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A preliminary study on digital quantification of ocular attributes in cattle as potential non-invasive indicators of anemia

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Abstract

Background Various non-invasive indicators such as ocular attributes have been tested and validated for the assessment of anemia and vitamin A deficiency in human medical sciences with promising results. However, regarding veterinary diagnostics/prognostics, there is limited literature for photometric assessment of anemia in cattle. The present preliminary study is the first account of digital quantification of various ocular attributes in cattle ($n = 36$) carried out with an objective to unearth the potential of these attributes (RGB analysis and morphometry of eyeballs, and color of palpebral conjunctiva) as non-invasive predictors of RBC count, hemoglobin (Hb) and packed cell volume (PCV).

Results The results showed that green ($r = 0.571$), blue ($r = 0.706$), yellow ($r = 0.624$), black ($r = 0.712$) and whiteness ($r = 0.778$) of cattle eye were positively and significantly ($P \leq 0.05$) correlated with Hb with 67.0% predictability for overall model. Similarly, red (r= -0.536), green (r= -0.565), magenta (r= -0.409), yellow (r= -0.563), black ($r = 0.700$) and whiteness (r= -0.805) were highly correlated ($P \leq 0.05$) with Hb with a strong overall model predictability of 67.6%. The associations with RBC count were, however, weaker and non-significant (23% predictability).

Conclusions It is concluded that various ocular attributes of cattle, particularly blue, yellow, black, whiteness and lightness, could serve as non-invasive indicators of Hb and PCV, assisting in detecting of anemia. The palpebral conjunctiva color chart developed through this preliminary data could function as an on-field point-of-care testing (POCT) tool to predict Hb and PCV levels in cattle.

Keywords Anemia, Non-invasive indicators, Ocular attributes, Anemia, Point-of-care-tests

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Background

The health and well-being of cattle are critical to the livestock industry. Identifying reliable diagnostic and prognostic indicators is essential for early detection and management of numerous health conditions. This becomes even more critical for the livestock being reared under tropical pastoralism where the shortage and poor quality of feedstuff allied with abundant vectors (mosquitoes and ticks) results in a higher incidence of various pathologies, in particular the blood-borne diseases (theileriosis, babesiosis and anaplasmosis) and resultant anemia [1, 2]. Within the blood picture/complete blood count, the red blood cell count (RBC), hemoglobin (Hb) and packed cell volume (PCV) are considered to be solid indicators of general health and anemia in cattle. For hematological assessment, 3-part and 5-part, highly sensitive automated veterinary hematology analyzers are being used which provide an authentic result. However, their use is limited in the resource-poor geographies of the world as they are expensive, need periodic maintenance, expensive chemicals and requirement for trained personnel. Their placement at distantly located laboratories further aggravates the issue of transit of blood samples from far-fetched animal herds to the laboratory [3, 4]. In addition, blood sampling in field conditions is a tedious and laborious task which needs appropriate restraint of the animal. There is an urgent need to develop and validate non-invasive, on-field, point-of-care tests (POCTs) for assessing animal health in general, and anemia in specific [5–7].

Various ocular attributes, being non-invasive and easy to monitor, have been tested and validated for assessment of anemia [8, 9] and vitamin A deficiency [10, 11] in human medical sciences with promising results. However, regarding veterinary diagnostics/prognostics, there is a paucity of literature with a single report on qualitative scoring of palpebral conjunctiva color of cattle for photometric assessment of anemia [12]. A few other researchers have focused mainly on eye white percentage of cattle as a predictor of various behavioral aspects [13–16]. To the best of knowledge, there is a complete lack of a diagnostic/prognostic chart for anemia detection in cattle. The present preliminary study is the first of its kind being reported for cattle with an objective to evaluate the potential of various ocular attributes (RGB analysis, color of palpebral conjunctiva and morphometry) as non-invasive predictors of RBC count, Hb and PCV for detecting anemia in cattle, while also examining the influence of sex, breed and age on these ocular attributes. The palpebral color grading chart developed through this preliminary data could function as an on-field point-of-care testing (POCT) tool for predicting RBC count, Hb and PCV levels in cattle.

Results

The results regarding the effect of sex, breed and age on studied ocular attributes are presented in Figs. 1, 2 and 3, respectively. None of the studied ocular attributes were statistically different ($P \geq 0.05$) in any of the groups. Based on these findings, it was statistically justified to combine the data into a single group to increase sample size and statistical power for subsequent analyses.

A weak but statistically significant relation was noticed between RBC count and blue (r= -0.255, $p \leq 0.05$), yellow (r= -0.204, $p \leq 0.049$) and black ($r = 0.347$, $p \leq 0.038$) attributes of cattle eyes. However, all other ocular attributes failed to significantly predict RBC count, and the overall model explained 79.5% of the variance with a low adjusted r-square of 23% (Table 1).

Regarding the regression analysis between Hb and studied ocular attributes, it was noticed that green ($r = 0.571$, $p \leq 0.050$), blue ($r = 0.706$, $p \leq 0.037$), yellow ($r = 0.624$, $p \leq 0.044$), black ($r = 0.712$, $p \leq 0.033$) and whiteness ($r = 0.778$, $p \leq 0.050$) of cattle eye were positively correlated with Hb and significantly predicted Hb. The adjusted r-square value of 0.67 attained for overall model indicated substantial predictability of 67.0% (Table 2).

