Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 44 (1987)
Heft: 1

Artikel: Identification and selection of cattle naturally resistant to African trypanosomiasis

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DOI: https://doi.org/10.5169/seals-313819

Acta Tropica *44*, 55–66 (1987)

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Identification and selection of cattle naturally resistant to African trypanosomiasis

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Summary

Cattle were exposed to natural trypanosome challenge in an area of high *Glossina* density (Samandéni, Burkina Faso) for various periods of time during 1982, 1983, 1984 and 1985. All of 30 Zebu proved to be sensitive to trypanosomiasis i.e. they died or were treated in extremis in 10±4 weeks. Twenty-one (31%) Baoulé were as sensitive as the Zebu while 47 (69%) were resistant i.e. they survived in good condition. Twenty Ndama/Baoulé crosses, indigenous to Samandéni were all resistant. Weekly blood samples were taken (2,317 in total) for the determination of parasitaemia and packed cell volume (PCV) as a measure of anaemia, the most important pathological feature of cattle trypanosomiasis. In both Zebu and sensitive Baoulé 59% of the blood samples showed positive parasitaemia, of which 38% and 52% respectively were *T. congolense* the major cattle pathogen in the area considered. In resistant Baoulé and Ndama/Baoulé 11% and 10% of the samples were positive for trypanosomes of which only 4% and 2% were *T. congolense* respectively. PCV decreased from 35 to 20 in Zebu, 39 to 20 in sensitive Baoulé and 40 to 34 in resistant Baoulé, there was no change in the indigenous Ndama/Baoulé.

Six Ndama/Baoulé indigenous to Samandéni remained resistant to trypanosomiasis when moved to another area of high *Glossina* challenge. Seven Ndama/Baoulé calves, conceived in Samandéni but born and kept for 2½ years in a *Glossina*-free area, also proved to be resistant to challenge. Twelve Baoulé calves, born from cattle selected under natural field challenge, and which had not come in contact with trypanosomes for the first 10 months of their life, proved to be resistant when exposed in the field.

These observations show that some, *but not all,* cattle from the Baoulé breed are naturally resistant to African trypanosomiasis, that this resistance does not need repeated exposure to trypanosomes early in life but appears to be inherited

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and functional against many types of antigenically different trypanosomes. Thus, selective breeding of trypanoresistant animals and their successful introduction, without trypanocidal drug protection, into areas of high *Glossina* density appears feasible.

Key words: trypanosomiasis; *Trypanosoma congolense;* cattle crosses; Baoulé breed; inherited resistance.

Introduction

It is generally admitted that African trypanosomiasis impedes the use of 7×10^6 km^2 of land adequate for cattle raising in intertropical Africa and constitutes the major constraint to increasing protein production in that area. Vector eradication, albeit successful in certain locations, cannot be realistically extended to the whole area infested (Jordan, 1985). Trypanocidal drugs have helped maintaining cattle in areas of *Glossina* infestation but under high challenge the frequency of prophylactic or curative interventions rapidly leads to drug resistance (reviewed in Holmes and Scott, 1982; Küpper and Wolters, 1983; Pinder and Authié, 1984; Authié, 1984). The extreme antigenic variability of trypanosomes makes development of a vaccine unlikely (reviewed in Turner 1982; Donelson, 1984; Vickerman, 1984; Roelants and Pinder, 1984). Thus efforts are made to study and promote some West African breeds of cattle which appear naturally resistant to trypanosomiasis (ILCA, 1979; Murray et al., 1982; Roelants and Pinder, 1984).

We have shown that some individuals of the Baoulé breed of taurine cattle (*Bos taurus*) were able to withstand natural *Glossina* challenge while others died as fast as Zebu cattle (*Bos indicus*) (Roelants et al., 1983). We present data confirming this first report by extending it to a larger number of animals observed over a period of 4 years. We also present evidence on the genetic nature of resistance.

Materials and Methods

Cattle were kept in 4 different areas. a) The CRTA farm located in *Banankeledaga,* 15 km north of Bobo-Dioulasso, which was free of *Glossina* but wich harbours tabanids and where parasitaemia was detected occasionally. b) *Samandéni,* 50 km north of Bobo-Dioulasso, where there is a ranch of the "Service de l'Elevage et des Industries Animals (Station Bovine de Samandéni)" located near the Black Volta river wich at the time had a high *Glossina* challenge. Animals there had to walk 10–20 km a day to feed on poor pasture and find water at the river. These are the type of conditions herdsmen find when introducing their cattle into *Glossina* infested areas. c) *Karankasso,* 40 km south of Bobo-Dioulasso, where the animals were kept in the bush and found water at the Koba river which was a zone of high *Glossina* challenge isolated from Samandéni as far as exchange of tsetse flies was concerned. d) *Bobo-Dioulasso* where some animals were kept in a fly-proof cattle facility at the CRTA.

