Trypanotolerance, an option for sustainable livestock production in areas at risk from trypanosomosis

G.D.M. d'leteren (1), E. Authié (2), N. Wissocq (1) & M. Murray (3)

(1) International Livestock Research Institute (ILRI), P.O. Box 30709, Nairobi, Kenya
(2) Seconded to International Livestock Research Institute (ILRI) by the Centre de coopération internationale en recherche agronomique pour le développement (CIRAD), and supported by the Institut français de recherche scientifique pour le développement en coopération (ORSTOM), France
(3) Department of Veterinary Clinical Studies, University of Glasgow Veterinary School, Bearsden, Glasgow G61 1QH, United Kingdom

Summary
Trypanosomosis is one of the major constraints on animal production in areas of Africa which have the greatest potential for significant increases in domestic livestock populations and livestock productivity. While the eradication of trypanosomosis from the entire continent is an unrealistic goal, considerable effort has been invested in the control of this disease through the use of trypanocidal drugs, management of the vector and exploitation of the genetic resistance exhibited by indigenous breeds. There is little hope that a conventional, anti-infection vaccine will be produced in the near future. Drug resistance is developing faster than generally thought. The control of the tsetse fly has been attempted over many decades. The decreasing efficacy of available trypanocidal drugs and the difficulties of sustaining tsetse control increase the imperative need to enhance trypanotolerance through selective breeding, either within breeds or through cross-breeding.

Trypanotolerance has been defined as the relative capacity of an animal to control the development of the parasites and to limit their pathological effects, the most prominent of which is anaemia. A major constraint on selection for trypanotolerance in cattle, for both within-breed and cross-breeding programmes, has been the absence of practical reliable markers of resistance or susceptibility. Distinct humoral immune response to trypanosome infection is the major feature of bovine trypanotolerance. The role that these responses play in the control of infection or disease is being addressed by ongoing research, but remains a matter of speculation at present.

Results in recent years have shown that packed cell volume (PCV) in particular and parasitaemia, the two principal indicators of trypanotolerance, are strongly correlated to animal performance. However, although direct effects of trypanosome infections on PCV and growth are obvious, more sensitive diagnostic methods for reflecting parasite control are required so that individual animals can be categorised reliably for their parasite control capability. One key finding is the major contribution made by each of the indicators evaluated to the overall trypanotolerance variance. Preliminary genetic parameters for PCV provide evidence that trypanotolerance is not only a breed characteristic but is also a heritable trait within the N'Dama population; this brings new opportunities for improved productivity through selection for trypanotolerance. More reliable estimation of genetic parameters of the indicators may well show that these parameters must be handled simultaneously for optimal progress. This would require diagnostics for assessing parasite control capability that identify trypanosome species more accurately, especially in mixed infections.
A major advantage of trypanotolerant livestock, particularly N'Dama cattle, is the resistance or adaptation of this breed to many of the important pathogens which prevail in the sub-humid and humid tropics. Research on practical indicators of resistance to these conditions will be required to establish relevant integrated strategies based on disease-resistant livestock. Selective breeding will require the integration of the traits that farmers hold important for their production systems.

Keywords
Breeding - Cattle - Disease resistance - Genetics - Immune response - Naturally acquired immunity - Parasites - Production - Trypanosomosis - Trypanotolerance - Tsetse.

Introduction
The African continent is faced with the challenge of satisfying a dramatic increase in demand for livestock products, in particular for milk and meat. Domesticated species play an important role in supporting human populations and in generating income and economic activity. The sub-Saharan African domestic ruminant population is composed of 162 million cattle, 127 million sheep and 147 million goats. Africa has 11% of the total world cattle population and 26% of the world small ruminant population. Eighty-two percent of the total livestock biomass in Africa is ruminant, thus the ruminant population forms the most important group on the continent (117).

The areas with the greatest potential for significant increases in livestock population and livestock productivity are the sub-humid and the non-forested parts of the humid zones. The sub-humid zones, which contain 22% of the land, account for 20% of the ruminant population, whereas the humid zones, which cover 19% of the land, account for only 6% of the ruminant population. Although the area suitable for grazing is not included, the non-forested part of the humid zone has a reservoir of biomass that only ruminants would be able to utilise. Thus, large areas of natural grassland could be exploited better to support the increasing demand for livestock products if constraints to - and opportunities for - their increased contribution to market economies were understood and overcome. Animal diseases, particularly those caused by parasites, are major constraints to animal production in these areas, and trypanosomosis is arguably the most important of these. Jahnke et al. considered that a total increase in cattle of 33 million heads might be possible and would lead to an additional production of 495,000 metric tonnes of meat per year (assuming productivity of 15 kg/head/year) and an increase in milk production of 1.26 million metric tonnes per year (using estimates of 38.3 kg milk/head/year) if eradication or sustainable control of trypanosomosis were achieved over the entire tsetse fly-affected area (7 million km²) in the sub-humid and humid zones (50). The potential benefit of sustainable control of trypanosomosis is considerable for the 40 countries in Africa affected by this disease.

Pathogenic species of salivarian trypanosomes are present throughout vast areas of Africa, Asia, Latin America and the Middle East, and these cause disease in cattle, sheep, goats, water buffalo, pigs, equidae, camels, wildlife and man. Recent relevant reviews that should be consulted include those by Murray et al. (72, 73). In Africa, the major pathogenic trypanosome species for livestock are transmitted by the tsetse fly (genus Glossina) and include Trypanosoma congolense, T. vivax, T. brucei brucei and T. simiae. The subspecies of T. brucei, T. b. rhodesiense and T. b. gambiense cause sleeping sickness in man. Non-tsetse fly-transmitted forms of trypanosomosis also occur in Africa, as well as in the Middle East, Asia and Latin America. The most important pathogen, under these circumstances, is T. evansi. This parasite can cause severe disease in horses and camels and can lead to significant losses in production and performance in cattle and water buffalo (72). The fact that domestic animals and wildlife also act as reservoir hosts for the human pathogens T. b. rhodesiense and T. b. gambiense and that trypanosomosis in humans is an important health constraint to rural development in large areas of Africa, must also be noted. No other continent appears to be dominated by one disease to the same extent that Africa is dominated by tsetse fly-transmitted trypanosomosis. This disease not only results in severe losses in production in domestic livestock due to poor growth, weight loss, low milk yield, reduced capacity for work, infertility and abortion, but also impairs the development of animal agriculture in zones which constitute 41% of the land but which carry only 26% of the ruminant population. The annual loss in meat production alone was estimated at United States (US) $5 billion in 1984 (70), but this figure excludes milk, hides and mixed agriculture. In Africa, 80% of traction power is non-mechanised. A six-fold increase in agricultural output as a result of the availability of a draught ox to a family unit has been calculated (62). Furthermore, the manure provided by livestock is essential for the production of food and cash crops and is a potential source of energy in the form of biogas. In view of this, and despite the continental impact of the disease, assessment of the global impact of
trypanosomosis is difficult and costly, due to the need to carry out such impact studies at the production system level to take into consideration the many complex interactions that affect farm outputs and in which trypanosomosis risk is only one component: hence the scarcity of available, reliable quantitative indicators of the direct and indirect impact of trypanosomosis and of its control.

Many factors indeed contribute to the magnitude of the problem of African trypanosomosis. One major factor is the complexity of the disease itself: for example, the multiplicity of species of trypanosomes which cause the disease, either individually or jointly. These trypanosomes are transmitted cyclically by the tsetse fly, of which there are some 36 species and subspecies, each adapted to different climatic and ecological conditions (39). While the tsetse fly is not the only vector of African trypanosomes, cyclical transmission of infection represents the most important problem because the tsetse fly, once infected, remains infective for a long period, in contrast to the ephemeral nature of non-cyclical transmission. At the same time, trypanosomes infect a wide range of hosts, including wild and domestic animals. The former do not suffer severe clinical disease but become carriers and constitute an important reservoir of infection. The success of the trypanosome as a parasite is due largely to the ability to undergo antigenic variation, i.e., to change a single antigen types expressed by a single parasite, each trypanosome species comprises an unknown number of different strains or serodemes, all capable of elaborating a different repertoire of variable antigen types (VATs) (114).