Similarly, ocular attributes viz. red (r= -0.536, $p \leq 0.016$), green (r= -0.565, $p \leq 0.030$), magenta (r= -0.409, $p \leq 0.05$), yellow (r= -0.563, $p \leq 0.013$), black ($r = 0.700$, $p \leq 0.022$) and whiteness (r= -0.805, $p \leq 0.008$) were highly correlated with PCV (Table 3). The overall model was strong with a high predictability of 67.6%.

The visual inspection, mean gray value (MGV), and pixel grouping of palpebral conjunctivae revealed that three predominant color patterns were being shown in the studied cattle being (a) pale to light pink ($n = 05$), (b) pink to deep pink ($n = 11$), and (c) reddish to pink ($n = 20$). The RBC count, Hb, and PCV showed a positive trend across the palpebral conjunctiva color groups with highest values seen in reddish-pink group (Fig. 4). As per this grouping, relevant reference intervals for RBC count, Hb and PCV were allocated to them and a palpebral conjunctiva color chart was developed (Fig. 5).

Discussion

The resource-poor regions, including Pakistan, face significant challenges in diagnosing and prognosing livestock due to factors such as distantly located laboratories, expensiveness of diagnostic/prognostic tests, and other allied issues. Furthermore, invasive methods of blood sampling imply stress upon animals resulting in erroneous outcomes. The non-invasive POCTs on the other hand, provide cost-effective, on-field, quick and reliable diagnosis/prognosis, thus relieving all the issues. The present preliminary study, for the first time, incorporated various ocular attributes (RGB analysis and morphometry of eyeballs, and color of palpebral conjunctiva) of

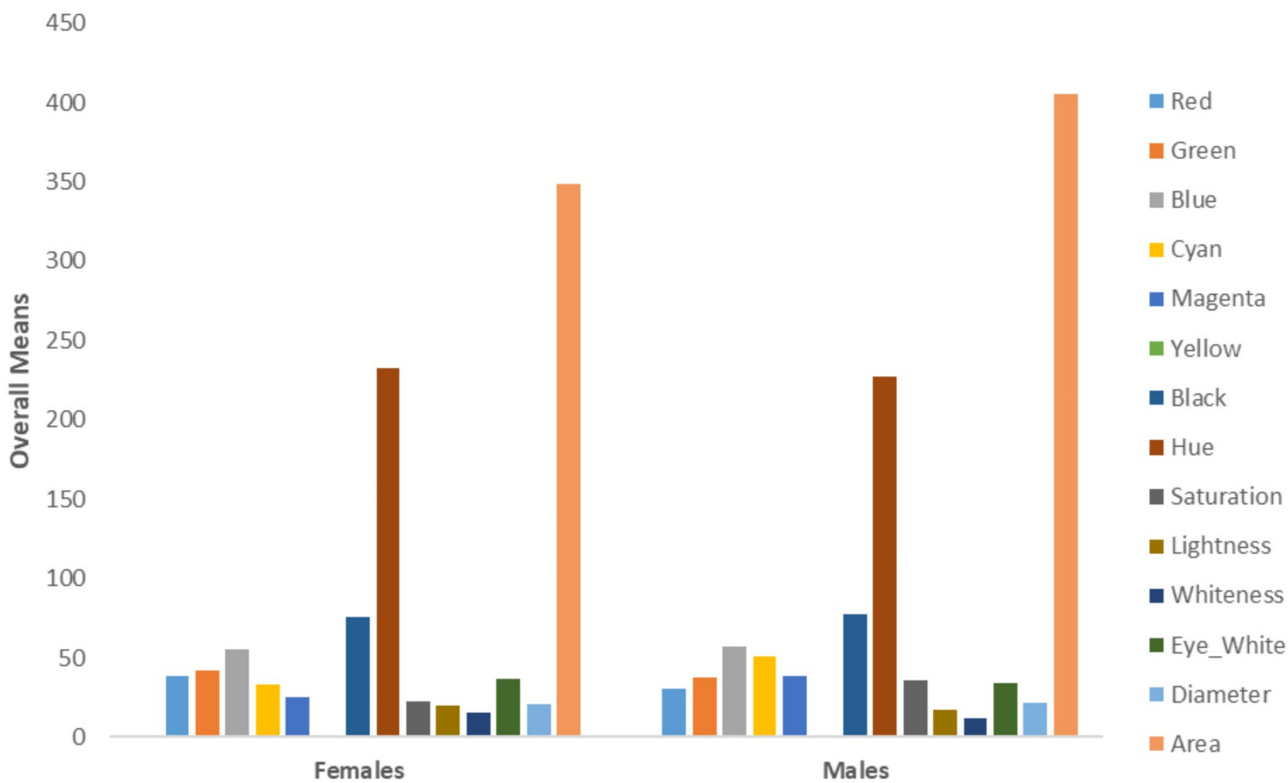

Fig. 1 Effect of sex on various eye attributes of cattle

cattle to be assessed as non-invasive indicators of three hematological parameters i.e. RBC count, Hb and PCV. These three are considered gold standard for assessing anemia. The palpebral conjunctiva color chart with its relevant reference intervals for RBC count, Hb and PCV developed in the present study could be a non-invasive and useful tool for on-field assessment of anemia in cattle.