Groups of adult Zebu (30 in total) and Baoulé (68 in total) were transferred from Banankeledaga to Samandéni at the beginning of the years 1982, 1983, 1984, and 1985. Adult Ndama/Baoulé crosses (13 in total), indigenous to Samandéni were observed concomitantly.

Some Ndama/Baoulé females from Samandéni were moved to Banankeledaga while in gestation and delivered their calves there. These calves stayed in Banankemedaga until they were 2½ years of age and 7 of them were then exposed to *Glossina* in Samandéni in 1985.

Six adult Ndama/Baoulé were moved from Samandéni to Karankasso together with 4 indicator Zebu from Banankeledaga.

Finally, calves were obtained by crossing one resistant or one sensitive Baoulé bull with females of unknown status. The calves were allowed to receive the colostrum from their mother for 24 h and were then kept in the fly-proof facility at Bobo-Dioulasso for 10 months. Subsequently, they were transferred to Banankeledaga to adapt to the open air and, after 5 months, transferred to Samandéni for selection. Twenty-six newborn Baoulé were put in the fly-proof facility but useful data on trypanosomiasis was obtained on only 13 of them in Samandéni, the rest having died of other causes in the stable (2), in Banankeledaga (4) and at Samandéni (7). It should be noted that mothers were selected in Samandéni only after producing these calves.

The first and most common manifestation of trypanosome infection in cattle is, by far, a progressive anaemia associated with a persistant fluctuating parasitaemia and all of the three common trypanosome species can produce this disease syndrome (Morrison et al., 1985). Thus, once a week, all *Glossina* exposed animals were examined for their general health status and jugular blood was taken for determination of parasitaemia and packed cell volume (PCV), following the method of Murray et al. (1977).

All animals were sprayed against ticks once or twice a week, depending on the season, with either Supona (Shell) 0.5‰ or Procigam (Shell) 2 ml/l. Blood smears were made for the detection of thick-borne disease when the PCV was falling 4 points or more. Blood from the first wave of parasitaemia was injected in NMRI mice to isolate trypanosomes. Subinoculation into mice was also used to attempt to detect subpatent *T. brucei* or *T. congolense*, particularly in animals showing a heavy *T. vivax* infection.

Results

The susceptibility to trypanosomiasis of 10 Zebu and 10 Baoulé moved from Banankeledaga to Samandéni in 1982, and of 7 Ndama/Baoulé indigenous to Samandéni was reported previously (Roelants et al., 1983). Five of these Baoulé and all 7 Ndama/Baoulé rarely showed parasitaemia or anaemia and remained in good condition; they were thus classified as resistant to trypanosomiasis. The remaining 5 Baoulé and all the Zebu showed patent parasitaemia, severe anaemia and died of trypanosomiasis; these were classified as sensitive.

In the present study a further 65 adult taurine cattle have been selected at Samandéni using the same criteria. Individual weekly parasitaemia readings and time to death are presented in Figs. 1–4. A group of 10 Baoulé and 7 Zebu were exposed at Samandéni in 1983. The results were similar to those obtained in 1982, 4 Baoulé were resistant and the 6 others were as sensitive as the 7 Zebu (Fig. 1). All the animals exposed in 1982 and 1983 were males. In 1984, another 41 Baoulé were put in Samandéni, 35 of which were females (Fig. 2). Ten were found to be sensitive (8 females and 2 males), and the other 31 resistant (27 females and 4 males). Five indicator Zebu were all sensitive. The same type of observation was performed on 7 more Baoulé, 3 females and 4 males in 1985 (Fig. 3). All appeared to be resistant and 8 indicator Zebu were sensitive.

