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Chapter 1 : African Trypanosomiasis

This book is, in many ways, characteristic of its senior author, reflecting a wholly justifiable concern with standardization of procedures, reproducibility of techniques and a quantitative approach.

Parasite Biology Causal Agents Protozoan hemoflagellates belonging to the complex Trypanosoma brucei. Two subspecies that are morphologically indistinguishable cause distinct disease patterns in humans: A third member of the complex, T. Life Cycle During a blood meal on the mammalian host, an infected tsetse fly genus Glossina injects metacyclic trypomastigotes into skin tissue. The parasites enter the lymphatic system and pass into the bloodstream. Inside the host, they transform into bloodstream trypomastigotes , are carried to other sites throughout the body, reach other body fluids e. The entire life cycle of African trypanosomes is represented by extracellular stages. The tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected mammalian host ,. The cycle in the fly takes approximately 3 weeks. Humans are the main reservoir for Trypanosoma brucei gambiense, but this species can also be found in animals. Wild game animals are the main reservoir of T. The distribution of T. Clinical Presentation Infection occurs in 3 stages. A trypanosomal chancre can develop on the site of inoculation. This is followed by a hemolymphatic stage with symptoms that include fever, lymphadenopathy, and pruritus. In the meningoencephalitic stage, invasion of the central nervous system can cause headaches, somnolence, abnormal behavior, and lead to loss of consciousness and coma. The course of infection is much more acute with T. Image Gallery Trypansoma brucei ssp. The two Trypanosoma brucei subspecies that cause African trypanosomiasis, T. A typical trypomastigote has a small kinetoplast located at the posterior end, a centrally located nucleus, an undulating membrane, and a flagellum running along the undulating membrane, leaving the body at the anterior end. Trypomastigotes are the only stage found in patients. Trypanosoma brucei in a thin blood smear stained with Wright-Giemsa. The trypomastigote is beginning to divide; dividing forms are seen in African trypanosomes, but not in American trypanosomes. Laboratory Diagnosis Laboratory Diagnosis The diagnosis rests upon demonstrating trypanosomes by microscopic examination of chancre fluid, lymph node aspirates, blood, bone marrow, or, in the late stages of infection, cerebrospinal fluid. A wet preparation should be examined for the motile trypanosomes, and in addition a smear should be fixed, stained with Giemsa or Field , and examined. Concentration techniques can be used prior to microscopic examination. For other samples such as spinal fluid, concentration techniques include centrifugation followed by examination of the sediment. Isolation of the parasite by inoculation of rats or mice is a sensitive method, but its use is limited to T. Antibody detection has sensitivity and specificity that are too variable for clinical decisions. In addition, in infections with T. The CDC currently does not offer any serologic or molecular tests for African trypanosomiasis. Treatment Information Treatment information for African trypanosomiasis can be found at: Enter Email Address DPDx is an education resource designed for health professionals and laboratory scientists. For an overview including prevention and control visit www.

