

Investigation of relationship between coat colour and presence of trypanosome parasitaemia in Small East African Zebu cattle in South-East Uganda.

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Abstract

A study to investigate the relationship between coat colour and presence of trypanosome parasitaemia in Small East African Zebu in South-East Uganda was conducted. 521 cattle from eight villages in two districts were photographed and these images classified both manually and by computer analysis. The relationship between levels of red, green and blue in cattle coat colour and presence of parasitaemia was investigated using a regression analysis model. This showed that an increase in the level of red or blue in the coat colour resulted in an increased number of months in which an animal would be predicted to be parasitaemic (with blue being statistically most significant). An increase in the level of green however resulted in a decrease in the number of months in which an animal would be predicted to be parasitaemic. Mean red, green and blue values were calculated for each of six colour categories (white, fawn, light brown, dark brown, grey and black). It was found that light brown and fawn coloured cattle were predicted to have a significantly lower number of months of trypanosome parasitaemia than cattle of all the other colour categories.



Introduction

Trypanosomiasis is caused by parasitic trypanosomes. In Africa several species of trypanosomes are transmitted by tsetse flies of the genus *Glossina*. Trypanosomes can infect humans, domestic livestock and wild mammals. *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* are responsible for the human disease sleeping sickness (Fèvre and others 2001). *T.b.rhodesiense* is zoonotic, with wild animals and domestic livestock acting as reservoirs of infection. Wild animals such as the warthog, bush pig and buffalo are often asymptomatic carriers. In domestic livestock the disease is known as 'nagana', a word derived from the Zulu 'nakane' meaning tsetse fly disease (Coetzer and others 1994). Infection of cattle is of the most economic importance. Three species of trypanosome infect cattle: *T. vivax*, *T. congolense* and *T. brucei*. The disease causes anaemia, loss of condition, infertility, abortion and depressed milk production and is therefore a major constraint to livestock production in affected areas (Brown and others 1990).

Cattle are infected with trypanosomes by the bite of an infected tsetse fly. The disease associated with trypanosomes therefore occurs in tsetse fly infested areas. The level of disease occurring depends on the degree of contact between cattle and tsetse flies. Different species of tsetse fly differ in their ability to transmit pathogenic trypanosomes. For example *Glossina fuscipes* is a poor transmitter of *T. congolense* compared with *G. morsitans* and *G. pallidipes* but is a good transmitter of *T. vivax*. Different species of tsetse also have different preferred habitats. Forest tsetse flies are of less significance to domestic livestock as this habitat is generally unsuitable for grazing animals. Riverine and savannah tsetse flies however infest areas that are valuable to livestock for watering and grazing (Brown and others 1990).

Due to the economic importance of trypanosomiasis, much research has been performed with the aim of minimising infection. Only eradication of the tsetse fly vector would abolish this disease. In the past this has been seen as impossible with over ten million square kilometres of land infested. Methods to control tsetse flies and control trypanosomiasis have been attempted: - minimising contact between livestock, wild animals and tsetse infestation; using tsetse traps; diagnosing disease and using chemotherapy or chemoprophylaxis; exploitation of trypanotolerant breeds of cattle and immunological approaches (Leak 1999). It is now being proposed to achieve final eradication of local tsetse populations by use of the sterile insect technique. This would involve first suppressing the tsetse fly population in an area. The release of factory reared, male tsetse, sterilized by radiation, would then compete with any remaining wild male tsetse to mate with the female tsetse to cause a dramatic reduction in the tsetse birth rate (Kabayo 2002). However, some view this approach with much caution on historical, ecological, logistical and financial grounds (Rogers and Randolph 2002).

In order to transmit trypanosomiasis infected tsetse flies must land on and bite the animal. Much research has been done to investigate host seeking behaviour of tsetse flies. Endogenous factors of the tsetse influence host seeking such as circadian rhythm of activity, starvation level, age, sex and pregnancy status of flies. Exogenous factors are also of importance including temperature, vapour pressure deficit, mechanical, olfactory and visual stimuli (Leak 1999).

