Indian Journal of Animal Sciences **91** (3): 235–238, March 2021/Short communication
https://doi.org/10.56093/ijans.v91i3.114150

Revealing inheritance of white markings in Nili Ravi buffalo

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Received: 8 September 2020; Accepted: 13 July 2021

Keywords: Inheritance, Nili Ravi buffalo, Phenotypic classification, White markings

Buffalo has a multiple utility and is considered as leader livestock species in terms of both dairy and meat production in India. Majority of the buffalo populations in India are black in colour with Nili Ravi buffalo breed being an exception. Nili Ravi due to its five characteristics, viz. white markings on forehead, muzzle, tail, legs and walled eye, is known as 'Panch or PanjKalyani'. However, the nature of markings is variable but most common are the markings on head, and tail region along with wall-eye. These white markings vary in degree and sometimes over white or under white animals appear even in a systematic mated population. Coat colour is reported to be having effects on production and reproduction traits of cattle under adverse climatic conditions of high temperature and solar radiation (Becerril *et al*. 1993). Some authors have found significant interactions between coat colour and calving season for days open and services period in cows (Becerril *et al*.1993, Cilek and Tekin 2007). The effects of white colour patches has been found to be significant and have a positive linear association is with the milk production, fat and protein percentages in cattle (Becerril *et al*. 1993, Godfrey and Hansen 1994, Maia *et al.* 2005). There are reports indicating interactions between colour and environment for physiological variables in Holstein cows grouped by white patches, concluding that white cows' conception in Holstein exposed to sun showed smaller changes in the physiological variables and less depression in milk production (Becerril *et al.* 1993, Godfrey and Hansen 1994). Heritability estimated for white coat colour through DFREML method in Holstein seems to be high and is reported as high level (0.770) by Becerril *et al.* (1994), indicating its importance in a breeding programme.

Coat and skin colour is governed by the production of melanin pigment, formed by oxidation followed by polymerization of amino acid tyrosine in the melanocytes (Seo *et al.* 2007). Genetic basis of colour patterns has been widely studied in different domestic animal species including cattle, dog and horse. Polymorphism/mutations in *KIT* and other melanin synthesis genes have been associated with coat colour/white patches in cattle, horse, dog and other species (Mau *et al*. 2004, Schmutz *et al*. 2009). Studies in cattle have shown the mutations in *KIT* gene of Angus, Hereford and Holstein having a role in determining the coat colour in the Hereford, but without any evidence of selective sweep in Holstein, indicating that complex mechanisms or other genes might also be involved in the spotted phenotype in this breed (Fontanesi *et al.* 2010). Genetically, expressivity and epitasis may affect/modify coat colour (Miko 2008).

Over whiteness of Nili Ravi buffalo is assumed as a negative trait by their owners because of their decreased market price. White markings extending above hock and knee and over the neck and body constitute a serious disqualification. Under-white markings in Nili Ravi also make it standing out of the breed characteristics. The pattern of inheritance of white patches of skin in Nili Ravi buffaloes has not been investigated so far and impact of white patches on physiology and production-reproduction performance not yet studied, whether if whiteness of skin affects its economic value. In this study, data generated on white marking patterns of various sire, dam and their progeny has been analysed to understand the inheritance of white markings in Nili Ravi buffalo at an organized farm.

Nili Ravi animals available at Central Institute for Research on Buffaloes, Nabha sub-campus were included in the study. Black skin colour, walled eyes and white markings on the forehead, face, muzzle, feet, and tail are the typical features of Nili Ravi buffaloes. Based on the data available on the degree of white markings on the skin, animals were classified into three groups, viz. typical, over-white and under-white Nili Ravi, using the criteria given in Table 1.