In the present study, the group-wise results revealed that sex, breed and age had no effect on any of the studied ocular attributes. These results suggest a universal applicability of these findings across different zebu cattle populations of the world. This is an encouraging result, indicating that the studied ocular attributes could be applied broadly, irrespective of the specific demographic characteristics of the cattle. As no such study was found after a thorough literature review, therefore comparison with any prior study is not possible. As the number of animals is less per group, hence these results may not be considered conclusive. A key limitation of this study is the relatively small sample size ($n = 36$), which may limit its statistical power, particularly for detecting small-to-moderate effect sizes. A power analysis was conducted to assess the adequacy of the sample size for the independent t-tests used in group comparisons. The results indicate that while detecting large effects (Cohen's $d = 0.8$) requires approximately 51 samples, detecting moderate ($d = 0.5$) or small effects ($d = 0.2$) would require 128 and 787 samples, respectively. Given that our t-test results showed no significant differences ($P \geq 0.05$) between sex and breed groups, we combined the data to enhance statistical power in analyzing ocular attributes relative to hematological parameters.

Our results demonstrated significant positive correlations between Hb and the blue, yellow, black, whiteness, and lightness of cattle eyeballs, while a strong negative correlation was found with the redness of the eyes. These correlations align with previous studies in human medicine, which have also shown ocular attributes to be predictive of various health conditions such as anemia and vitamin A deficiency [10, 11]. However, there is a dearth of such research in the veterinary field, particularly for large animals such as cattle, and our study appears to be the first of its kind for cattle.

The results regarding regression models between various ocular and hematological attributes showed that the predictability of PCV (67.6%) and Hb (67.0%) was higher as compared to that for RBC count (23.0%). A 61% predictability has been reported earlier for cattle eye fundus in predicting vitamin A deficiency [17]. In a pilot study conducted on non-invasive Hb detection using redness of the palpebral conjunctiva (photometric assessment), a higher predictability of 98% has been reported [12]. The lower model predictability for RBC count in our study

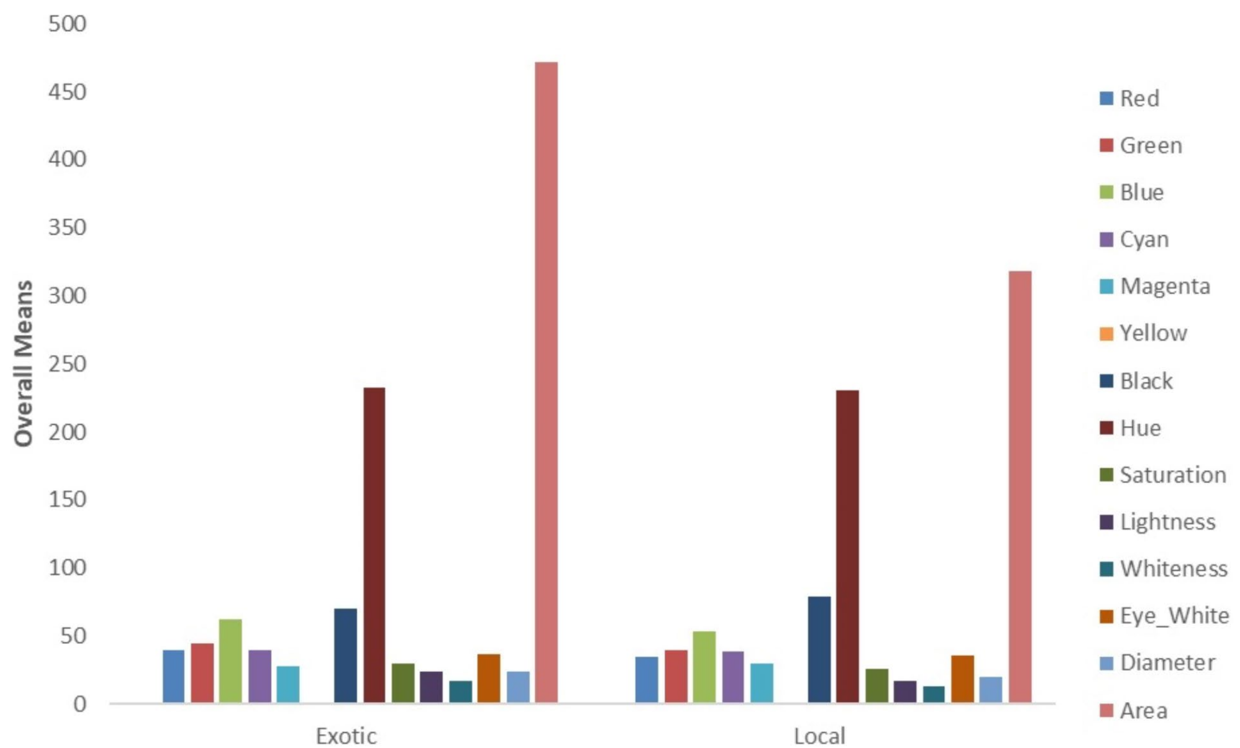

Fig. 2 Effect of breed on various eye attributes of cattle

may be due to the complex regulatory mechanisms governing RBC counts compared to Hb concentration and PCV, both of which reflect the oxygen-carrying capacity of blood more directly [18]. As compared to Hb and PCV, RBC count is influenced by intricate physiological mechanisms of the body including erythropoiesis, destruction and blood volume regulation [19, 20]. This may explain why ocular attributes, which are more closely linked to changes in blood color and oxygenation, are better suited for predicting Hb and PCV, rather than RBC count.