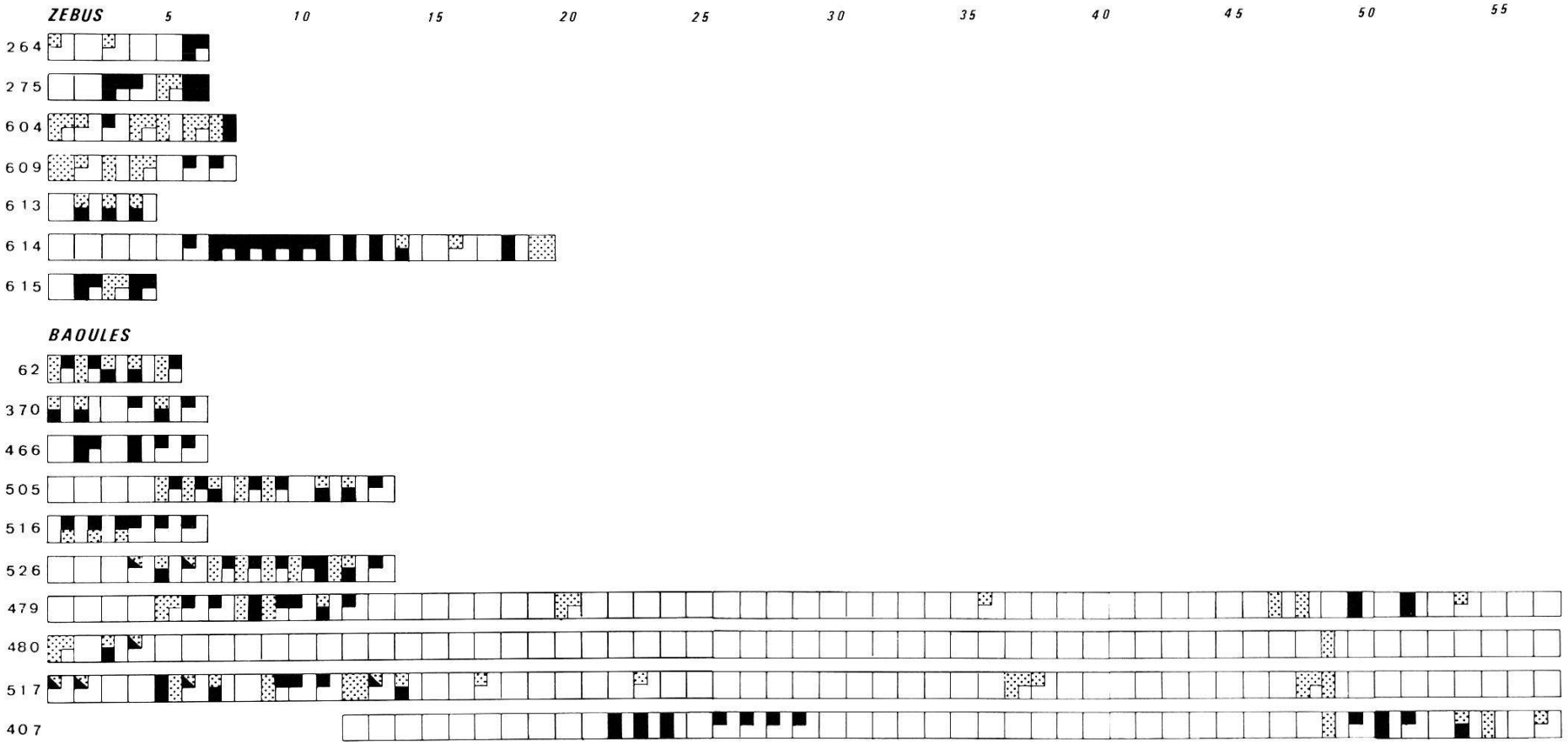

Fig. 1. Selection of cattle in Samandéni in 1983. Boxes represent weekly examinations, interruption of the boxes means that the animal has died or has been treated in extremis. The observation was stopped after 57 weeks.
Parasitaemia in jugular blood:

- □ = below the level of detection: $<10^3$ organisms/ml
- ▨ or ▩ = 10^3–10^5/ml *T. vivax* or *T. congolense*
- ▨ or ▩ = 10^5–10^6/ml
- ▨ or ▩ = 10^6–10^7/ml
- ▨ or = $>10^7$/ml