This study aims to investigate the visual stimuli of different coat colours of cattle. Previous work on visual stimuli has been done in relation to tsetse traps. These are a more acceptable way of controlling tsetse than ground or aerial spraying of insecticides, which have ecological and environmental impacts. Tsetse traps have been used since the early 20th century with the first attempts taking place in 1910. Sticky traps were used then on the backs of plantation workers on the island of Principe (Leak 1990). Nowadays tsetse traps are odour baited, insecticide impregnated cloth targets of specific shape, size and colour to maximize catches.

Tsetse flies perceive shape, size, contrast with background, colour and movement. These stimuli are important in attracting flies to a target once they are in visual range and also have a role in the landing responses of flies. It has been found that larger objects are more attractive to tsetse flies than smaller ones (Hargrove 1980). Compact shapes such as squares and circles are more attractive than elongated ones (vertical or horizontal rectangles) (Torr 1989). Several studies have been performed to test colours for their attractiveness to various tsetse species. All colours divert tsetse from their usual upwind direction path (Torr 1989). Colours that promote initial attraction of the tsetse have been studied to determine the best colour to use on the exterior of traps. Black, red and blue have been found to be attractive (Green 1993). Phthalogen blue traps catch significantly more tsetse than other colours as blue is highly attractive (Gibson and Torr 1999). White is moderately attractive (50% attraction compared to black). Green is less attractive to tsetse flies and the least number are attracted to yellow (Vale 1982). The strongest landing responses were found to be on black surfaces (Green 1986). Consequently traps are made to be phthalogen blue on the outside to attract the maximum number of tsetse flies and black on the inside to maximise the proportion of tsetse that land on the entrapment area. If impregnated screens are used these include large rectangles of black to maximise landing and killing (Gibson and Torr 1999). A recent study has shown that a simple design based on minimal blue and black rectangular panels, with a trap body consisting of a configuration of netting is very effective (Mihok 2002). A laboratory study of landing responses to



Appendix 1 - click on the map to open a larger (pdf) version

black and white patterns showed that *Glossina morsitans morsitans* showed a nine-fold greater preference for black over white. This species also landed twice as frequently on a vertical black stripe as a horizontal one (Brady 1988). A field study of the visual response of tsetse to striped targets (Gibson 1992) showed that tsetse are less attracted to stripes than solid colours and that they avoid horizontal patterns. This has supported the suggestion that zebras are protected from being fed on by biting flies, including tsetse, by their striped pattern in particular the horizontal stripes on the lower part of the animal, which is where tsetse would normally feed (Gibson 1992).

Materials and Methods

Study area:

The study was carried out in eight villages in Southeast Uganda. See Map in Appendix 1 (below). In the Busia district four villages were investigated: Kubo, Sitengo, Nanjeho and Buyimini. In Tororo district, Hitunga, Bughaji, Magoje and Ojelai were investigated. This area receives 1200-1500mm rainfall annually. The rainfall is bimodal with two wet seasons (March-May and September-November) and two dry seasons (December-February and June –August) and the daily mean temperatures range between 15° C (minimum) and 27° C (maximum) (Magona and others 2000). These villages are in areas of subsistence farmland, with the Tororo villages all close to wetland/swamp areas. The Busia villages are close to rivers, Lake Victoria or woodland. These are suitable habitats for the tsetse fly species *Glossina fuscipes fuscipes*, a riverine tsetse species that infests these districts.

DFID Project:

In each village eighty cattle have been randomly tagged for the purpose of a Department for International Development (DFID) Animal Health Programme Project. Glasgow and Edinburgh Universities are undertaking the project and there are four main objectives:

- To design a hand held device to enable rural farmers without access to veterinary advice to improve differential diagnosis of sick cattle.
- To investigate the prevalence of tick-borne diseases.
- To investigate the prevalence of trypanosomiasis within cattle in the area and the species of trypanosomes involved.
- To investigate human sleeping sickness caused by *Trypanosoma brucei rhodesiense* that can be carried in cattle and transmitted to people by tsetse flies.