**Options for trypanosomosis control**

Besides the complexity of the disease and its epidemiology, other factors contributing to the failure to contain and reduce the problem of trypanosomosis include the enormous geographical area affected and the limitations of methods currently available for extensive control. The sustained public efforts and resources required to achieve definitive solutions for large-scale vector eradication are paradoxical in an era when the public sector is less involved than ever before (as a result of the privatisation of animal health/veterinary services), and when sustainability is increasingly believed to rely on private initiative and resources. Recent reviews of trypanosomosis control elaborated on the possible causes of the difficulties encountered in the sustainable economic development of trypanosomosis-affected areas (42, 72, 73, 83). While the eradication of trypanosomosis is an unrealistic goal for most of Africa, considerable effort has been invested in control of this disease by the use of trypanocidal drugs, management of the vector and exploitation of the genetic resistance exhibited by indigenous breeds, such as N'Dama cattle and Djallonké sheep.

The use of trypanocidal drugs is well established and represents the most widely adopted approach to control trypanosomosis. However, estimates for 1984 revealed that only 25 to 30 million curative or prophylactic treatments are given every year for the 48 million cattle exposed to trypanosomosis; thus there is scope for increased use (70). Considering the number of recent reports of trypanosome resistance to current drugs, drug resistance can be assumed to be developing faster than generally thought. Not only individual cases are recognised (83), but regional distribution is increasingly reported in East and West Africa (16, 29, 86). For example, in an area of northern Côte d'Ivoire where tsetse fly was controlled, D'Eternet et al. (29) demonstrated possible resistance after a screening of recurrent infections following the systematic treatment of infected animals. There was evidence for resistance to the three most commonly used trypanocidal drugs (diminazene acetate, isometamidium chloride and homidium bromide), not only in cattle resident in the north of the country but also in cattle that had been imported earlier from southern Mali and southern Burkina Faso. Both T. congolense and T. vivax expressed resistance to these three drugs. In the tsetse fly-controlled area of northern Côte d'Ivoire, the control of the residual T. vivax population might thus be difficult (19). Further south, in areas where N'Dama cattle are predominant, there was less evidence of resistance to the three trypanocidal drugs, except in one isolated herd where a large number of relapses occurred in relation to homidium bromide, although without influence on cattle health and performance. The ability of the N'Dama to effect a self-cure may have prevented the detection of drug-resistant trypanosomes in the bloodstream of these cattle (29). As there appears to be little hope for developing new trypanocidal drugs to benefit smallholder farmers in the short term, major consideration is now being given to better use of the existing drugs, with appropriate guidelines for delaying the development of drug resistance (40). Given the actual or potential problem of drug resistance in many areas, drug usage clearly cannot be relied upon continuously as the sole method of trypanosomosis control.

Attempts to control tsetse flies have been made over many decades. Initially, these attempts included eradication of wildlife, clearing of fly barriers to prevent the advance of the vector, widespread bush clearing to destroy breeding habitats, and ground and aerial insecticide spraying. The release of sterile males is still used for the eradication of tsetse flies in isolated areas. The main method currently employed to control tsetse flies is the use of synthetic pyrethroid insecticides to impregnate traps and screens, baited or not with odour attractants (52). More recently, live animals which have been impregnated through spraying, dipping or by pour-on treatments have been used as live targets (13). The last approach offers a major possibility of reducing
expressed that, as resistance of ticks to these pyrethroids is public-private good (94). However, fears are already being control (12) and may be well accepted by farmers as a mixed maintenance costs and of combining tick control with tsetse flies. In some countries, pyrethroids have already been banned for use in tick control (E.M. Nevill, personal communication), and therefore cannot be employed for tsetse fly control. Pharmaceutical industries set major priorities for developing drugs which are active on ectoparasites, but action on tsetse fly is considered only as an interesting side effect. Indeed, none of the recently developed drugs which act on ectoparasites and tsetse fly were targeted primarily for tsetse fly-control: the effect on tsetse fly was often explored long after these drugs had been registered as acaracidal drugs. The limits of these techniques still need to be assessed before a wider adoption is promoted. Holmes reviewed some of the factors which determine the probability of success: for example, cattle densities required to ensure effective control, the scope for treating only a proportion of the cattle, the level of fly-cattle interactions as measured by fly blood meal analyses, etc. (42).

One of the major components of sustainability of the recently developed tsetse fly control methods is the active participation of the majority of communities contributing to a relevant production system in a given environment or region. In this perspective, major research efforts are being conducted into the development of methodologies for reliable prediction of the willingness of traditional farmers to contribute to the different possible trypanosomosis control methods, either as private or public goods (95). Major economic incentives are thus required for these techniques to be accepted by farmers for collective action, compared to methods of a more private nature, such as the use of curative or prophylactic drugs or trypanotolerant livestock. If part of the relative success of the live bait technology can indeed be attributed to the semi-private nature of such an undertaking, the very private nature of breed choices and breeding strategies for parasite/disease resistance traits can explain the major success of trypanotolerant cattle as a component of, or as a single option for, livestock production under trypanosomosis risk. The exploitation of disease resistance traits to trypanosomosis and dermatophilosis – two very important diseases of humid Central and West Africa – has certainly been seen by farmers as an integral part of their production approaches to the alleviation of disease constraints for many decades (28).

Trypanotolerant livestock, an option for sustainable livestock production

Trypanotolerance, the ability of some livestock breeds to survive, reproduce and remain productive under trypanosomosis risk without the aid of trypanocidal drugs, was recognised and exploited by farmers long before research on trypanotolerance began. The exploitation of trypanotolerant breeds is practised as a major (if not the only) option for sustainable livestock production in nineteen countries in the most humid parts of West and Central Africa. In eleven countries, trypanotolerant cattle (mainly N'Dama) were either moved into the highest risk areas or were imported from other countries. For example, in countries where policy encouraged the exploitation of N'Dama cattle, there was an increase in numbers of this breed between 1979 and 1985 at an annual rate of 9%. There are now N'Dama herds in nearly all West and Central African countries; these could be the source of genetic material for further dissemination (28). Trypanotolerance in cattle is well documented, particularly in N'Dama cattle, the most numerous trypanotolerant breed, and in the West African shorthorn. While significant differences in resistance to trypanosomosis occur also among various zebu (Bos indicus) types (31, 47, 74, 78), most Bos indicus cattle in tsetse fly-infested areas require regular treatment or are found only on the fringes of fly belts. Exotic breeds cannot be maintained even in areas of low tsetse fly risk without intensive trypanocidal drug therapy and veterinary care.

There is a continued perception that because of their small size, trypanotolerant livestock are less productive than other breeds (42). The International Livestock Centre for Africa (ILCA) demonstrated, however, that in areas where the tsetse fly risk was low or zero, the productivity of N'Dama and West African shorthorn cattle was equal to that of the physically larger trypanosusceptible zebu (44). As relevant long-term contemporary breed comparisons are not usually available, an approach developed by ILCA was used in two in-depth productivity studies carried out in two production systems with similar beef-orientated objectives (37, 104). Cattle production efficiency was expressed by relating total annual output back to the unit of cow metabolic weight, and herd productivity was expressed by the cow productivity index multiplied by cow viability (44). Using this approach, herd productivity of N'Dama cattle raised under very high
trypanosomosis risk was demonstrated to be equal to the herd productivity of grade Boran cattle also maintained under high disease risk but with permanent chemoprophylaxis (137.3 kg of 8-month weaner calf produced per 100 kg N'Dama cow metabolic weight per herd per year versus 137.5 kg for the Boran cow herds). Trypanotolerant N'Dama cattle thus compare very well with Boran cattle, which are regarded as one of the best beef cattle breeds in Africa, with the added advantage that N'Dama cattle are not dependant on trypanocidal drugs, whereas Boran cattle would not survive without the drugs. Similarly, Agyemang et al. demonstrated that when milk extracted from N'Dama cattle for human consumption was taken into consideration, their overall productivity was superior to that of zebu breeds maintained under similar traditional systems in the absence of tsetse fly challenge (1).

While it is generally accepted that trypanotolerance as a breed characteristic is under genetic control, there is evidence that the stability of trypanotolerance can be affected by environmental factors, such as overwork, intercurrent disease and repeated bleeding, pregnancy, parturition, suckling and lactation. Probably the single most important factor is nutrition. In the first large-scale attempt to evaluate the effect of trypanosomosis risk (defined rather subjectively, but using relevant information available at that time) on performance of N'Dama and West African Shorthorn cattle at 30 different locations, research performed by ILCA, the Food and Agriculture Organisation and the United Nations Environment Programme demonstrated that although these breeds remain productive under trypanosomosis risk, their outputs were affected by increasing risk (44).