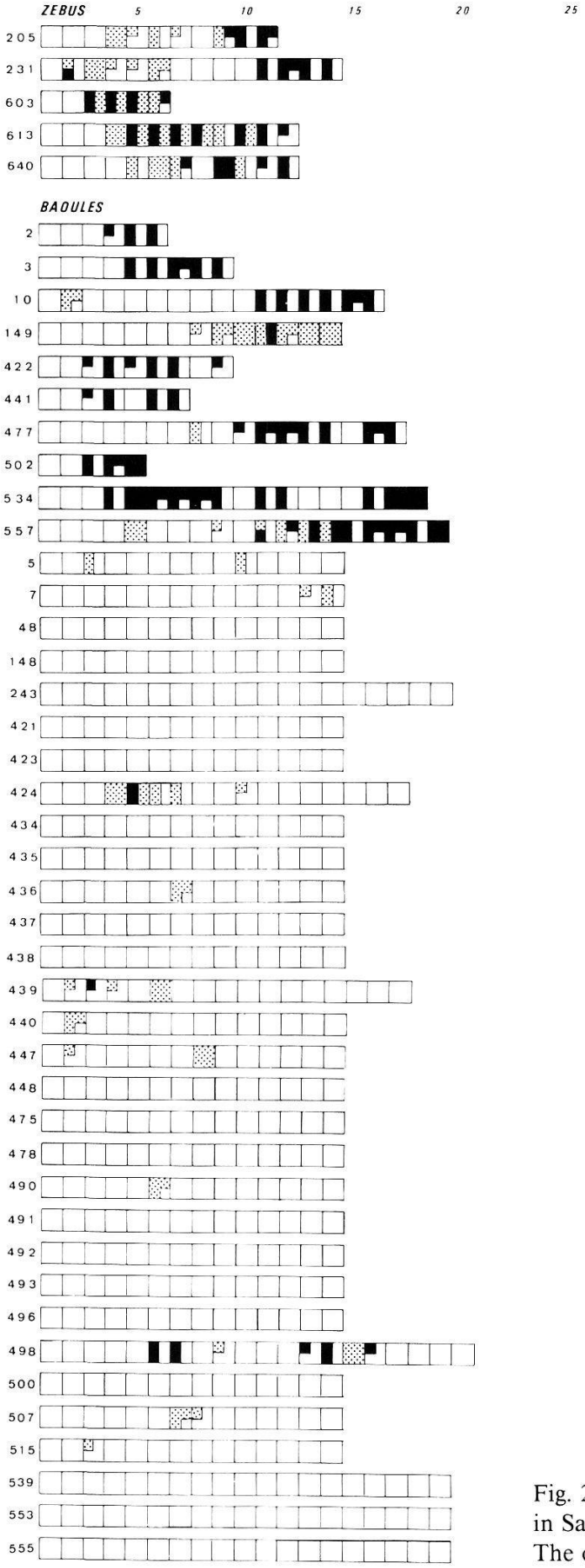

Fig. 2. Selection of Zebu and Baoulé cattle in Samandéni in 1984. Legend as in Fig. 1. The observation was stopped after 19–20 weeks.

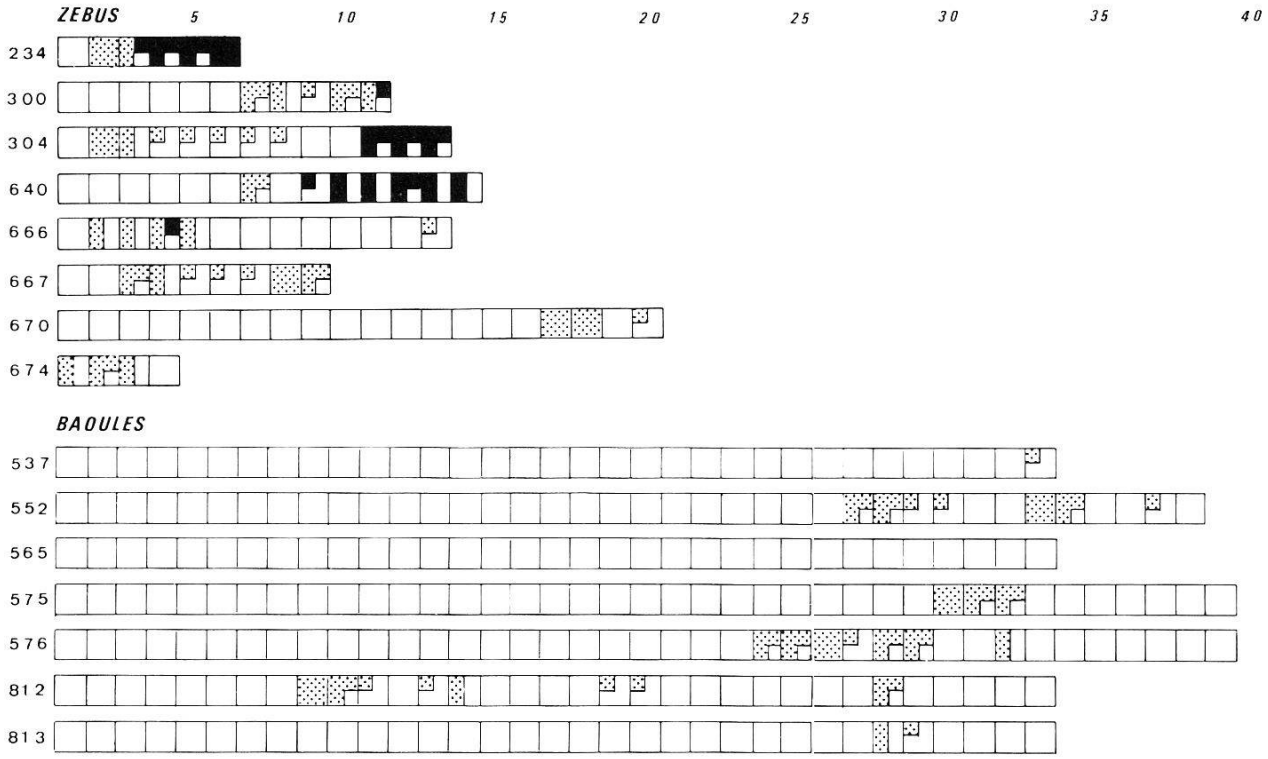

Fig. 3. Selection of Zebu and Baoulé cattle in Samandéni in 1985. Legend as in Fig. 1. The observation was stopped after 34–39 weeks.