In our results, eye white percentage and eyeball's morphometry (diameter and area) could not prove to be reliable indicators for any of the hematological attributes under consideration (RBC count, Hb and PCV). On the contrary, previous few studies have endorsed that the eye white percentage of cattle is a reliable indicator of animal welfare, emotional state and stress level of the animal [13–15]. However, these studies had used still/stationary video cameras installed in animal pens for taking images whereas our study utilized mobile phone. This difference in image capturing methodology could be attributed to decreased predictability of eye white percentage and morphometric parameters in predicting any of the hematological attributes. Furthermore, prior studies on eye white percentage of cattle have been conducted to solely assess emotional/behavioral attributes whereas our study incorporated hematological attributes.

For grouping the animals on the basis of palpebral conjunctiva color, visual inspection, RGB assessment, and MGV of fixed pixel in images of palpebral conjunctivae was carried out. It was noticed that three predominant color patterns were shown in the studied cattle being (a) pale to light pink, (b) pink to deep pink, and (c) reddish to pink. Only five of the total animals had pale to light pink palpebral conjunctiva. As per this grouping, relevant RIs for RBC count, Hb and PCV were allocated to them and a palpebral conjunctiva color chart was developed for on-field use to ascertain anemia which may be a notable contribution of this study. The reference intervals revealed that they tended to increase exponentially being lowest in pale to light pink and highest in reddish pink group. However, they were within the physiological range for Pakistani cattle reported elsewhere [21, 22]. Five of the total animals having pale to pink palpebral conjunctiva had lowest values for RBC count, Hb and PCV indicating mild anemia. However, comparison with prior studies indicated that a wide range of these blood parameters has been reported from Pakistan [21]. And as these animals showed no signs of diseases/anemia, hence may be considered normal. In addition, data correspondence of

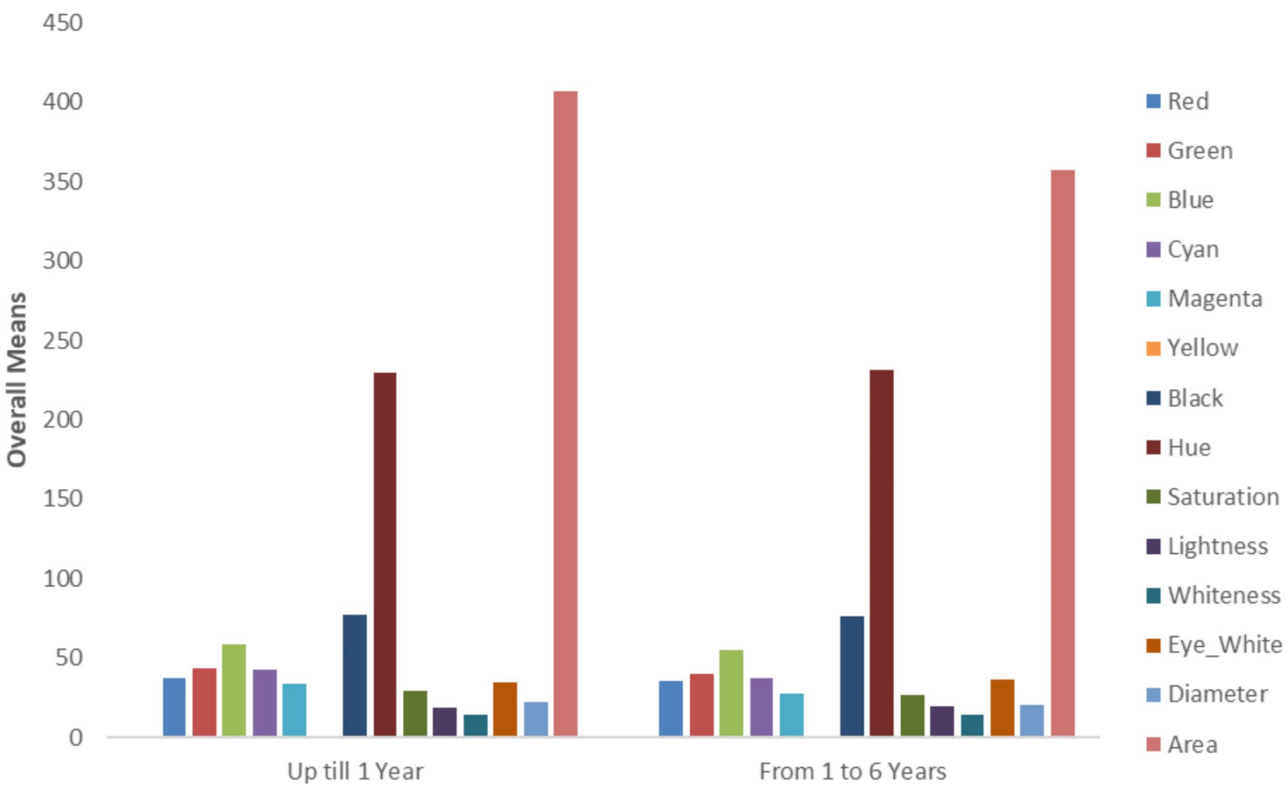

Fig. 3 Effect of age on various eye attributes of cattle

Table 1 Multiple regression analyses between RBC count and various ocular attributes in cattle