Later studies attempted to provide more accurate evaluation of the links between trypanosomosis risk components and animal performance. In the study cited earlier in this paper, Feron et al. compared the productivity of herds of N'Dama cattle maintained in the different ranches situated in low- and high-risk areas (37). Reproductive performance differed by 15.8%, with 85.7% in the lower risk areas and 74.0% in the higher risk areas, respectively. In studies where disease situation was investigated more accurately within the herds by regular examination for parasites, the infection frequency of individual animals was used to study the direct effect of parasite infections on performance. For example, in a traditional village production system where feeding constraints are common, Agyemang et al. demonstrated that cows which had been detected as parasitaemic once or twice during the post-partum period had a 56% calving percentage, compared with 63% for those never detected as parasitaemic, and six month lactation milk offfakes of 140 kg and 176 kg, respectively (1). In a beef-oriented system located in one of the highest tsetse fly/trypanosomosis risk areas recorded by ILCA (46), Trail et al. demonstrated a higher growth rate of 13.5% for post-weaners which had never been detected as parasitaemic over a two year period compared to those that had been detected as parasitaemic (175 g/day versus 155 g/day) (113).

However, conclusions should not be drawn too rapidly on the general relationships between performance and risk measured only by non-species-specific trypanosome detection. Indeed, trypanosome species, and their relative pathogenicity in given geographical areas, are among the many factors influencing animal susceptibility to the parasites/disease and the effect of disease on productivity. A good example is a survey of 186 breeding cows (with a total of 436 completed calving intervals) reported by Wissocq et al., which demonstrated that only high levels of T. congolense infection had a significant effect on cow reproductive performance, whereas low levels of T. congolense infection and all levels of T. vivax infection had no significant effect (118). In this particular situation, both trypanosome species had a similar prevalence.

These findings have major strategic implications. In higher risk situations, where tolerant livestock are maintained without the aid of trypanocidal drugs, productivity can be improved by strategic interventions, e.g., by the use of trypanocidal drugs at critical times, through vector control or through enhancing livestock disease resistance by selective breeding. There are very few reports evaluating the effect of trypanocidal drugs or vector control on the productivity of trypanotolerant cattle. Diall et al. (27) indicated that under high trypanosomosis risk in Mali, N'Dama cattle had to be maintained with the support of trypanocidal drugs. In some extensive livestock production systems of central Côte d'Ivoire, the use of a single dose of a prophylactic trypanocidal drug, isometamidium chloride, has been routinely given at weaning to N'Dama calves, although the longer-term benefits of this treatment have not been demonstrated (53). In a study carried out in Gabon, ILCA demonstrated that the use of prophylactic trypanocidal drugs prior to the three-month breeding season was beneficial in improving the calving percentage of cows stressed by lactation from 52% to 64% (45). The cows which benefitted most from the strategic use of a trypanocidal drug were clearly those with the lowest capability to limit the development of anaemia, the latter factor being regarded as the most reliable indicator of the level of trypanotolerance (72). Thus, the profitability of, and justification for, the application of such strategic alternative trypanosomosis control on farms with trypanotolerant livestock can be expected to decrease as the disease resistance levels of animals increase. This observation can be related to a similar interaction reported by Itty et al. on susceptible East African zebu cattle in Kenya, i.e., the change of comparative profitability of two trypanocidal drug regimes - curative diminazene aceturate, or prophylactic isometamidium chloride - as disease risk changed (48). These two examples indicate how much interactions between risk, drugs and treatment regimes, livestock breeds and levels of disease resistance can influence farm outputs and the profitability of interventions.
The evaluation of the interactions between the components of trypanosomosis risk and the different disease control methods deserves further attention in order to design sustainable strategies which are tailored more to the specific needs, constraints and opportunities of individual production systems. There is no one solution that will be valid for all production systems, ecological zones, or regional or national markets.

The decreasing efficacy of the trypanocidal drugs available and the difficulties of sustaining tsetse fly control increase the imperative need for enhancing trypanotolerance through selective breeding, either within breeds or through cross-breeding. This last option is possibly the most appropriate for dairy-oriented production systems (21, 23).

These conclusions support the analysis by Holmes that more integrated strategies need to be developed (42). Livestock production under trypanosomosis risk will have to focus increasingly on the integrated control strategies which are more reliant on trypanotolerant livestock, on methods for increasing disease resistance and/or on improved vector control techniques, and possibly on less (or more careful) use of trypanocidal drugs.

Understanding trypanotolerance

Although farmers have exploited trypanotolerant traits through their breed choices, a major constraint to selection for trypanotolerance, both for within-breed and cross-breeding programmes, has been the absence of reliable markers of resistance or susceptibility. Practical indicators of resistance should arise from a better understanding of the biological mechanisms that underlie trypanotolerance.

Trypanotolerance has been defined as the relative capacity of an animal to control the development of the parasites and to limit their pathological effects, the most prominent of which is anaemia (43, 69, 71). While a general relationship exists between the parasite load and the severity of anaemia, the mechanisms involved in each component of trypanotolerance control of parasite development and mitigation of anaemia appear to be distinct and to operate independently (72, 73). Elucidating these mechanisms and their genetic control has been a major research goal over the past twenty years. Firstly, understanding these mechanisms might lead to identification of markers that could be used in selection for disease resistance. Secondly, this knowledge might provide new means of controlling the disease by conferring to susceptible cattle a similar capacity to that identified in resistant cattle, and by optimising this capacity in trypanotolerant breeds.

Considerable insight into the immunological events which follow trypanosome infection has been gained from the study of rodent models (61). At the same time, the genetic basis of trypanoresistance is being unravelled in inbred strains of mice (54). However, the development of infections, the associated pathology and the immunological events involved differ between laboratory models and the natural disease of cattle. Thus, no rodent model has led to identification of a mechanism that was later confirmed as a contributor to bovine trypanotolerance so far. A good example can be given with tumour necrosis factor-alpha (TNF-α): this cytokine appears to play a key role in trypanoresistance of C57BL/6 mice (F. Iraqi, personal communication), but initial studies carried out in cattle have provided conflicting results and have failed to indicate an involvement of this cytokine in trypanotolerance (89). A detailed review of the immunological events described or suspected to occur in trypanosome-infected mice and cattle has been published recently (98). The current paper will focus on the differences that have been observed in the immune responses of trypanotolerant and trypanosusceptible cattle.

A number of early studies in cattle were carried out in field situations, as reviewed by Authié (5). Although natural challenge gives the best assessment of the ability of an animal to cope with infection and disease, in experiments in which cattle of various origins, unknown history and different physiological status were infected with undefined doses of uncharacterised stocks of parasites the results were often conflicting and thus the mechanisms of resistance remained poorly understood. Over the last ten years, controlled experimental infections with cloned, cyclically transmitted T. congolense have been conducted simultaneously in naïve zebu and Taurine cattle, and comparative studies on the immune responses of these animals were carried out. These experiments have confirmed that, while parasitaemia and anaemia do not greatly differ between the two breeds in the initial stages of a primary infection, the capacity of Taurine cattle to limit parasitaemia and to resist anaemia becomes obvious 30 to 50 days after infection (32, 80). However, very few mechanisms have been identified so far to account for the superior control of parasitaemia and/or anaemia in trypanotolerant cattle.

Recent studies in N'Dama and Boran (zebu) cattle have aimed at dissecting the cellular component of the immune response to trypanosomes, addressing both T-cell and monocyte/macrophage functions (58, 59, 99, 101). Importantly, these studies are improving the understanding of some of the immunosuppressive mechanisms associated with trypanosome infection, whether these mechanisms are beneficial or deleterious to the host (98). However, none of the cellular events identified appear to differ fundamentally between Boran and N'Dama cattle. Studies which focused on the early immunological events at the site of infection, through examination of chancre development in tolerant and susceptible cattle (2) or by cannulation of afferent and efferent lymphatics of the draining lymph node have also failed to reveal differences in the ways in which infection is established, parasite antigens are presented to the immune system or in the cell subsets involved in and the cytokines patterns
triggered by the initial responses (V. Lutje, personal communication).

Since trypanosomes in Africa are mainly intra-vascular and extra-cellular parasites, the major immunological differences associated with trypanotolerance are likely to exist at the level of humoral immunity. The capacity of trypanotolerant cattle to generate sustained antibody responses to trypanosome antigens is probably the most prominent immunological feature that has been identified so far. Following infection, the bovine host develops trypanosome-specific immunoglobulin M (IgM), which is directed to the VSG and to non-variant sub-surface antigens, as well as IgM that is not trypanosome-specific. The trypanosome-specific IgM are transient and their levels are similar in both trypanotolerant and trypanosusceptible cattle (9, 116). A distinct population of IgM consists of antibodies of low specificity, which react with both trypanosome and non-trypanosome antigens (116). These ‘polyspecific’ antibodies, which may involve the auto-antibodies reviewed by Taylor (98), are likely to mediate pathology rather than protection. Interestingly, this group of antibodies has been detected in susceptible Boran but not in trypanotolerant NDama cattle (116).