Ndama/Baoulé calves, 3 females and 4 males, were conceived in Samandéni and born in Banankeledaga. They were exposed to *Glossina* in Samandéni at 2½ years of age. They all were resistant (Fig. 4).

To examine if the resistance to trypanosomiasis is limited to one set of antigenic variants found in a single area, 6 male Ndama/Baoulé were moved in 1983 from Samandéni to Karankasso an area which is also highly infested with *Glossina.* They all remained resistant to trypanosomiasis (Fig. 5). After 17 weeks they were returned to Samandéni where their resistance status did not change (Fig. 5).

Table 1 presents the analysis of time to death of sensitive animals, anaemia as drop in PCV and parasitaemia in all adult Zebu, Baoulé and Ndama/Baoulé observed from 1982 to 1985 in Samandéni. No difference was found between male and female.

It can be seen that the sensitive Baoulé have the same average time to death as Zebu (10 weeks). As reported in the literature (Murray, 1979; Morrison et al., 1985), the most important pathological feature in these animals is anaemia. PCV values dropped drastically in Zebu, from 35 to 20, and in sensitive Baoulé, from 39 to 20; values also dropped somewhat in resistant Baoulé from 40 to 34 but not in Ndama/Baoulé during the period of observation. All resistant animals maintained themselves in excellent general condition. Even sensitive, highly parasitized and anaemic animals, although obviously distressed, dit not loose much body weight. As an example, the weight of Baoulé selected in 1984 was,

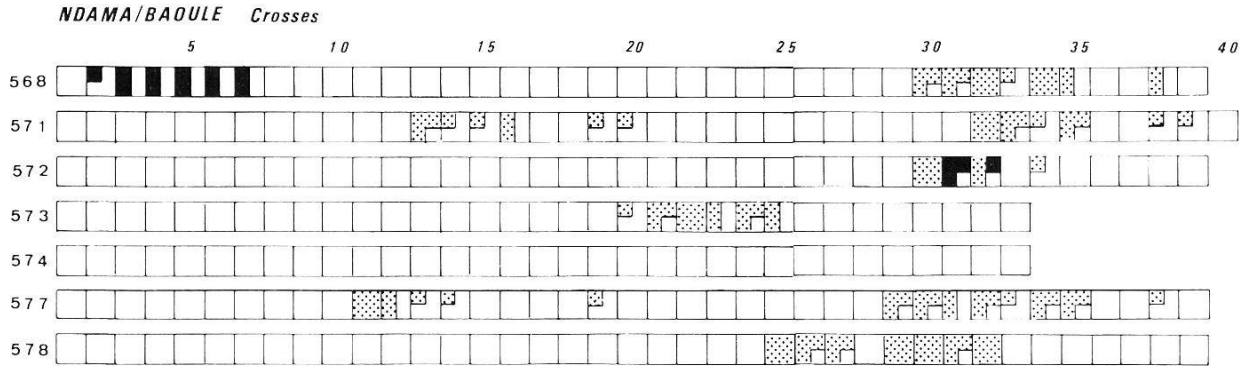

Fig. 4. Selection of Ndama/Baoulé cattle in Samandéni. Legend as in Fig. 1. The observation was stopped after 33–40 weeks.