The cattle are not all owned by the same people but most families tend to have a few cattle each. If an animal is lost from the study at any time because it is sold, dies or is killed another animal is tagged in its place to keep the sample population for each village at eighty.

The same cattle used for the DFID project were photographed to enable investigation of the association between coat colour and trypanosomiasis infection. During the study period of a month it was not possible to photograph all of these animals because each village was only visited twice and some animals were away grazing or being used for ploughing etc.

Cattle population:

The cattle used in this study are all small East African Zebu, a *Bos indicus* breed kept under traditional management. This breed is used for milk and meat production and work purposes (Payne and Hodges 1997). Zebu cattle were introduced into Africa around 2000B.C. Some came directly from India or Arabia and some via Egypt (Felius 1985). A second wave of Zebu cattle from Asia was introduced in the 7th century A.D. and these short-horned Zebu are now the dominant breed in East Africa (Maule 1990). It has generally been assumed in East Africa that *Bos indicus* cattle are highly susceptible to trypanosomiasis, in contrast to West African breeds such as N'Dama that possess the trait of trypanotolerance (Coetzer and others 1994). There is however epidemiological evidence to suggest that in some areas a degree of tolerance has been developed. Indeed thousands of Zebu cattle survive around the shores of Lake Victoria despite being continuously exposed to tsetse. A study based in south-west Kenya (Mwangi and others 1998) also showed Maasai Zebu to be less susceptible to infection than Galana Boran (another *Bos indicus* cattle breed).

Management:

In these villages very little, if any selective breeding is performed. Most of the bulls are entire and only since the DFID project has started, have any castrations of bulls or young bullocks been done. This may be why there is such a variation of colours within the breed unlike in domestic European breeds when there is a definite breed standard e.g. Friesians are black and white. The cattle are tied up around the homesteads at night and grazed on communal pastures during the day, often in swamp areas where tsetse flies are prevalent. Young calves are usually tethered by the homestead all the time.

Data Collection:*A: Images of cattle*

During the study period as many tagged cattle were photographed as possible. This was done using a digital camera. The colour balance was set by focussing the camera on a piece of white card prior to the photographs being taken at each site within the village. Two pictures were taken of each animal (one for each side), with the sun on them and as few shadows as possible. These images were then classified according to colour.

B: Diagnosis of trypanosomiasis

Once every four weeks all the tagged cattle are clinically examined and data is collected for each animal. Body condition, coat condition, mucous membrane colour, rectal temperature, pre-scapular lymph node size, number of boophilus ticks present, number of rhipicephalus ticks present and haemoglobin content of a blood sample from the ear vein are all noted. Thick and thin films are made from the ear vein sample. A jugular blood sample and faecal sample are also obtained from every tagged animal. To detect trypanosomes in the blood of these cattle, the examination of thick and thin Giemsa stained films (Stephen 1986) and Buffy coat examination using the Woo technique is performed. The Buffy coat is obtained by centrifugation of a heparinised micro-haematocrit centrifuge tube. The spun tubes are then directly observed when mounted on a slide and immersed in oil. The parasites are visible at the Buffy coat/plasma junction. These tubes are then cut with a diamond pen and the Buffy coat and a small amount of plasma tapped out onto a slide, covered with a cover slip and viewed using the dark ground/phase contrast setting on the microscope (Fèvre 2001).

Unfortunately, due to the fluctuating nature of the parasitaemia, failure to demonstrate presence of trypanosomes in the blood sample does not mean that the animal is not infected. PCR is therefore also used for diagnosis but the results of PCR diagnostics are not available at this time.