Phenotypic coat colour data of 1122 Nili Ravi buffalo sired by 21 bulls and 381 dams and 720 of their offspring were included in the study (out of which 325 were female and 395 were male progenies) for the inheritance pattern analysis. Based on the degree of phenotypic white coat colour markings, 351 typical, 201 over-white and 168 under-white Nili Ravi buffalo calves were included. Sire,

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Table 1. Classification criteria based on phenotypic coat colour in Nili Ravi animals

Phenotypic classification	Criteria for classification
Typical Nili Ravi	Walled eyes (Bilateral), white markings on the forehead, tail, muzzle/chin, legs (all four/ fore or hind legs)
Over-white Nili Ravi	Walled eyes (Bilateral), white markings on the forehead, extending up to the face, neck region, white tail, with white legs above fetlock and up to belly region, and white spots on the other parts of body
Under-white Nili Ravi	Walled eyes (Bilateral), white markings on the forehead, tail, with low intensity or spots missing on forelegs/muzzle/chin

Table 2. Number of animals included for inheritance pattern analysis based on phenotypic classification

Characteristic	No. of sires	Progeny per sire	No. of dams	Progeny per dam
Typical Nili-Ravi	8	44	222	2
Over-white Nili-Ravi	11	18	74	3
Under-white Nili-Ravi	2	84	85	2
Total	21	146	381	7

dam and progeny included for phenotypic classification are listed in Table 2.

Sire to progeny coat colour inheritance and dam to progeny coat colour inheritance patterns were studied and their significance was tested by Chi-Square statistical analysis using SPSS software (IBM SPSS statistics version 21.0). A *P* value < 0.05 and *P* value < 0.01 was considered statistically significant. Likelihood ratio and Mc-NemarBowker test were also performed to confirm the results (Smith and Ruxton 2020).

Buffaloes were classified for the present study into three groups based on the degree of white markings, namely, typical, over-white and under-white Nili Ravi. A sample size of 1122 animals of Nili Ravi buffaloes were included in the study sired by 21 bulls and 381 dams and their 720 offspring were included in the study, out of which 325 were female and 395 were male progenies. Ratios were calculated in typical over-white coloured skin relative to under-white skin coloured buffaloes. Coat colour inheritance pattern from sire and dam to progenies of both the sexes were determined.

Study revealed that when the phenotype (white markings) of sire was 'typical' then the progenies born were more 'typical' followed by over-white and least numbers of under-white progenies of the sire were present in the herd (Table 3). The progeny per sire varied from 13 to 58. Segregation ratios with respect to 'typical' coloured sire to its progeny were 2:1:1 (typical white: over-white: under-white). When the sire was over-white then the progenies born were equal for 'typical' and over-white groups. Segregation ratios with respect to over-white colour sire to its progeny were 2:2:1 (typical white: over-white: under-white). When the sire was under-white then the progenies born were more 'typical' and equal in numbers for over-white and under-white skin colour. Segregation ratios with respect to under-white colour sire to its progeny were 2:1:1 (Typical white: Over-white: Under-white). Statistical significance for Likelihood ratio showed a significant result ($P<0.05$), Mc-NemarBowker test showed a highly significant result ($P<0.01$), while Chi-Square test showed a non-significant result ($P<0.05$). The inheritance of phenotypic skin colour white markings from sire to its progeny was not inherited true and the ratio varied, i.e. typical to typical and over-white to over-white was not observed, so a definite conclusion cannot be drawn from the present inheritance study in Nili Ravi. The probable reason could be the small sample size and also skin colour is not governed by a single gene. However, based on the phenotypic classification of skin colour, it can be inferred that the typical Nili Ravi has more blackness per animal compared to over-white Nili Ravi animal. Thus, inheritance pattern indicate the black colour may be dominant over white colour in Nili Ravi buffaloes.