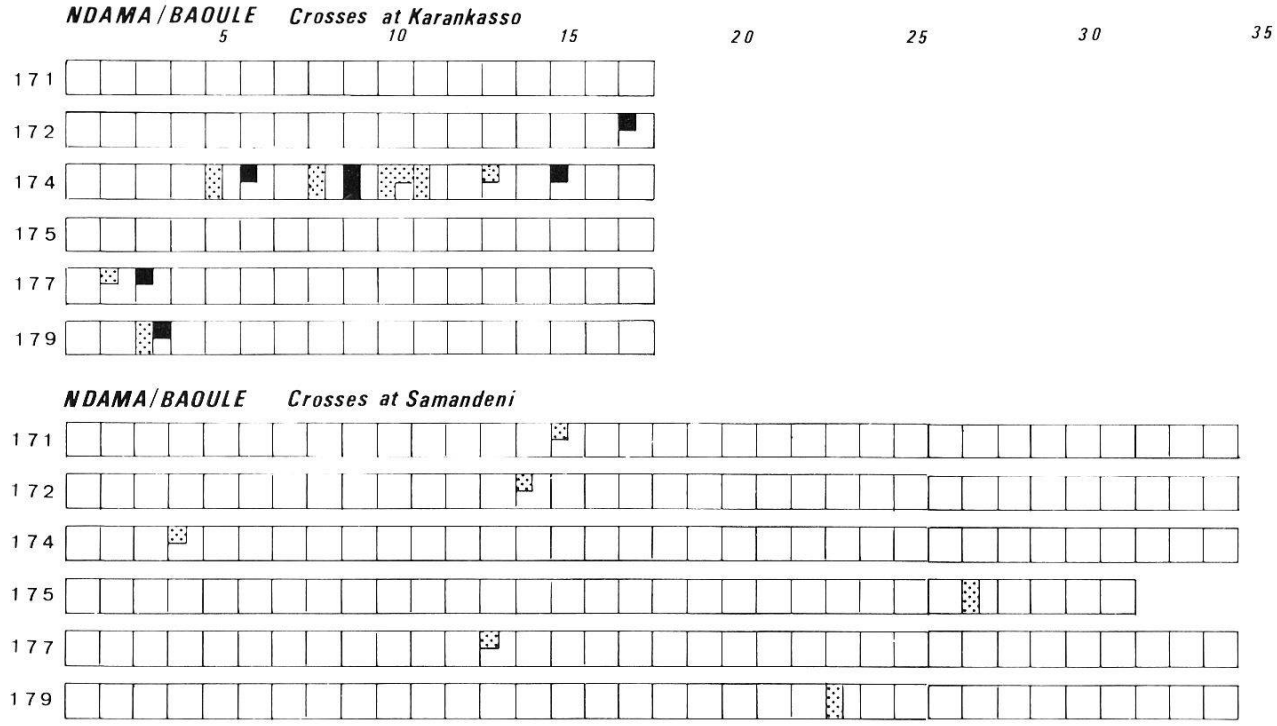

Fig. 5. Selection of Ndama/Baoulé cattle first in Karankasso, then in Samandéni. Legend as in Fig. 1. The observations were stopped after 17 and 30–33 weeks.

before selection, 248 kg±42 for the animals which were later shown to be sensitive and 258 kg±32 for the resistant ones. After selection the weights were 225 kg±25 for the sensitive cows which had been treated and evacuated and 240 kg±31 for the resistant ones.

It was essential to prevent or treat tick-born diseases in order to study trypanosomiasis itself. Three major outbreaks of anaplasmosis, with some cases of babesiosis, occurred during the 4th–14th weeks and 48th–52th weeks of the 1983 observation period and the 28th–34th weeks of the 1985 observation of adult Baoulé and Zebu. This was accompanied by anaemia and patent parasitaemia even in resistant animals. After treatment of anaplasmosis with tetracy-

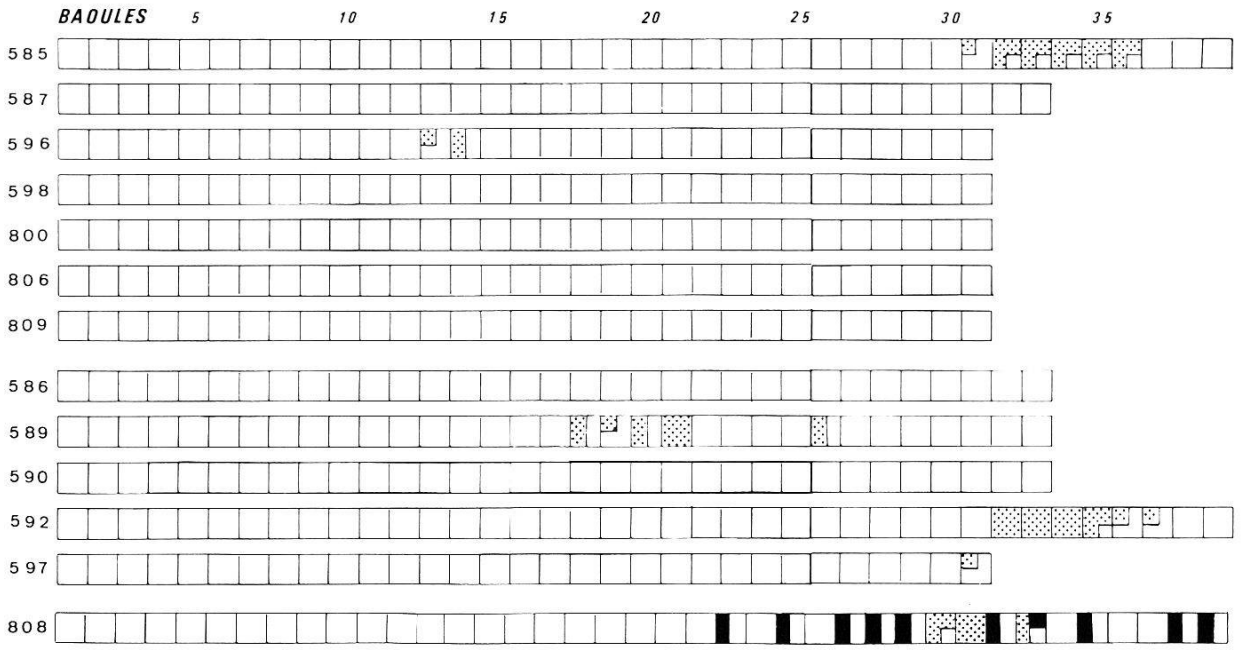

Fig. 6. Selection in Samandéni of Baoulé calves born from parents of known sensitivity.
R×R 586, 589, 590, 592, 597
R×S 585, 598, 800, 806, 809
S×R 587
S×S 808
?×S 596
Legend as in Fig. 1. The observation was stopped after 31–39 weeks.