A trypanosome-specific IgG response is elicited in infected cattle almost coincidentally with the IgM response, and this is predominantly of the IgG1 isotype (9). However, while high and sustained levels of specific IgG1 are detected in NDama, only low and transient IgG1 are detected in Boran cattle (9, 116). The difference is not the mere reflection of differential absorption of antibody by specific antigen present in plasma during infection, but lies at the B cell level (100). The ‘suppression’ of specific IgG in Boran cattle is reversed by drug therapy, which is usually followed by a sharp increase in antibody titres (E. Authié, unpublished findings). As the difference in specific IgG production between tolerant and susceptible cattle appears to increase during rechallenge infections (9), this is likely to be a prominent feature of cattle maintained under field challenge.

The failure of susceptible cattle to develop a sustained IgG response to trypanosome antigens can be considered as one manifestation of immunosuppression, the causes and the consequences of which remain unknown. Of particular interest is the possibility that some of this IgG may play a role in the control of infection. Early studies indicated that more resistant cattle might develop higher levels of neutralising antibodies to trypanosomes during the first peak of parasitaemia than fully susceptible cattle (2, 85). Studies in NDama and Boran cattle exposed to primary infections failed to confirm these findings. If the antibody response to cryptic epitopes of the VSGs shows the isotypic characteristics described above, the antibodies to exposed epitopes (variant-specific antibodies), which are thought to mediate clearance of trypanosomes (41), surprisingly do not follow this rule and do not exhibit higher levels in NDama than in Boran cattle (116). These findings infer that, in primary infections, the superior control of parasitaemia in NDama cattle is unlikely to operate through anti-VSG responses (116). To conclude on this question, however, an examination of the extent and the rapidity of immune recognition of the various VATs of a given repertoire will be necessary, and this is a difficult point to address. In contrast to primary infections, the superior control of parasitaemia in trypanotolerant cattle following secondary (homologous) infections is associated with more efficient anamnestic responses to VATs (81, 115). The hypothesis that acquired immunity contributes to exacerbating resistance in trypanotolerant cattle is in agreement with early field observations (26).

The superior humoral response of NDama cattle may also mediate neutralisation of parasite products that are responsible for pathology, therefore contributing to increased resistance of the host to the disease. Neutralisation can be effected directly, or through formation of antigen-antibody complexes that are cleared by phagocytic cells. Speculation regarding the identity of the parasite factors which cause pathology have been based on their biological effects in vitro and their role in other parasite models, but the determination of the pathogenic role of any single parasite molecule has not been possible.

Many scientists have hypothesised that trypanosomes release immunomodulatory molecules which depress T-cell responses in the infected host. In the mouse model, soluble extracts of trypanosomes have shown a suppressive effect in vitro, either by inducing cluster of differentiation 8* (CD8*) T cells to secrete interferon (IFN)-gamma (11, 22), a cytokine which activates macrophages and suppresses T-cell proliferation, or by directly activating macrophages to release TNF-alpha (60), nitric oxide and/or prostaglandins which are mediators of suppression (98). In trypanosome-infected cattle, mechanisms other than nitric oxide and the release of prostaglandins appear to determine T-cell suppression (100). No immunosuppressive molecule, whether of host or parasite origin, has been confirmed to play a role in bovine trypanosomosis. There is also very little indication of differing cytokine patterns in resistant versus susceptible cattle.

Degradative enzymes, such as proteases and neuraminidases (sialidases), have been considered as potentially harmful to the host (103). Interestingly, the sialic acid content of bovine erythrocytes has been found to decrease during T. vivax infection, thus causing abnormal ageing of the cells and subsequent erythropagocytosis that might contribute to anaemia (34). The erythrocytes of NDama cattle were found to contain more sialic acid than those of zebu cattle, and may thus be less sensitive to damage by parasite sialidases (35, 36). However, no sialidase has been purified and characterised from bloodstream forms of African trypanosomes so far.

Certainly, the best characterised of the trypanosome-degradative enzymes are cysteine proteases (18). One such
enzyme, congopain, which has been isolated from *T. congolense* bloodstream forms (7, 66), is of particular interest due to its hydrolytic activity over a wide range of protein substrates at physiological pH, its presence in the circulation of infected cattle (8, 51) and the fact that it was identified through comparative studies of immune responses in resistant and susceptible cattle. Congopain is highly antigenic during primary infections of N'Dama cattle and in other trypanotolerant breeds, whereas cattle of susceptible breeds develop only transient IgM response followed by low or undetectable levels of specific IgG (8). IgG elicited by trypanotolerant cattle during infection, as well as Fab fragments generated from this IgG, exhibit an inhibitory effect on congopain (Authié et al., manuscript in preparation). Ongoing experiments aimed at generating neutralising antibody through immunisation with recombinant congopain should yield definitive conclusions regarding the pathogenicity of this enzyme, the role of antibody-mediated protease inhibition in trypanotolerance and the potential of congopain-specific antibody to modulate the disease.

Besides having a greater ability to develop specific humoral responses, trypanotolerant cattle have been found to maintain higher complement levels during trypanosome infection than susceptible zebu cattle (6). Hypocomplementaemia is a feature of bovine trypanosomiasis and, in susceptible cattle, patent infection is associated with a severe and continuous decrease in C3 and complement haemolytic activity (55, 77, 88, 96). After three to four weeks of infection, C3 level and complement activity may be reduced to 10% to 20% of normal values in zebu cattle (6). This initial decrease is less pronounced in trypanotolerant cattle, which subsequently tend to regain normal levels. Within the trypanotolerant Boulé breed, a correlation was found between the ability of an individual to maintain complement levels, the ability to maintain packed cell volume (PCV) values and survival under natural challenge (6). Whether the higher complement levels in trypanotolerant cattle result from lower parasite loads, lesser consumption/activation of complement by immune complexes, or from a capacity to produce more complement factors in response to infection, is not known. This phenomenon might, however, have important consequences on the control of infection and disease, since C3 plays a crucial role in the generation of protective immune responses. Opsonisation of trypanosomes by C3 might facilitate subsequent clearance of the trypanosomes by phagocytic cells (23). More generally, C3 is required for antigen processing and presentation (24), for isotype switching from IgM to IgG and for the generation of memory B cells (14). Most of the immunological defects identified in trypanosusceptible cattle and reviewed above might, thus, be related to the drastic C3 depletion that these animals experience early in infection.

The evidence of a role for immune mechanisms in trypanotolerance does not rule out the contribution of other non-immunological mechanisms reviewed by Authié (5). As suggested by studies on resistance to trypanosomiasis in wildlife, serum factors other than antibodies may conceivably modulate the viability, multiplication or differentiation of the parasites (67). However, no such factor has been identified in the serum of either susceptible or tolerant cattle that could explain the difference in parasite loads between the two breeds. The intrinsic capacity of the bone marrow to respond to anaemia by efficient haematopoiesis should also be considered. Recent studies in *T. congolense*-infected cattle have provided evidence of an early bone marrow impairment in susceptible Boran cattle and also indicated that N'Dama cattle may compensate more effectively than Boran cattle for the degree of anaemia (3, 4). Finally, the differential attractiveness of cattle for tsetse flies remains a pending question (38, 56).

In summary, distinct humoral immune responses to trypanosome infection are the major feature of bovine trypanotolerance. The role that these responses play in the control of infection or disease is being addressed by ongoing research, but remains at present a matter of speculation. The capacity to mount highly efficient secondary responses must be considered as an important contributor to the overall level of resistance that trypanotolerant cattle express in the field, and a clearer understanding of trypanotolerance would be gained by investigating memory immune responses at both the humoral and cellular levels. The immunological observations made so far, particularly regarding antibody responses and complement factors, might be useful for defining new markers of trypanotolerance. Their relevance under field situations, however, remains to be confirmed. In order to evaluate the immunological parameters associated with trypanotolerance, the development of practical tests that can be used on a large scale, such as enzyme-linked immunosorbent assay (ELISA), is necessary.