Inheritance based on dam path: In this path, the progeny per dam varied from 1 to 5. When the phenotype of dams' skin colour was 'typical' then the progenies born was more number of 'typical' followed by an equal proportion of over-white and under-white progenies (Table 4). Segregation ratios with respect to the typical white dam to its progeny were 2 : 1 : 1 (typical white : over-white : under-white). When the dam is over-white then the progenies born was more number of typical-white followed by over-white and less number of under-white progenies. Segregation ratios

Table 3. Skin colour inheritance pattern (sire to its progeny path) based on variable white colour markings in Nili-Ravi buffalo

Phenotypic classification Sire (21)	Progeny coat colour ratio Typical : Over-white : Under-white	Tests for significance (*P* value)		
		Chi-Square	Likelihood ratio	McNemar–Bowker
Typical Nili-Ravi (8)	4 : 05 : 01 (139 : 174 : 38)	0.067	0.044*	0.000**
Over-white Nili-Ravi (11)	9 : 13 : 01 (77 : 115 : 9)			
Under-white Nili-Ravi (2)	3 : 04 : 01 (63 : 85 : 20)			

Table 4. Skin colour inheritance pattern (dam to its progeny path) based on variable white colour markings in Nili-Ravi buffalo

Phenotypic Classification Dam (381)	Progeny coat colour ratio Typical : Over-white : Under-white	Tests for significance (P value)		
		Chi-Square	Likelihood Ratio	McNemar–Bowker
Typical Nili-Ravi (222)	4 : 01 : 01 (219 : 72 : 60)	0.000**	0.000**	0.000**
Over-white Nili-Ravi (74)	4 : 02 : 01 (117 : 57 : 27)			
Under-white Nili-Ravi (85)	5 : 01 : 03 (101 : 19 : 48)			

Numbers in parenthesis indicate number of animals. **(P<0.01) and *(P<0.05).

with respect to over-white colour dam to its progeny were 4 : 3 : 1 (typical white : over-white : under-white). Interestingly, when the dam is under-white then the progenies born was an equal proportion of typical white, over-white, and under-white progenies and segregation ratios were 1 : 1 : 1 (typical white : over-white : under-white). Statistical analysis for these inheritance patterns with respect to the dam to progeny path was analyzed using the Chi-Square test, Likelihood ratio test, Mc-NemarBowker test. All three tests were showed a highly significant ratio at 99% level of significance. These results thus indicate that white-skin colour is not inherited true and it deviates from dam to its progeny, however, the progeny production of under-white or black skinned dam path, indicates that may be the one of the genes governing the skin colour is influenced by the sex in Nili Ravi buffalo.

White markings and spotting patterns that usually allow identification of breeds are predominantly under genetic control and display genetic variation (Liu *et al.* 2009). Results indicated that typical Nili Ravi being the predominant coat colour pattern followed by over-white Nili Ravi and under-white Nili Ravi that was inherited from the parent to progeny. All the possible combinations of crossing patterns showed significant effect of coat colour inheritance from sire to progeny and dam to progeny, i.e. there is a significant difference in sire and its progeny and dam and its progeny skin colour, and the skin colour is not same in the parental and progeny generations.

Overall the results indicate variable inheritance patterns from sire to progeny and dam to progeny based on phenotypic skin/coat colour ratios and no definite conclusion could be drawn from the study. Genomic studies however are warranted to further reveal the genetic basis of inheritance patterns of white markings in Nili Ravi buffalo.

SUMMARY

Among registered Indian buffalo breeds, Nili Ravi is well known for its production potential as well as unique typical five white markings, because of which it is also known as Panch-Kalyani. In this study an attempt was made to understand the inheritance pattern of white markings in 720 progenies sired by 21 bulls and belonging to 381 dams at an organized farm. Based on extent of white markings, animals were classified into typical, under and over white categories and significance of inheritance was tested by Chi-Square, Likelihood ratio and Mc-NemarBowker tests to confirm the results. The results showed distribution of inheritance pattern from sire to progeny, and dam to progeny was that the identical in white markings classes ratios and inheritance of skin/coat colour patterns could not be explained. Further, studies at genomic and epigenetic levels needed to reveal the genetic/methylation differentiation basis of white markings variation in Nili Ravi buffalo.

ACKNOWLEDGEMENTS

Authors are also thankful to the Director, ICAR-CIRB and Director, ICAR-NDRI for providing their help and support in completing this work. Authors acknowledge the financial support received from ICAR-NBAGR, under institute funded project, to carry out the work.

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