Table 1. Survival, anaemia and parasitaemia in adult bovids exposed to field challenge

	Time to death weeks $\bar{x}$±SD	Samples examined n	PCV			Parasitaemia (% of samples)				
			Start $\bar{x}$±SD	End[a] $\bar{x}$±SD	P (t test)	Total[d] positive	*T. cong.* only	*T. viv.* only	*T. cong.* and *T. viv.*	Total *T. cong.*
Sensitive										
Zebu (n = 30)	10±4[b]	292	33±5	20±5	<0.001	59	23	21	15	38
Baoulé (n = 21) ...	10±5[c]	217	39±9	20±5	<0.001	59	29	7	23	52
Resistant										
Baoulé (n = 47) ...	survive	1061	40±7	34±6	<0.001	11	2	7	2	4
Ndama/Baoulé (n = 20)	survive	747	34±5	31±5	>0.5	10	2	8	<1	2

[a] week of death or end of observation period
[b] animals actually died (13) or were treated when moribund (17)
[c] animals actually died (8) or were treated when moribund (13)
[d] *T. brucei* was detected occasionally in 1982 by mouse subinoculation but has not been detected since.

clin and babesiosis with Acaprin (Bayer) trypanosomes disappeared spontaneously from the blood of resistant animals without any trypanocidal treatment.

Rodgers (1985) discussed quantitation of trypanosomiasis "risk" or "challenge" and showed that the Berenil Index of cattle, i.e. the interval between

Table 2. Comparison of PCV and parasitaemia in 217 samples taken simultaneously in sensitive and resistant Baoulé (1982–1983–1984)

	Decrease in PCV	Parasitaemia (% of samples)				
		Total positive	*T. cong.* only	*T. viv.* only	*T. cong.* and *T. viv.*	Total *T. cong.*
Sensitive (n = 21)	19 points	59	29	7	23	52
Resistant (n = 40)	6 points	16	3	7	6	9

patent parasitaemia of animals treated with Berenil every time they show patent parasitaemia whilst exposed to tsetse challenge, shows a linear relationship with challenge, as does also the prepatent period of previously uninfected cattle taken into tsetse areas. The prepatent period of uninfected Zebu exposed in Samandéni was 3.5±3.0 weeks which by Rodger's classification corresponds to high challenge. The prepatent period of sensitive Baoulé was 3.8±2.2 weeks.

The frequency of positive parasitaemia, in weekly examinations, was identical (59%) in Zebu and sensitive Baoulé and almost identical in resistant Baoulé and Ndama/Baoulé 11% and 10% (Table 2). *T. congolense* is the most important cattle pathogen in the areas used for observation. *T. congolense* positive samples were 38% in Zebu, 52% in sensitive Baoulé but only 4% in resistant Baoulé and 2% in Ndama/Baoulé.

Resistant Baoulé have been observed for longer periods of time than sensitive ones and more blood samples were analysed in the former (1,061) than in the later (217). To see if this did not introduce a bias in the results, the above parameters were compared on samples taken simultaneously from sensitive and resistant Baoulé (Table 2). One can see that parasitaemia and fall in PCV are not significantly different from those reported for all the samples in Table 1.

The observation that 7 Ndama/Baoulé calves born and raised in Bananke-ledaga were resistant when exposed at Samandéni (Fig. 4) indicates that the trait may be inherited. However, this trait may need repeated exposure to trypanosomiasis in early life to be expressed and there are trypanosomes at Banankeledaga. Thus a second group of calves was raised in a fly-proof facility where there was no risk of exposure.