Finally, the possibility of finding a solution to the enigma of bovine trypanotolerance through the molecular genetic approach should be mentioned (102). However, the search for genetic markers, and subsequently for the genes controlling resistance, is hampered by the difficulty of phenotyping trypanotolerance accurately under experimental conditions. PCV, parasitaemia and performance, the currently available indicators that have proved useful in the field, might not be powerful enough to reveal the maximum of the variation observed in the field during a single experimental primary infection of limited severity. Thus, the correlation between natural and experimental fly challenge, questioned by Pinder et al. (84), will have to be studied further and the experimental conditions for optimising the methods of phenotyping trypanotolerance determined. There are also complex interactions between the genotype and the environment that make the interpretation of genetic analysis difficult. However, continued efforts towards understanding the phenomenon and the pathogenesis of bovine trypanotolerance remain vital, since better exploitation of trypanotolerant cattle, as well as alternative control strategies...
for trypanosomosis, in the long term, will be likely to arise from both immunological and genetic approaches.

From understanding to better exploitation

Further to understanding the mechanisms underlying trypanotolerance, the exploitation of resistance traits relies on the characterisation of these traits in the field and their practical measurement. The successful use of any criteria for identification of trypanotolerant breeds of cattle and/or superior animals within these breeds depends on the practicality of their measurement, on the strength of the linkage of the criteria with the economically important production traits such as viability, reproductive performance and growth, and on the associated genetic parameters.

In susceptible livestock, trypanosomosis is known to have a dramatic effect on reproductive performance – one of the most economically important components of productivity – through abortion and long post-partum anoestrus. Despite initial observation by Lorenzini et al. that artificially infected Boran cattle stopped cycling while N'Dama did not (57), the reasons why trypanotolerant cattle are much less affected than susceptible zebu cattle have not been investigated fully.

Quantification of the relative importance for different age groups of the phenotypic and genetic components of each parameter in a range of trypanosome infection situations is a pre-requisite to the practical and sustainable utilisation of such parameters. Experimental and field studies have clearly demonstrated that trypanotolerance is associated with the capacity to control the intensity, prevalence and duration of parasitaemia and the capacity to resist the development of severe anaemia, as measured by PCV.

In field studies, the degree of anaemia can be quantified easily by measuring PCV. Although the measurement of PCV is very accurate, the biological interpretation of PCV variation in the field is meaningful (as far as accurate trypanotolerance measurement is concerned) only if other factors affecting PCV levels are well identified, quantified or controlled.

In contrast, the degree of parasitaemia is not so easily quantified, and quantification has depended on demonstration of trypanosomes in peripheral blood by parasitological techniques. The most sensitive practical field approach has been to detect the presence of trypanosomes by the dark ground/phase contrast buffy coat technique (BCT) (68) and to quantify the intensity of the infection as a parasitaemia score (82). Although this method has contributed to major progress in characterising trypanotolerance in the field, parasite detection techniques have limitations. A high proportion of infections go undetected, as many are chronic. In addition, parasitaemia often fluctuates markedly and may be below the limit of detection, particularly in trypanotolerant livestock.

Relation between criteria of trypanotolerance and livestock performance

Results in recent years have shown that PCV in particular and parasitaemia, the two principal indicators of trypanotolerance, are strongly correlated with animal performance, especially post-weaning growth, reproductive performance and overall cow productivity (28, 109, 111, 113).

In a ranch in Mushie, Democratic Republic of the Congo, N'Dama cows raised under a high natural tsetse fly/trypanosomosis challenge were recorded over their calving intervals for several years. Their offspring were also recorded from birth until placement in different weaning herds where they were kept until between 24 and 36 months of age. Regular blood samples (either monthly or two-weekly) were examined to detect the degree of anaemia and the presence of trypanosomes, the species of trypanosomes and score. Other possible causes of anaemia were controlled; control of ticks was conducted by weekly dipping and that of internal parasites was achieved through a pasture management system involving extensive grazing, the absence of night paddocks and regular burning of pastures.

In one study of 64 cows monitored for 3.5 years, the comparative influences of frequency of parasitaemia, parasitaemia intensity and PCV were measured in relation to calving interval, calf weaning weight and cow productivity (weight of weaner calf per cow per annum) (106). Cows more frequently detected as parasitaemic had a 14% shorter calving interval and a 15% higher productivity than contemporaries that were parasitaemic less frequently. The effects of parasitaemia intensity were not significant. Animals maintaining a high PCV had an 11% shorter calving interval, a 9% heavier calf weaning weight and a 24% higher cow productivity than those maintaining a low PCV value. Simultaneous evaluation of the relative effects of PCV and parasitaemia – in both the preweaner calf and dam – on calf performance (weaning weight), showed that calf PCV values were at least as important as dam PCV values. Monthly trypanosome prevalences were similar in calves and dams (9.2% and 9.5%, respectively). Calves which were able to maintain a high PCV value when highly parasitaemic had a weaning weight which was 9.9 kg (7.7%) higher; similarly, a dam maintaining a high PCV had an 11% shorter calving interval and a 15% higher productivity than contemporaries (9.2% and 9.5%, respectively). Calves which were able to maintain a high PCV value when highly parasitaemic had a weaning weight which was 9.9 kg (7.7%) higher; similarly, a high PCV value measured in the dam increased the calf weaning weight by 8.8 kg (6.1%).

In another study quoted earlier, in which the same basic protocol was implemented over the lifetime careers of 186 breeding cows having completed a total of 436 calving intervals, Wissocq et al. reported the effect of the same criteria on time to conception (118). The species of trypanosomes detected were included in the analyses. An effect of
Trypanosome infection on time to conception was only significant when two or more T. congolense infections were detected during the eight-month post-partum period, whereas there was no significant effect with either a low level of T. congolense infection or any level of T. vivax infection. The times to conception (including standard error) were as follows:

- no infection: 156 ± 16.1 days
- one T. vivax infection: 174 ± 26.5 days
- one T. congolense infection: 172 ± 20.3 days
- two or more T. congolense infections: 214 ± 22.4 days.

Thus, the inclusion of trypanosome species in the analysis allowed the identification of the relationship between species, level of infection and effect on reproductive performance. Similarly, only when cows were highly infected by T. congolense during the eight months post-partum period was a significant relationship between average PCV and time to conception observed within groups of cows, classified according to the numbers and species of trypanosomes. In this case, trypanosome effect on conception was strongly associated with anaemia control: there was indeed a significant reduction of 15.3 days in time to conception per unit increase in average PCV. This study highlighted the importance of determining trypanosome species for studying relationships between infection, anaemia and performance.

Approaches to measuring parasite control aspects more accurately

Initially, control of anaemia development (as measured by average PCV value) appeared to be the criterion of trypanotolerance most closely linked to overall productivity, in production systems where other possible causes of anaemia were systematically controlled. However, although the direct effects of trypanosome infections on PCV and growth are obvious, a more sensitive method than BCT for reflecting parasite control is required so that individual animals can be reliably categorised for parasite control capability, as a high proportion of infections (particularly in trypanotolerant livestock) are not detected by BCT analysis. The importance of parasite control in the assessment of disease resistance has been well documented in tick (90) and helminth (10) resistance research, with a high level of genetic control sometimes demonstrated (92). Based on these relative difficulties, further work has been carried out to explore other approaches to the characterisation of parasite control using newly developed diagnostic techniques. One assumption was that trypanosome antigen detection tests would give a better indication of an ongoing infection than the BCT analysis. Initial work evaluated the possible contribution of antigen-detection to measure the infection control component of trypanotolerance more accurately. Animals which gave positive results for parasite presence by the antigen ELISA technique, but which were able to control parasites below a level detected by BCT, might be more tolerant than those which gave positive results for both tests (109, 110). Another justification of the work came from the demonstration that, while PCV can be measured accurately in the field, the precise use of PVC as a trypanotolerance criterion (after other possible causes of anaemia had been controlled systematically) depended greatly on the accuracy of measurement of trypanosome infection status.

A situation with high natural challenge was therefore identified, with large numbers of post-weaners of both sexes and a range of ages maintained, in different pastures/herds representing different levels of disease risk. These animals were monitored regularly for PCV, parasitaemia, antigenaemia, the number of trypanocidal drugs required and weight. Other anaemia-causing pathogens were controlled as described earlier. Animals with severe clinical indications of trypanosomosis were treated with diminazene accurate at 3.5 mg/kg. Trypanosome antigens were detected by ELISA techniques according to Nantulya and Lindqvist (75, 76), the only test available for use on a large scale at this time.

