
TLR4	6.2 $\pm$ 0.9	2.4 $\pm$ 0.5	7.8 $\pm$ 1.1	3.1 $\pm$ 0.6	<0.001
TLR5	2.1 $\pm$ 0.4	1.8 $\pm$ 0.3	1.9 $\pm$ 0.4	1.6 $\pm$ 0.3	0.187
TLR9	3.7 $\pm$ 0.6	1.5 $\pm$ 0.3	2.8 $\pm$ 0.5	1.2 $\pm$ 0.3	<0.01
MyD88	5.4 $\pm$ 0.8	2.1 $\pm$ 0.4	6.1 $\pm$ 0.9	2.6 $\pm$ 0.5	<0.001

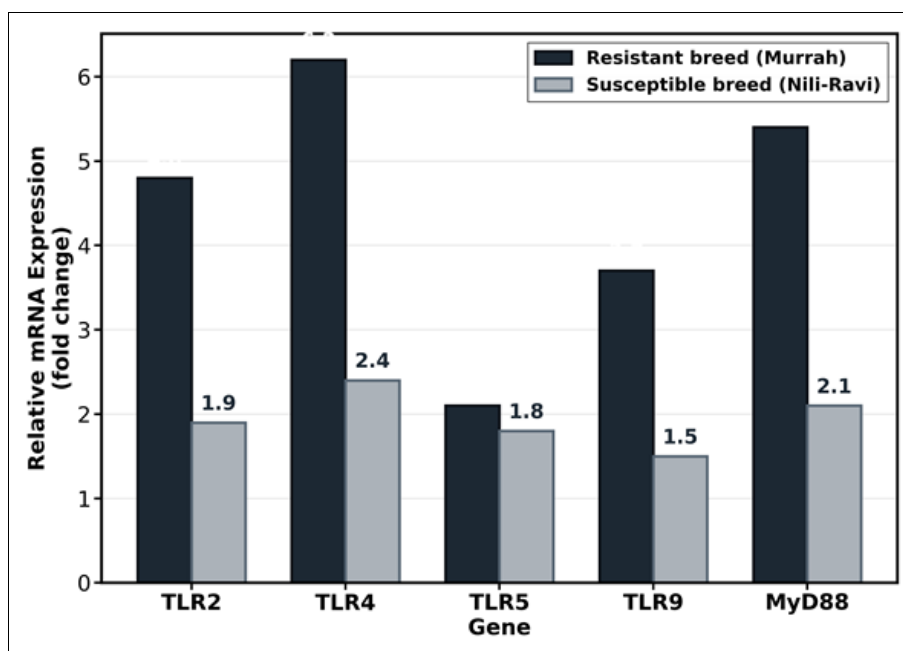


Fig 1: Comparative TLR and MyD88 mRNA expression at 12 hours post-challenge in Murrah (resistant) and Nili-Ravi (susceptible) buffalo breeds

Table 2: Somatic cell counts ($\times 10^3/\text{mL}$) by breed and time post-challenge

Time	Murrah <i>S. aureus</i>	Nili-Ravi <i>S. aureus</i>	Murrah <i>E. coli</i>	Nili-Ravi <i>E. coli</i>
0 h	142 $\pm$ 34	156 $\pm$ 41	138 $\pm$ 28	149 $\pm$ 37
6 h	312 $\pm$ 58	487 $\pm$ 74	428 $\pm$ 67	684 $\pm$ 92
12 h	486 $\pm$ 72	892 $\pm$ 114	624 $\pm$ 89	1,082 $\pm$ 148
24 h	384 $\pm$ 64	1,240 $\pm$ 168	298 $\pm$ 54	1,468 $\pm$ 192

Table 3: Pro-inflammatory cytokine mRNA expression at 12 hours (fold change)

Cytokine	Murrah <i>S. aureus</i>	Nili-Ravi <i>S. aureus</i>	Murrah <i>E. coli</i>	Nili-Ravi <i>E. coli</i>
TNF- α	8.4 $\pm$ 1.2	3.6 $\pm$ 0.7	11.2 $\pm$ 1.6	4.8 $\pm$ 0.9
IL-1 β	6.7 $\pm$ 0.9	2.8 $\pm$ 0.5	8.9 $\pm$ 1.3	3.4 $\pm$ 0.6
IL-8	12.3 $\pm$ 1.8	5.1 $\pm$ 0.8	14.6 $\pm$ 2.1	6.2 $\pm$ 1.0