These animals were born from crosses between known resistant (R) and sensitive (S) parents: 5 R ♀ × R ♂, 5 R × S, 1 S × R, 1 S × S and one animal born from a sensitive bull and a female which could not be selected in Samandéni because of lameness. They were kept for the first 10 months of life in a fly proof facility and were then exposed in Samandéni for 33–39 weeks. All the animals, but the S × S cross, showed scanty parasitaemia which were exclusively *T. vivax* and were almost entirely confined to 3 animals infected with anaplasmosis (585

between the 31st and 36th week of exposure, 589 between the 18th and 21st week and 592 between the 32nd and 37th week [Fig. 6]). The PCV of these animals dropped from an average of 30±3 to 25±3 during the observation. The S×S cross (808) was the only animal showing *T. congolense* infection but only after 23 weeks of exposure and its PCV dropped from 30 to 21 at 39 weeks of exposure. Five indicator Zebu (234, 304, 666, 667, 670) introduced at the same time as the Baoulé died after 6, 13, 13, 9, 20 weeks of exposure, 3 other Zebu (300, 640, 674) introduced at the 16th week of Baoulé exposure died at 11, 14 and 4 weeks of exposure. They had *T. vivax* and *T. congolense* infections. The PCV of these Zebu dropped from an average of 34±8 to 21±3.

Discussion

The results reported here confirm on a larger number of animals that some West African taurine cattle, but not all, are resistant to trypanosomiasis. We find that about 2 out of 3 Baoulé exposed to high *Glossina* challenge maintain themselves in good health while 1 out of 3 show disease and die unless treated with trypanocidal drugs. These results agree with those in the literature concerned with other West African taurine breeds, essentially Ndama and Muturu, which also show ⅔ resistance and ⅓ mortality to trypanosomiasis under heavy fly challenge (reviewed in Roelants and Pinder, 1984; Roelants, 1986). There is thus no doubt that many Baoulé can survive *without trypanocidal treatment* under hard conditions, where they have difficulty to find water and food, in areas of high *Glossina* density, where all Zebu die within 10 weeks on average.

Anaemia, as measured by the PCV, has been described as a reliable marker for the severity of the disease in cattle trypanosomiasis (Murray, 1979; Morrison et al., 1985). Besides death itself, resistance and sensitivity were also reflected by PCV which decreased just as much (from 39 to 20) in sensitive Baoulé as in Zebu (from 35 to 20) but only slightly in resistant Baoulé (40 to 34). The PCV of the resistant Baoulé at the end of the observation period was similar to that of the indigenous Ndama/Baoulé (31–34).

Of 1,808 tests for parasitaemia in resistant animals 196 (11%) were positive and only 63 (3%) showed *T. congolense* the major pathogen in the area under consideration. No patent parasitaemia was ever detected in 27 out of these 67 resistant animals. This is in sharp contrast to sensitive animals, whether Zebu or Baoulé, where 59% (300/509) of the blood samples were positive, of which 44% (225/509) showed *T. congolense.*

The difference in infection rate raises the question of whether sensitive cattle are bitten more often by *Glossina* than resistant ones. Our preliminary studies appear to indicate that Zebu might be more attractive to tsetse flies than Baoulé but that there is no difference between sensitive and resistant Baoulé. It was also shown that all animals, irrespective of their resistance or sensitivity in the field, became heavily parasitized when cyclically infected by captured flies

and that this type of infection does not mimic the field situation (Pinder M., Bauer F., Fumoux F., Roelants G. E.: submitted).

Trypanoresistance was maintained in animals transferred from one *Glossina* infested area to another. This is important in showing that the resistance appears to be generalized and not specific for certain antigenic types of trypanosomes. Indeed no exchange of *Glossina* takes place between the two area chosen (Samandéni and Karankasso) and it was shown that the antigenic variants of *T. congolense* were different in each area (G. Duvallet, personal communication).

It is of crucial importance to know whether the resistance seen here is hereditary. Seven products of resistant parents born and raised in a tsetse-free area were resistant when exposed to *Glossina* at 2½ years of age. Twelve animals of which at least one of the parents was resistant were also resistant. Unfortunately, only one sensitive by sensitive cross was available. Thus, selective breeding of trypanoresistant animals appears possible but more data has to be gathered for proper genetic analysis. A genetic basis for trypanoresistance is also supported by the fact that analysis of blood proteins polymorphisms show differences between resistant and sensitive Baoulé e.g. animals homozygotes AA for albumin have 6.5–13.7 times more chances, depending on the statistical evaluation used to be resistant than heterologous AB or homozygous BB (Queval R., Fumoux F., Roelants G. E.: submitted). It is interesting that the later 12 resistant animals were raised for the first 10 months of their life in a fly-proof facility. Thus, repeated progressive trypanosomal infection early in life is not a prerequisite for the expression of resistance.

Acknowledgments

We thank the "Direction des Services de l'Elevage et des Industries Animales" of Burkina Faso for allowing access to the ranch and animals of Samandéni, Mr. T. Palé for technical assistance, Mrs. S. Adjibadé for typing the manuscript and Mr. R. Lacotte for the artwork. This work was supported by the "Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH" PN 77.2227.5 and the "Institut d'Elevage et de Médecine Vétérinaire des Pays Tropicaux, département du Centre de Coopération Internationale en Recherche Agronomique pour le Développement (IEMVT-CIRAD)", Maisons-Alfort, France.

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