In one study involving 568 animals, 69.7% showed at least one case of parasitaemia during the period, whereas all showed antigenaemia. The average proportion of time for which an animal had detectable parasitaemia over the 21-week period was 11%, while for antigenaemia the proportion of time was 69% (119). Looking separately at the direct independent effects of both parameters on anaemia and growth, there was a very significant effect of detected parasitaemia on both PCV and growth: animals that had never been infected had higher PCV (30.0% versus 27.7%) and gained on average 64 g more per day (180 g/day versus 116 g/day) than those which had been infected. Significant relationships between antigen detection and average PCV and daily weight changes over the test period were also observed: animals with a below-average proportion of detected antigenaemia had a 2.5% unit higher PCV (29.8% versus 27.8%) and gained 38 g more per day (159 g/day versus 121 g/day) than those with an above-average proportion.

To analyse the effect of antigenaemia on PCV and weight gain, analyses of covariance, stratified by parasitaemia status, were conducted for both parasitaemic and non-parasitaemic animals. There were significant decreases in PCV and weight gain with each unit increase in antigenaemia (Table I).

Thus, the serial antigen tests carried out over the 21-week period proved to be useful in trypanotolerance research and to determine the relationships between parasitaemia and antigen tests. Such testing allowed determination of the antigen detection patterns of an animal through which the infection status of that animal could be classified more accurately. The testing also indicated that these patterns were associated to a significant extent with anaemia, the other trypanotolerance criterion, and with performance as indicated by growth rate.
parasitaemic in one herd, 54.2% in the second herd and prevalence, ranged from 28.8% of animals detected as were monitored weekly over a 21-week period in three herds infections. However, this finding needs to be confirmed in no corresponding significant decrease in proportion of time animal age in the two higher trypanosomosis risk herds, but parasitaemic with T. vivax, corresponding to increasing the proportion of time for which animals were detected as 90.6% in the third herd. There were significant decreases in trypanosomosis risk, as measured by trypanosome infection and the possible linkage between this infection, but apparently not following T. congolense. This is regarded as strong evidence for the ability of more resistant to, infections of both T. vivax and T. congolense. There were no significant age trends in the proportion of time detected as parasitaemic with either species of parasite in the lowest trypanosomosis risk herd. The proportion of time for which post-weaners of different age groups were detected as parasitaemic with each trypanosome species was estimated and the trends showed major differences in the proportions of T. vivax and T. congolense found at 12 months of age compared to those found at 45 months. The change in T. vivax:T. congolense proportions was most marked in the herd under the highest trypanosomosis risk.

The importance of the ability to acquire resistance to specific aspects of trypanosome infections

The evolution of the pattern of trypanosome infections over time was evaluated in calves from birth to maturity and thereafter in the different age groups of their dams (112). One month after weaning, animals were infected with both T. vivax and T. congolense. From that time until 42 months of age, the proportion of time for which an animal was infected with T. vivax gradually decreased in relation to T. congolense infection levels. In the dams, this trend continued from four years to at least eight years of age, by which time the level of T. vivax infection was only one-third of that of T. congolense infection. This is regarded as strong evidence for the ability of N'Dama cattle, in this region of Africa, to acquire significant control of the development of parasitaemia following T. vivax infection, but apparently not following T. congolense infections. However, this finding needs to be confirmed in other areas. The data also confirmed the belief that pre-weaner calves grazing with their dams are more protected from, or more resistant to, infections of both T. vivax and T. congolense.

The importance of the ability to acquire resistance to trypanosome infection and the possible linkage between this ability, anaemia control and growth of trypanotolerant N'Dama cattle was further assessed by Wissocq et al. on 464 post-weaner N'Dama cattle aged from 12 to 24 months, which were monitored weekly over a 21-week period in three herds exposed to different levels of risk (119). The levels of trypanosomosis risk, as measured by trypanosome prevalence, ranged from 28.8% of animals detected as parasitaemic in one herd, 54.2% in the second herd and 90.6% in the third herd. There were significant decreases in the proportion of time for which animals were detected as parasitaemic with T. vivax, corresponding to increasing animal age in the two higher trypanosomosis risk herds, but no corresponding significant decrease in proportion of time for which animals were detected as parasitaemic with T. congolense. There were no significant age trends in the proportion of time detected as parasitaemic with either species of parasite in the lowest trypanosomosis risk herd. The proportion of time for which post-weaners of different age groups were detected as parasitaemic with each trypanosome species was estimated and the trends showed major differences in the proportions of T. vivax and T. congolense found at 12 months of age compared to those found at 45 months. The change in T. vivax:T. congolense proportions was most marked in the herd under the highest trypanosomosis risk.

The linkages between animal age and growth whilst parasitaemic with T. vivax, T. congolense or a mixture of the two parasites in animals ranging from 12 to 24 months of age under a high level of trypanosomosis risk were further evaluated, and the results are indicated in Table II.

With increasing age, the effect of T. vivax parasitaemia on animal growth was reduced significantly in comparison to the effect of T. congolense parasitaemia. Thus, not only is the ability to acquire some control of the development of parasitaemia over time following a T. vivax infection confirmed, but the deleterious influence of this parasite on animal performance also appears to be reduced. Further research will have to clarify if, and how, these findings could be put to practical use in selection decisions for trypanotolerance levels.

<table>
<thead>
<tr>
<th>Parasitaemia status</th>
<th>No. of animals tested</th>
<th>Packed cell volume (%) ± standard error</th>
<th>Growth (g/day) ± standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression in animals not detected as parasitaemic (−)</td>
<td>229</td>
<td>−0.18 ± 0.044</td>
<td>−3.5 ± 1.8</td>
</tr>
<tr>
<td>Regression in animals detected as parasitaemic (+)</td>
<td>339</td>
<td>−0.33 ± 0.034</td>
<td>−5.3 ± 1.37</td>
</tr>
</tbody>
</table>

Subsequent work will explore the importance of the determination of trypanosome species antigen detection as a predictor of tolerance and for estimating anaemia control more accurately. In addition, as more data become available the interactions between parasitaemia, antigenaemia, anaemia control and growth can be explored more fully.

Ability to acquire resistance to specific aspects of trypanosome infections

The genetic components of these trypanotolerance measurements are being determined progressively using a qualitative approach in longitudinal studies of well-monitored production systems, in which the necessary identification and/or quantification of environmental and genetic effects are possible. Blood typing for parentage identification and/or quantification of environmental and well-monitored production systems, in which the necessary measurements are being determined progressively using a quantitative approach in longitudinal studies of trypanosomosis risk.

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Heritability values for, and genetic and phenotypic correlations between, growth, average PCV and lowest PCV over a three-month testing period have been reported by Trail et al. for N'Dama cattle under moderate challenge (107). When all environmental and parasitaemia information was

Table I
Analysis of covariance of changes in average packed cell volume (%) and growth (g/day) per unit change in antigenaemia stratified by parasitaemia status

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Genetics of trypanotolerance measurements

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Table II
Least squares regression of daily liveweight change on age in months when detected as parasitaemic with *Trypanosoma vivax*, *T. congolense* or a mixture of both

<table>
<thead>
<tr>
<th>Infection type</th>
<th>Number of animals</th>
<th>Parasitaemia measures in model ± standard error</th>
<th>Anaemia control added to parasitaemia measures in model ± standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. vivax</em></td>
<td>43</td>
<td>6.92 ± 2.09 (a)</td>
<td>6.18 ± 2.05</td>
</tr>
<tr>
<td><em>T. congolense</em></td>
<td>29</td>
<td>−2.15 ± 3.00</td>
<td>−0.98 ± 3.00</td>
</tr>
<tr>
<td><em>T. vivax</em>/<em>T. congolense</em> mixture</td>
<td>92</td>
<td>2.04 ± 1.37</td>
<td>2.31 ± 1.35</td>
</tr>
</tbody>
</table>

(a) Regressions of daily liveweight change on age in months differ significantly between *T. vivax* infections and *T. congolense* infections (P < 0.05)

Quantification of the relative importance of trypanotolerance criteria

With the selection criteria for trypanotolerance already available, or in the process of becoming so, the design of selection programmes is thus becoming possible. Having established how such criteria relate to all other economically important traits for given production systems, the next step will be to establish the most appropriate relative weightings between these criteria and all economically important traits, based on economic importance, heritability and phenotypic and genetic correlations, in order to develop appropriate and relevant selection indices.

Quantification of the relative importance of trypanotolerance indicators requires longitudinal studies in well-characterised production systems with the simultaneous recording of all possible criteria along with the relevant production parameters.