For this study results from the first year of the DFID project have been used. This project began in July 2001 in Tororo district and September 2001 in Busia district. Ian Anderson and the staff at the Livestock Health Research Institute (LIRI), Tororo collected this data. The author would like to thank them for this work.

C: Data Analysis

The cattle images collected were analysed in two ways:

I) Manual Colour Classification:

A manual colour classification was performed which involved stating for each animal the main colour. This was done by looking at all the images and putting the colours into the categories: black, grey, dark brown, light brown, fawn or white. ([See Appendix 2 for examples of colours](#)) It was decided to restrict the number of colour categories to six. If a second colour was present this was also stated with the approximate percentage of second colour present noted.

II) Computer Analysis of Colours:

All coloured images consist of red, green and blue pixels. A computer programme which analyses the proportion of red: green: blue pixels in a specified area was used over as large a rectangular area as possible on each animal's body (without including shadows, background, mud patches or rope in that area). This generated two sets of results for each animal (one for each side). This programme was designed by Mark Eisler and coded by Graeme McComb (both of Glasgow University) to whom the author is very grateful.

The data from both these methods of colour classification was entered into a Microsoft Excel Spreadsheet. The Gen Stat programme was then used to analyse this data.

Results

During the period of study 521 cattle were photographed, 286 from Busia district and 235 from Tororo District. Of these cattle the numbers in each colour category (with only main colour being considered) are shown in table 1.

TABLE 1

District	Village	Light Brown	White	Black	Fawn	Dark Brown	Grey	Grand Total
Busia	Buyimini	22	15	9	13	7	5	71
	Kubo	16	23	10	15	6	5	75
	Nanjeho	21	5	9	9	11	6	61
	Sitengo	31	15	16	7	8	2	79

Busia Total		90	58	44	44	32	18	286
Torono	Bunghaji	23	10	17	11	7	6	74
	Hitunga	23	7	7	9	6	1	53
	Magoje	9	10	8	3	4	1	35
	Ojelai	23	24	12	11	3		73
Torono Total		78	51	44	34	20	8	235
Grand Total		168	109	88	78	52	26	521

Table 1: Numbers of cattle in each colour category, in each of the villages in the study.

A regression analyses model was generated using all the data from the computer programme. Both sides of each animal are therefore included. The data fits a Poisson distribution and has been transformed using a log link function. This model describes the effect of varying amounts of red, green and blue colour in the cattle's coats on the number of months in which trypanosome parasitaemias (of any salivarian species) were detected during the one year study, allowing for differences between district and village. Figures 1,2 and 3 illustrate the effect each individual colour has on the number of times cattle are positive for trypanosomiasis. ([Click here](#) to open a pdf containing figures 1- 4.)

The model takes into account all three colours at the one time and all three colours have a significant effect on the model:

- An increasing level of blue results in an increased number of infections predicted by the model.
- An increasing level of red results in an increased number of infections predicted by the model.
- An increasing level of green results in a decreased number of infections predicted by the model.
- Blue has a greater effect than red on the number of infections predicted by the model.
- Statistically the results generated for blue are most significant (for blue p value < 0.001, for green p=0.004 and for red p=0.026).

Figure 4 shows the number of months in a year in which trypanosomes would be expected to be found in the blood of cattle of each of the colour categories. This chart is based on the regression analyses model. Cattle of only one colour are considered. For each colour category an average of all the red, green and blue values has been taken so that the level of infection for a 'typical' light brown cow for example can be predicted. ([Click here](#) to open a pdf containing figures 1- 4.)

This shows that light brown and fawn cattle are significantly less likely to have infections than dark brown, black, white or grey cattle. There is no significant difference between light brown and fawn cattle. There is also not a significant difference between dark brown, black, white or grey cattle. For all the colours there is significantly more infection in Busia district than Tororo district. When the average red, green and blue values for each colour category are looked at (See Table 2) it can be seen

that light brown cattle have the lowest level of blue and fawn cattle have a high level of green. White and grey cattle have high levels of blue and green. For these colour categories it appears that the effect of the blue increasing the level of infection is greater than the effect of green reducing the level of infection.