Attributes	*r*-value	*r*-square	Adj. *r*-sq	Prediction Equation	Sig.
Red	-0.392	0.153	0.093	8.72+(48.6x)	0.114
Green	-0.326	0.106	0.043	6.10+(51.3x)	0.064
Blue	-0.255	0.065	0.02	3.74+(53.7x)	0.05*
Cyan	0.301	0.091	0.02	5.20+(52.2x)	0.485
Magenta	0.376	0.142	0.08	8.14+(49.3x)	0.160
Yellow	-0.204	0.042	0.02	2.40+(55.1x)	0.049*
Black	0.347	0.120	0.05	6.91+(50.5x)	0.038*
Hue	-0.042	0.002	0.07	0.10+(57.4x)	0.753
Saturation	0.308	0.095	0.03	5.45+(52.0x)	0.991
Lightness	-0.401	0.161	0.10	9.24+(48.2x)	0.090
Whiteness	-0.410	0.168	0.10	9.64+(47.8x)	0.106
Eye White (%)	0.078	0.006	0.06	0.35+(57.1x)	0.962
Diameter (mm)	-0.292	0.085	0.020	4.89+(52.6x)	0.552
Area (mm^2)	-0.307	0.094	0.029	5.41+(52.0x)	0.494
Overall Model Summary	**0.892**	**0.795**	**0.23**	**-**	**0.396**

these five animals with age revealed that these three animals were above 11 years old. The senile-based anemia has been well documented both in humans and animals as with progressing age, the erythropoiesis decreases [23, 24]. Such charts have been successfully used in human medicine for the detection of anemia [8, 9]. Similarly, FAMACHA eye chart for detection of anemia in sheep has also been reported to be a valid and authentic tool [25–27]. Our laboratory also devised and published a serum color chart for estimation of Hb and bilirubin in Pakistan's indigenous Sipli breed of sheep [28]. The adaptation of this technique/chart for cattle could significantly improve animal health management in regions with limited resources.

Conclusion

In summary, the ocular attributes, particularly image attributes of the cattle eyeballs such as blue, yellow, black, whiteness and lightness, could serve as non-invasive indicators of Hb and PCV, assisting in detecting of anemia.

Table 2 Multiple regression analysis between hemoglobin and various ocular attributes in cattle

Attributes	*r*-value	*r*-square	Adj. *r*-sq	Prediction Equation	Sig.
Red	0.458	0.210	0.153	5.52+(20.8x)	0.067
Green	0.571	0.138	0.076	3.63+(22.7x)	0.050
Blue	0.706	0.093	0.029	2.46+(23.9x)	0.037
Cyan	0.337	0.113	0.050	298+(23.3x)	0.564
Magenta	0.384	0.147	0.086	3.88+(22.4x)	0.117
Yellow	0.624	0.050	0.018	1.32+(25.0x)	0.044
Black	0.712	0.170	0.111	4.48+(21.8x)	0.033
Hue	0.108	0.012	0.059	0.30+(26.0x)	0.594
Saturation	0.312	0.098	0.033	25.57+(23.8x)	0.594
Lightness	0.463	0.214	0.158	5.64+(20.7x)	0.218
Whiteness	0.778	0.228	0.173	6.02+(20.3x)	0.050
Eye White (%)	0.216	0.047	0.021	1.23+(25.1x)	0.421
Diameter (mm)	0.317	0.100	0.036	2.64+(23.7x)	0.232
Area (mm^2)	0.331	0.110	0.046	2.89+(23.4)	0.210
Overall Model Summary	**0.897**	**0.805**	**0.670**	**-**	**0.371**

Table 3 Multiple regression analysis between packed cell volume and various ocular attributes in cattle

Attributes	*r*-value	*r*-square	Adj. *r*-sq	Prediction Equation	Sig.
Red	-0.536	0.113	0.050	13.32+(104.7x)	0.016
Green	-0.565	0.027	0.042	3.19+(114.8x)	0.030
Blue	-0.489	0.008	0.063	0.93+(117.0x)	0.015
Cyan	0.392	0.154	0.094	18.17+(99.8x)	0.352
Magenta	0.409	0.044	0.025	5.14+(112.8x)	0.051
Yellow	-0.563	0.215	0.159	25.35+(92.6x)	0.013
Black	0.700	0.040	0.028	4.73+(113.2x)	0.022
Hue	-0.405	0.164	0.104	19.33+(98.6x)	0.212
Saturation	0.288	0.083	0.018	9.80+(108.2x)	0.444
Lightness	0.253	0.064	0.003	7.55+(110.4x)	0.404
Whiteness	-0.805	0.093	0.028	10.97+(107.0x)	0.008
Eye White (%)	0.203	0.041	0.027	4.84+(113.1x)	0.710
Diameter (mm)	-0.273	0.075	0.008	8.80+(109.2x)	0.941
Area (mm^2)	-0.270	0.073	0.007	8.58+(109.4x)	0.994
Overall Model Summary	**0.956**	**0.914**	**0.676**	**-**	**0.102**

The palpebral conjunctiva color chart developed through this data could function as an on-field POCT tool for predicting Hb and PCV levels in cattle. This preliminary study suggests that future research should involve a larger sample size and include healthy as well as diseased cattle to validate these findings. Furthermore, additional non-invasive indicators/tools for use in veterinary diagnostics/prognostics such as thermography and infrared imaging of cattle eye may be integrated in future research to improve accuracy of anemia detection. The use of artificial intelligence for automated ocular image analysis could also be explored to streamline the process and enhance on-field applications.