A first such study on 255 N'Dama post-weaners (monitored with the standard protocol described above) has allowed the simultaneous quantification of four indicators of trypanotolerance. Those were as follows: species of trypanosome detected; length of time parasitaemic; parasitaemia score; and anaemic condition estimated by PCV (113). Each animal was recorded over a two-year period from weaning, at 10 months of age, to 34 months of age. Curative trypanocidal drug treatment was given when an animal had severe clinical signs of trypanosomosis. This examination did not take place at sampling times and was unrelated to infection and PCV measures. A single dose of diminazene aceturate at the rate of 3.5 mg/kg body weight was used. Animals were weighed, on average, once every six weeks.

The relative effects, on trypanocidal drug requirements and growth, of changes in the listed indicators were assessed. The effects of trypanosome species on drug requirements and growth were directly measurable. In the case of the other three indicators, the effects on drug requirements and growth that would be brought about by a change of one standard deviation in each indicator were calculated. This allowed comparison of similar sized changes in these three indicators, which are of necessity recorded in dissimilar units. These field data are unique because of the long period of continuous recording involved, the large number of serial sampling occasions per animal, the very high percentage of animals infected and the approximately equal proportions of *T. vivax* and *T. congolense* infections detected (since these are the two important species affecting cattle production).
A total of 41% of all animals required an average of 1.5 treatments with trypanocidal drugs over the two years. *T. vivax* and *T. congolense* infections had similar effects on number of trypanocidal drug treatments required. A reduction of one standard deviation in length of time infected reduced the number of treatments required by 0.23 (36%) and an increase of one standard deviation in PCV reduced the number required by 0.27 (43%) (Table III). Changes in the intensity of parasitaemia were not important for the number of treatments required.

Focusing on growth, the end product, the average growth rate was 150 g/day, an equivalent of a weight increase of 109 kg over the two years (Table III). A *T. congolense* infection reduced growth by 12.4 g/day, which equalled 8% more than a *T. vivax* infection. A reduction of one standard deviation in length of time infected increased growth by 9.8 g/day (6.5%), a reduction of one standard deviation in parasitaemia score increased growth by 9.0 g/day (6.0%), and an increase of one standard deviation in average PCV increased growth by 8.4 g/day (5.6%).

Quantitative phenotyping of N'Dama cattle in practical animal breeding and production schemes will, for economic reasons, be carried out on post-weaner animals (107). The necessity for simultaneously considering the four indicators, namely the infection aspects of trypanosome species, duration of parasitaemia and intensity of parasitaemia, and the anaemia measure of, average PCV, is illustrated above by the approximately equal effects of these indicators on the final performance trait of daily liveweight gain. Absence of information on any of these criteria would significantly reduce the accuracy of the estimate of the overall trypanotolerance phenotype of an animal and would reduce the progress possible by selection in N'Dama cattle in this central African situation.

### Future exploitation approaches

One key finding of this long-term study is the major contribution of each of the four indicators evaluated to the overall trypanotolerance variance. Once the genetic aspects of each indicator have been estimated reliably, the results may well demonstrate that all four must be handled simultaneously for optimal progress. This would require careful recording, with additional use of new diagnostics for assessing parasite control capability and precise identification of trypanosome species, especially in mixed infections.

Identification and utilisation of superior animals in breeding programmes will depend on accurate measurement of these criteria of trypanotolerance in a 'trypanotolerance test' (105, 108). The practical characteristics of such a test still need to be established. The impact will depend on the proportion of the variation associated with the trypanotolerance indicators that can be manipulated. However, genetic correlation between these indicators, the production traits and resistance to other economically important parasites/diseases must still be determined. New practical immunological markers (if and when available) could contribute to more comprehensive characterisation of trypanotolerance in the field. Likewise, molecular markers would greatly assist and expedite selection decisions based on the resistance traits (102), provided that these markers are associated with a sufficiently large proportion of the genetic variation observed in the field. Stear et al. identified one such influential gene, associated with resistance of sheep to the nematode *Ostertagia circumcincta*, which is the most significant gene identified for resistance to any parasite species, within or around the major histocompatibility complex (MHC), to date (91, 92).

### Table III

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Time infected (%)</th>
<th>Intensity of infection (score)</th>
<th>Average packed cell volume (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean measure</td>
<td>15.40</td>
<td>2.65</td>
<td>29.36</td>
</tr>
<tr>
<td>Standard deviation of measure</td>
<td>10.92</td>
<td>0.80</td>
<td>2.20</td>
</tr>
<tr>
<td>Regression coefficient of growth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In g/day</td>
<td>−0.90</td>
<td>−11.32</td>
<td>3.81</td>
</tr>
<tr>
<td>As a percentage of mean daily growth</td>
<td>−0.60</td>
<td>−7.51</td>
<td>2.53</td>
</tr>
<tr>
<td>Effect of change of one SD in measure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In g/day</td>
<td>9.8 b</td>
<td>9.0 b</td>
<td>8.4 b</td>
</tr>
<tr>
<td>As a percentage of mean daily growth</td>
<td>6.5</td>
<td>5.0</td>
<td>5.5</td>
</tr>
</tbody>
</table>

SD: standard deviation

a) Reduction of one SD for time infected and intensity of infection, increase of one SD for average packed cell volume

b) P<0.05
Tolerance to other economically important parasites and pathogens

In addition to their resistance to trypanosomosis, trypanotolerant cattle, and the N'Dama breed in particular, have other genetic advantages that contribute to their potential for use in livestock development programmes in the tropics. These cattle are reported to be resistant to several other important infectious diseases (73), including a number of tick-borne infections such as dermatophilosis (93), heartwater, bovine anaplasmosis and bovine babesiosis (33). Lower tick burdens have been reported recently in N'Dama cattle in comparison with zebu cattle (17, 64). Mattioli and Cassama suggested that tick resistance in N'Dama as compared with zebu cattle might interfere with larval development (65). Mattioli et al. also reported that N'Dama cattle showed lower prevalence of strongyle infections, and lower egg outputs when infested by strongyles, than the zebu Gobra cattle (63). More recent research at the International Trypanotolerance Centre, the Gambia, also provided evidence of the greater resistance to heartwater disease of N'Dama cattle compared to Gobra zebu (R.C. Mattioli, personal communication).

As noted earlier, the equal attribution of resistance to both trypanosomosis and dermatophilosis to the success of N'Dama cattle in the areas of the most humid part of West Africa and in Central Africa where they were introduced must be recognised. In those areas, dermatophilosis is endemic. The historic evidence indicates that the introduction of exotic breeds to this area had not been possible prior to the use of the N'Dama breed (for example, the failed introduction of susceptible cattle breeds such as the Africander cattle) because of the high risk of both trypanosomosis and dermatophilosis (G. Wissocq and J.M. Ribeaucourt, personal communications).

Breed comparison studies between N'Dama cattle and exotic breeds, such as the Sahiwal and the Nguni, as well as the crosses between these breeds, demonstrated higher productivity but intermediate levels of trypanotolerance in the crosses (15, 79). However, almost none of these crosses survived when active animal health management was not available; in fact, crossbreds could not be maintained in any of these situations because of the extensive losses due to dermatophilosis (G.D.M. d'leteren, personal observation).

In summary, a major advantage of trypanotolerant livestock, particularly N'Dama cattle, is the resistance or adaptation to many of the important pathogens prevailing in the sub-humid and humid tropics. Research on practical indicators of resistance to these conditions will be required to establish relevant integrated strategies based on disease-resistant livestock. Selective breeding must integrate the traits that farmers see as important for their production systems. More recent studies have indicated how highly farmers, who have knowledge about trypanotolerant cattle, rank disease resistance traits in choosing a cattle breed (49, 97).

Conclusion

While an increasing number of examples of innate resistance to disease are being identified in domestic livestock, trypanotolerance is one of the best recognised and most thoroughly investigated examples. Experimental and field studies reported in this paper are providing the basic tools with which the trypanotolerance trait can be identified and exploited. Evaluation of the degree of genetic determination of the different disease resistance traits, their heritability and their genetic correlations with each other and with animal performance traits should now be possible; this knowledge will allow progress to be made in the development of breeding programmes and policies.

There is increasing recognition that Africa possesses animal genetic resources probably unparalleled in any other continent. Evidence that these resources can provide sustainable and environmentally sound solutions for some of the vast disease problems currently confronting Africa is now being found. Thus, the natural innate resistance possessed by breeds of cattle, such as the N'Dama and the West African shorthorn, to trypanosomosis and to several other important infectious diseases is now accepted as an important component of national and regional disease control programmes. The fact that these breeds also possess considerable production potential and that their disease resistance traits could be exploited in crossbreeding offers an unparalleled opportunity to improve livestock production in the vast areas of Africa dominated by the tsetse fly, ticks and helminths, particularly as production systems evolve into more market-orientated production.