Colour	No of cattle	Red	Green	Blue
Light Brown	112	175.99	117.17	55.91
Fawn	67	167.98	133.37	80.18
Black	47	95.93	90.98	82.23
Dark Brown	42	125.18	101.94	75.65
Grey	18	139.42	132.89	104.89
White	37	168.31	156.26	125.04

Table 2: Average Red, Green and Blue values for each colour category of cattle.

Discussion

The collection of images for this study was done in a manner to fit in with collection of data for the DFID project. This meant that despite efforts to take images in a standardised fashion, some photographs had to be taken even when there were shadows or dirt over the body of the cattle. Each photograph was also taken in a different area of each village and so alterations in lighting and background occurred. The camera was adjusted at each site to set the colour balance against a white background. These images could be taken in a much more controlled way however if they were all taken against the same backdrop e.g. against a self-coloured background with lighting conditions maintained the same for all photographs. How feasible it would be to do this in the field however is questionable.

The images obtained were analysed by two different methods for this study. The first method by manual classification was subjective. It was decided to use just six colour categories because with any more than this it would become difficult to decide which category an animal would fit into. Only a main colour and presence of second colour were noted. This system allowed all the cattle to be classified relatively easily. The percentage of second colour noted was again subjective. In order to minimise the error in this method the same person classified all the cattle. With this being done manually any shadows, dirt or rope overlying the body of the cattle could be identified and this taken into account when allocating the animal to a colour category.

The second method of colour classification was objective. A computer programme was used to analyse each image and the ratio of red: green: blue pixels of a specified area over each animal's body was determined. This programme had to be used in a particular way to ensure only the animal's colour was analysed. If an animal had a large patch of dirt on its side a much smaller area had to be used to avoid including any dirt that was not representative of the true colour of the coat. This programme could only give a ratio of pixels and so this does not reflect areas of separate colours. For example a cow that is fifty percent black and fifty percent white would be given the same values under analysis as a cow that is completely grey.

The results of this study were analysed using a regression analysis model. This shows that an increasing level of blue or red in cattle coat colour results in an increase in predicted number of months in which cattle are positive for trypanosomiasis over a one year period, with the results for blue being statistically most significant. This is perhaps to be expected due to the extensive work that has been done to design tsetse traps. These studies show that blue is the colour most attractive to tsetse flies (Gibson and Torr 1999). The model also shows that an increasing level of green in the coat colour results in a decrease in predicted number of months in which cattle are positive for trypanosomiasis. Again studies have shown green to be less attractive to tsetse flies (Vale 1982).

When this model is applied to real cattle colours by using mean red, green and blue values for each of the colour categories in the manual colour classification, light brown and fawn coloured cattle are predicted to have significantly less infection than any of the other colour categories. Interestingly, when cattle of only one colour are considered, in the villages used in the study, light brown and fawn coloured cattle are present in higher numbers than the other colour categories. In the villages studied, it does not appear that the farmers have chosen to breed selectively for these colours because castrations of the bulls were not performed before the project started. A possible explanation for the predomination of these two colours could relate to the clinical signs of trypanosomiasis. This disease causes infertility and abortion. If, as the results of this study suggest, light brown and fawn coloured cattle have lower infection levels than other colours of cattle, then it is logical that these colours would be naturally selected for because their conception rates may be greater and abortion rates lower.

Conclusion

In this study it has been shown that an increasing amount of blue or red in cattle coat colour increases the number of months in which cattle are likely to be positive for trypanosomiasis (with blue being most statistically significant). An increasing level of green decreases the number of months of parasitaemia. The study has also shown that in the villages investigated, light brown and fawn coloured cattle are likely to be positive for trypanosomiasis less often than cattle of other colours.

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