Methods

Study location

The present study was carried out at the University Livestock Farm (for sampling and image capturing) and Post-graduate Laboratory, Department of Physiology (for hematological analyses), The Islamia University of Bahawalpur (IUB). The IUB is located in city of Bahawalpur which is one of the three main cities located at the outskirts of Cholistan desert. The desert has an area of 26000km^2 with dry arid weather. The livestock and the pastoral systems (nomadic and transhumanie) of the desert have been reviewed in detail earlier [4, 29]. In brief, as per the split herding, the young animals are kept at the pens located near man-made/natural water reservoirs ('Tobas' in local dialect) whereas the adult animals are sent far off for grazing under the surveillance of livestock herders [29, 30].

Study animals and image/video capturing

Apparently healthy Cholistani, Sahiwal and exotic (Friesian and Jersey) cattle breeds ($n = 36$) being reared under intensive farming system at the University Livestock Farm were incorporated in the study. The feeding and management pattern was similar for all animals at the

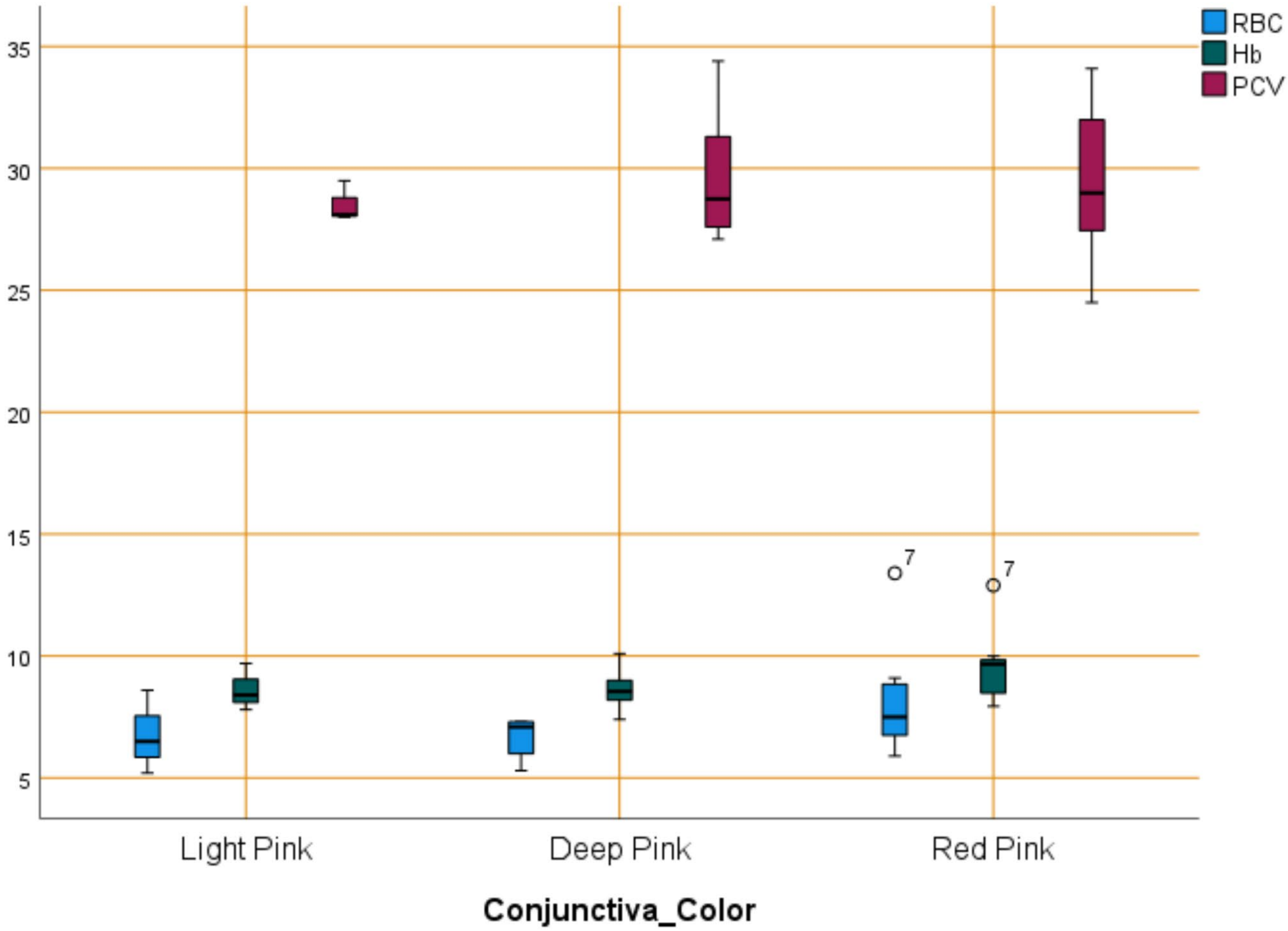

Fig. 4 Boxplot indicating the hematological attributes (Red blood cell count, Hemoglobin and Packed cell volume) across the three palpebral conjunctiva color groups in cattle

farm. All animals are regularly assessed for health attributes through clinical examination by veterinarians and laboratory tests mainly blood analysis. Periodic and systematic anthelmintic protocols are followed regularly. Water is provided ad-libitum. Health of each animal was ascertained through focal group discussion with farm personnel, farm records, and was confirmed through complete blood count. Before embarking upon each animal for image analysis and blood collection, temperature, pulse and respiratory rate were also determined.