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The authors are grateful to Dr J.C.M. Trail for his constructive comments on the earliest draft of this paper and to Dr J. McDermott for most helpful discussions of this manuscript. The work of the authors and the many studies carried out by ILRI and its partners which have been reported extensively in this paper were supported by the governments of Belgium, France, Germany, Switzerland, the United Kingdom and by the European Union through the European Development Fund.
La trypanotolérance : une option pour un élevage durable dans des zones menacées par la trypanosomose

G.D.M. d'Ieteren, E. Authié, N. Wissocq & M. Murray

Résumé
La trypanosomose est l’un des principaux obstacles auxquels se heurte l’élevage dans des régions d’Afrique présentant le plus fort potentiel d’accroissement important du cheptel et de sa productivité. Certes, l’éradication totale de la trypanosomose du continent africain tient de la gageure. Cependant, des efforts considérables ont déjà été consentis pour lutter contre cette maladie, notamment par l’administration de médicaments trypanocides, par le contrôle des vecteurs et par l’exploitation de la résistance génétique propre aux races indigènes. Il n’y a guère de chance pour qu’un vaccin conventionnel, anti-infectieux, puisse être mis au point dans un proche avenir. De plus, la résistance aux médicaments progresse plus rapidement que prévu. Quant aux essais de lutte contre la mouche tsé-tsé, ils se poursuivent déjà depuis plusieurs décennies. Compte tenu de la réduction de l’efficacité des médicaments trypanocides disponibles et des difficultés de la lutte à long terme contre le vecteur, une priorité doit être donnée à l’amélioration de la trypanotolérance par sélection génétique, soit au sein d’une même race, soit par croisement.

La trypanotolérance se définit comme la capacité relative d’un animal à limiter le développement de parasites et leurs effets pathologiques, notamment l’anémie. Le principal obstacle à la sélection de bovins trypanotolérants, que ce soit au sein d’une même race ou par croisement, était l’absence de marqueurs pratiques et fiables de la résistance ou de la sensibilité. La principale particularité de la trypanotolérance bovine réside dans la réponse caractéristique de l’immunité humorale à l’infection. Des études sont actuellement menées sur le rôle que jouent ces réponses dans la lutte contre l’infection ou la maladie, mais, pour l’instant, on en est toujours réduit aux hypothèses.

Les résultats obtenus ces dernières années montrent que les deux principaux indicateurs de la trypanotolérance, plus particulièrement les valeurs de l’hématocrite (packed cell volume : PCV), mais aussi de la parasitémie, sont fortement corrélés aux performances de l’animal. Toutefois, même si les effets directs des infections à trypanosome sur le PCV et la croissance sont évidents, il faudrait des méthodes de diagnostic plus sensibles pour révéler l’existence de différences dans le contrôle des parasites, de sorte que chaque animal puisse faire l’objet d’une classification fiable selon sa capacité à lutter contre ces parasites. L’une des découvertes importantes est la contribution majeure de chacun des indicateurs évalués à la variabilité globale de la trypanotolérance. Les premiers paramètres génétiques concernant le PCV montrent que la trypanotolérance n’est pas seulement une caractéristique raciale mais également un trait héritable au sein du cheptel bovin N’Dama, ce qui ouvre de nouvelles possibilités d’améliorer la productivité par la sélection d’animaux trypanotolérants. Une estimation plus fiable des paramètres génétiques de ces indicateurs pourrait bien montrer qu’il faut agir simultanément sur ces deux paramètres pour obtenir des résultats optimaux. Cela impliquerait que des techniques de diagnostic permettent d’évaluer les possibilités de contrôle des parasites grâce à une identification plus précise des espèces de trypanosomes, notamment lors d’infections mixtes.

L’avantage majeur des animaux trypanotolérants, en particulier les bovins N’Dama, est la résistance ou l’adaptation de cette race à nombre d’agents pathogènes qui prévalent dans les régions tropicales sub-humides et humides. Des travaux devront être menés sur des indicateurs pratiques de résistance dans ces régions, afin d’établir des stratégies intégrées appropriées, utilisant un bétail résistant aux maladies. La sélection génétique implique l’intégration des
caractéristiques jugées importantes par les éleveurs pour leur système de production.

Mots-clés

La tripanotolerancia, posible camino hacia una producción ganadera sostenible en zonas de alto riesgo de tripanosomosis
G.D.M. d’Ieteren, E. Authié, N. Wissocq & M. Murray

Resumen
La tripanosomosis es uno de los mayores problemas que lastran la producción animal en zonas de África por lo demás dotadas de un gran potencial para ampliar su población ganadera y aumentar la productividad del ganado. Aunque la erradicación de la tripanosomosis de todo el continente sigue siendo un objetivo inalcanzable, se han realizado considerables esfuerzos para contener la enfermedad, recurriendo para ello al uso de medicamentos tripanocidas, a la gestión del vector y al aprovechamiento de la resistencia genética que presentan algunas razas autóctonas. Hay pocos motivos para suponer que en un futuro próximo pueda fabricarse una vacuna antiinfecciosa convencional, y la resistencia a los medicamentos se desarrolla con más rapidez de la esperada. Hace muchas décadas que viene intentándose el control de la mosca tsé-tsé. La mengua de eficacia de los fármacos tripanocidas y las dificultades de mantener bajo control las poblaciones de mosca tsé-tsé hacen tanto más imperiosa la necesidad de potenciar los rasgos de tripanotolerancia mediante una selección, ya sea en raza pura o por cruzamiento.
Se ha definido la tripanotolerancia como la capacidad relativa de un animal para controlar el desarrollo del parásito y limitar el alcance de sus efectos patógenos, el más notorio de los cuales es la anemia. La ausencia de marcadores prácticos y fiables de resistencia o susceptibilidad ha sido uno de los grandes inconvenientes para aplicar programas de selección de tripanotolerancia en el ganado vacuno, tanto en raza pura como por cruzamiento. El rasgo principal de la tripanotolerancia bovina reside en una respuesta muy característica de inmunidad humoral a la infección tripanosómica. Aunque en la actualidad se está investigando el papel que desempeñan estas respuestas en el control de la infección o de la enfermedad, ello sigue constituyendo por ahora materia de especulación.
Los resultados de las investigaciones de los últimos años han demostrado que la parasitemia y, sobre todo, el hematocrito (packed cell volume, PCV), que son los dos principales indicadores de tripanotolerancia, guardan una estrecha correlación con el rendimiento del animal. Sin embargo, y aunque es evidente que las infecciones tripanosómicas tienen efectos directos sobre el PVC y el crecimiento, para poder tipificar con fiabilidad a cada animal en función de su capacidad para controlar al parásito son necesarios métodos de diagnóstico más sensibles, capaces de dar cuenta justamente del grado de control del parásito. En este sentido, un hallazgo fundamental es la notable contribución de cada uno de los indicadores evaluados a la varianza global de tripanotolerancia. Ciertos
parámetros genéticos preliminares ligados al PVC demuestran que, en el seno de poblaciones de bovinos N'Dama, la tripanotolerancia es no sólo una característica racial sino también un rasgo hereditario. Esta observación abre nuevos horizontes para conseguir una mayor productividad mediante la selección de rasgos de tripanotolerancia. Una estimación más fiable de los parámetros genéticos de los indicadores podría poner de manifiesto que la obtención de los mejores resultados posibles pasa por una gestión simultánea de dichos parámetros. Para ello serían necesarias técnicas de diagnóstico capaces de estimar la capacidad de controlar al parásito y de identificar a las especies de tripanosoma con mayor precisión, especialmente en los casos de infección mixta.

Una de las grandes ventajas que presenta el ganado tripanotolerante, en especial los bovinos N'Dama, es la resistencia o adaptación de esa raza a muchos de los principales agentes patógenos que subsisten en las regiones tropicales húmedas y subhúmedas. Para implantar estrategias integradas adecuadas basadas en la selección de ganado bovino resistente a la enfermedad será necesario seguir buscando indicadores prácticos de resistencia a esas afecciones. En los programas de selección habrá que tener en cuenta e incorporar aquellos rasgos que los criadores estimen importantes para sus sistemas de producción.

**Palabras clave**

**References**