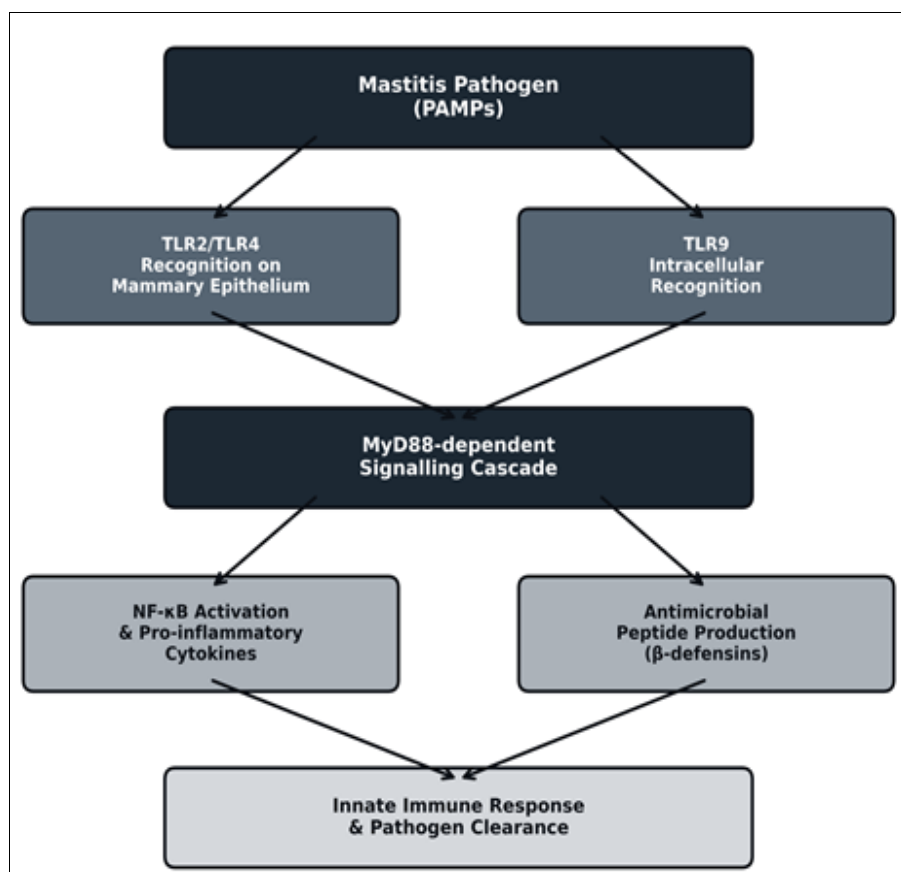


Fig 2: Schematic model of TLR-mediated innate immune signalling in buffalo mammary epithelium following mastitis pathogen challenge

Discussion

The 2.5- to 3.2-fold higher TLR2 and TLR4 expression in Murrah buffaloes provides a molecular basis for the breed's recognised mastitis resistance [6]. TLR4 is the principal receptor for *E. coli* lipopolysaccharide, while TLR2 recognises *S. aureus* lipoteichoic acid and peptidoglycan [3]. The correspondingly higher MyD88 expression in Murrah animals indicates that the breed advantage extends beyond receptor density to signalling pathway amplification, resulting in faster and more robust pro-inflammatory cytokine production [9, 10].

The SCC divergence at 24 hours—Murrah already declining while Nili-Ravi continued rising—suggests that rapid TLR-driven neutrophil recruitment enables earlier pathogen clearance, preventing the sustained inflammatory response that characterises clinical mastitis in susceptible animals [11]. TLR5, which primarily detects flagellin, showed no breed difference, consistent with its limited role in mammary defence against the non-flagellated pathogens used in this challenge [3].

Limitations

The experimental challenge model used fixed bacterial doses that may not replicate natural infection dynamics. Biopsy sampling at multiple time points may induce local inflammation. The research did not assess TLR protein expression or downstream NF-κB nuclear translocation. Breed comparisons using only two breeds cannot identify the specific genetic variants responsible.

Conclusion

Murrah buffaloes mount a substantially stronger TLR-mediated innate immune response to mastitis pathogens than Nili-Ravi buffaloes, characterised by higher TLR2, TLR4,

TLR9, and MyD88 expression and more rapid SCC resolution. These breed-level differences in innate immune receptor expression offer potential molecular markers for breeding programmes targeting mastitis resistance in *Bubalus bubalis*. Genomic studies to identify specific TLR polymorphisms underlying these expression differences are warranted.

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Contributions Not Qualifying for Authorship

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