For image capturing of cattle eyes, each animal was restrained in the cattle crush with its head in the working chute. Before image capturing, about 15 min were provided to the animals for acclimatization and calming down as per prescribed protocol [31]. A measured distance was strictly adhered to, while taking images. Same time of the day, same cattle crushes, and same personnel were maintained during the research to decrease chances of error. Small video clips were also recorded along with the images. Each eye was captured multiple times due to distractions causing the animals to blink or jerk. Care was taken so that whole eye along with its eye white/sclera is captured. For palpebral color, the animal handler slightly tilted the head of the animal, pressed the medial canthus and lower eyelid, exposing the palpebral conjunctive to be captured [32].

All images were taken using iPhone 12 ProMax (Apple Inc. USA) with fixed light, zoom and allied internal settings for monotonous imaging, mounted on a mobile stand. The specifications of the cell phone are given below:

Chip A-14 bionic chip, next generation neural engine.

Camera Ultra-wide f/2.4 aperture, 120° field of view, Wide f/16 aperture, Telephoto: f/2.2, 2.5X optical zoom, 12X digital zoom, LIDAR scanner, Lens: Main 12MP, Panorama 63MP, Saphire crystal cover, HEIF and JPEG Image formats.

Blood collection and analysis

After image capturing, each animal was given few minutes in the cattle crush so that stress may not hamper blood profile. Aseptic blood collection was carried out

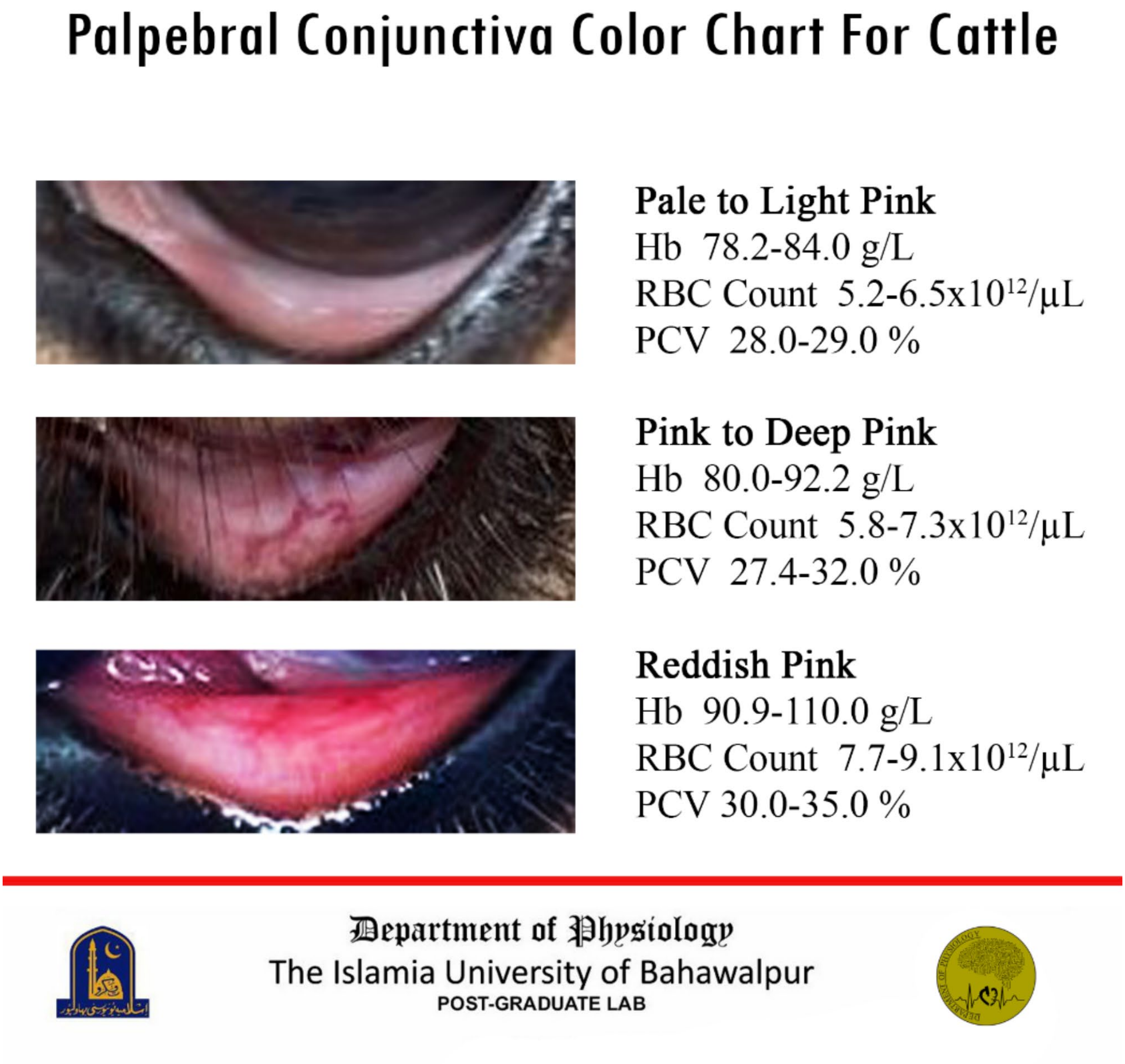

Fig. 5 Palpebral conjunctiva color chart for estimating hemoglobin, RBC count and packed cell volume in cattle. The values are 25th to 95th percentiles

using 10 cc disposable syringes. Blood was taken to the laboratory in purple-topped vacutainers having EDTA (Becton Dickinson, USA) as preservative within 15 min of collection.

Blood was analyzed for RBC count, Hb and PCV using previously validated, automated veterinary hematology analyzer (Rayto 7600, China) after a thorough mixing on a Roller Mixer (MixR-40, Daihan Scientific, Korea).

Image/Video processing and analysis

All the captured images/videos of cattle eyes were transferred to the computer, sorted for appropriateness, and cropped in different ways as to accomplish analysis of the area of palpebral conjunctiva, whole eye and eyeball (excluding sclera). The morphometric attributes of the eyeballs (diameter and area), eye white percentage and pixels of the area of palpebral conjunctiva were attained through Adobe Photoshop CS6 portable (Adobe Inc. USA). From the attained images of eyes, two orthogonal diameters of total visible eye and of iris/pupil were measured and eye white percentage was calculated as prescribed earlier [13, 14].

Image analysis for cattle eyeballs was carried out using an online software (www.imagecolorpicker.com). This Swiss software utilizes services from Brevo, Google Analytics, Trustpilot and Cloudfare Workers, and provides 11

RGB attributes namely red, green, blue, cyan, magenta, yellow, black, hue, saturation, lightness, whiteness.

Palpebral conjunctiva color

The color of palpebral conjunctiva was ascertained through three methods: on-site visual inspection by two examiners, visual assessment of images on computer screens by two examiners, and through MGV of a 10-pixel (fixed) area of palpebral conjunctiva attained through Adobe Photoshop CS6 portable (Adobe Inc. USA). The MGV values were also confirmed through Image J software (NIH, USA). Grouping on the basis of this color was accordingly carried out.

Statistical analysis

For the purpose of analysis, data was grouped as per sex (males, $n = 11$; females, $n = 25$), breed (local, $n = 21$; exotic, $n = 15$) and age (up till 1 year, $n = 11$; above 1 year, $n = 25$). Grouping as per palpebral conjunctiva color conducted visually and through MGV was confirmed through k-clustering technique implied upon the pixels of the palpebral conjunctiva keeping the number of clusters at 3. The attained data regarding hematological and ocular attributes was analyzed through Statistical Package for Social Sciences (SPSS for windows, v20, SPSS Inc., Chicago, IL, USA) and is presented as mean (± SE). Difference in these attributes within various study groups (age, sex and breed) was determined through independent t-test. Given the absence of significant differences, data from all animals were pooled into a single dataset. This approach was justified to increase statistical power and enhance model robustness for subsequent regression analyses of ocular attributes with hematological parameters. Multiple regression analysis was implied between the hematological and ocular attributes of the study and resultantly r-values, adjusted r-square values and regression prediction equations were computed. Given that multiple predictors were included in the regression models, multicollinearity was assessed using the Variance Inflation Factor (VIF) and tolerance values. The results indicated that all VIF values were below 5, suggesting an absence of severe multicollinearity and confirming that the predictors were independently contributing to the model. For deducing reference intervals of hematological attributes, 25th to 95th percentiles were deduced using Reference Value Advisor software (freeware v.2.1, http://www.biostat.envt.fr/reference-value-advisor), a validated statistical tool for veterinary reference interval determination. The non-parametric method (percentile-based approach, 2.5th to 97.5th percentiles) when data distribution deviated from normality, and parametric methods (95% confidence intervals) were used for normally distributed data. The selection of method was guided by Shapiro-Wilk normality tests and visual inspection of histograms.

Abbreviations

Hb	Hemoglobin
MGV	Mean gray value
PCV	Packed cell volume
POCT	Point-of-care-test
RBC	Red blood cell count

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Author contributions

The present study was designed and proposed by U.F., M.H.L., and DF. The lab protocol was carried out by M.A.A., M.I., A.A., F.N., and A.I. Data was analyzed by S.N., U.F., A.A., and M.I. The manuscript was written and reviewed by U.F., M.A.K., F.S., D.F., F.S.A. and K.A.S. Images were analyzed by M.A., A.A., and F.N. Funding and resources was managed by D.F., F.S.A., and K.A.S.

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Data availability

All the relevant data has been incorporated within the article.

Declarations

Ethics approval and consent to participate

The study is a part of a collaborative research project carried out by the Department of Zoology and Department of Physiology, IUB vide approval No. PHYSIO-92/2024-33 dated 05-05-2024.

Consent for publication

Consent for publishing this article has been attained from all co-authors.

Competing interests

The authors declare no competing interests.

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