Chapter 2 : CDC - DPDx - Trypanosomiasis, African

Infection in the human host begins when the infective stage, known as the metacyclic stage, is injected intradermally by the tsetse fly. The organisms rapidly transform into blood-stage trypomastigotes long, slender forms , and divide by binary fission in the interstitial spaces at the site of the bite wound. The buildup of metabolic wastes and cell debris leads to the formation of a chancre. Trypanosomes have a single specialized mitochondrion called a kinetoplast mitochondrion. Maxi-circle DNA contains sequences that, when edited, direct translation of typically mitochondrially-encoded proteins. In the vertebrate host, trypanosomes depend entirely upon glucose for energy and are highly aerobic, despite the fact that the kinetoplast-mitochondrion completely lacks cytochromes. Instead, mitochondrial oxygen consumption is based on an alternative oxidase that does not produce ATP. When in the insect vector, the parasite develops a conventional cytochrome chain and TCA cycle. The surface of the trypanosome has numerous membrane-associated transport proteins for obtaining nucleic acid bases, glucose, and other small molecular weight nutrients. None of these proteins react well with antibodies, because although they lie in exposed regions of membrane, they are shielded by allosteric interference provided by the variant surface glycoprotein VSG coat proteins. This flagellated stage enters the bloodstream through the lymphatics and divides further, producing a patent parasitemia. The number of parasites in the blood is generally so low that diagnosis by microscopic examination is often negative. At some point, trypanosomes enter the central nervous system, with serious pathological consequences for humans. Some parasites transform into the non-dividing short, stumpy form , which has a biochemistry similar to those of the long, slender form and the form found in the insect vector. The tsetse fly becomes infected by ingesting a blood meal from an infected host. These short, stumpy forms are pre-adapted to the vector, having a well developed mitochondrion with a partial TCA cycle. In the insect vector, the trypanosomes develop into procyclic trypomastigotes in the midgut of the fly, and continue to divide for approximately 10 days. Here they gain a fully functional cytochrome system and TCA cycle. When the division cycles are completed, the organisms migrate to the salivary glands, and transform into epimastigotes. These forms, in turn, divide and transform further into metacyclic trypanosomes, the infective stage for humans and reservoir hosts. The cycle in the insect takes days, depending upon the species of the fly, the strain of the trypanosome, and the ambient temperature. If tsetse flies ingest more than one strain of trypanosome, there is the possibility of genetic exchange between the two strains, generating an increase in genetic diversity in an organism that may not have a sexual cycle. Flies can remain infected for life months. Tsetse flies inject over 40, metacyclic trypanosomes when they take a blood meal. The minimum infective dose for most hosts is organisms, although experimental animals have been infected with a single organism. Infection can also be acquired by eating raw meat from an infected animal. In East Africa, this mode of transmission may be important in maintaining the cycle in some reservoir hosts.

Chapter 3 : Trypanotolerant livestock in the context of trypanosomiasis intervention strategies

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The first stage, known as the hemolymphatic phase, is characterized by fever, headaches, joint pains, and itching. Fever is intermittent, with attacks lasting from a day to a week, separated by intervals of a few days to a month or longer. Invasion of the circulatory and lymphatic systems by the parasites is associated with severe swelling of lymph nodes , often to tremendous sizes. The second phase of the disease, the neurological phase , begins when the parasite invades the central nervous system by passing through the bloodâ€"brain barrier. Parkinson -like movements might arise due to non-specific movement disorders and speech disorders. Individuals may also exhibit psychiatric symptoms such as irritability, psychotic reactions, aggressive behaviour, or apathy which can sometimes dominate the clinical diagnosis. An untreated infection with T. Trypanosoma brucei gambiense accounts for the majority of African trypanosomiasis cases, with humans as the main reservoir needed for the transmission, while Trypanosoma brucei rhodesiense is mainly zoonotic, with the occasional human infection. Trypanosoma brucei gambiense causes the diseases in west and central Africa , whereas Trypanosoma brucei rhodesiense has a limited geographical range and is responsible for causing the disease in east and southern Africa. In addition, a third subspecies of the parasite known as Trypanosoma brucei brucei is responsible for affecting animals but not humans. Wild game animals and cattle are the main reservoir of T. These parasites primarily infect individuals in sub-Saharan Africa because that is where the vector tsetse fly is located. The two human forms of the disease also vary greatly in intensity. Furthermore, trypanosomes are surrounded by a coat that is composed of variant surface glycoproteins VSG. These proteins act to protect the parasite from any lytic factors that are present in human plasma. However, from the several parasites present in the plasma, a small number of them will experience changes in their surface coats resulting in the formation of new VSGs. Thus, the antibodies produced by the immune system will no longer recognize the parasite leading to proliferation until new antibodies are created to combat the novel VSGs. Eventually the immune system will no longer be able to fight off the parasite due to the constant changes in VSGs and infection will arise. While taking blood from a mammalian host, an infected tsetse fly injects metacyclic trypomastigotes into skin tissue. From the bite, parasites first enter the lymphatic system and then pass into the bloodstream. Inside the mammalian host, they transform into bloodstream trypomastigotes, and are carried to other sites throughout the body, reach other body fluids e. The entire life cycle of African trypanosomes is represented by extracellular stages. A tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected mammalian host. The entire life cycle of the fly takes about three weeks. In addition to the bite of the tsetse fly , the disease can be transmitted by: Blood transfusion Sexual contact This may be possible [16] Horse-flies Tabanidae and stable flies Muscidae possibly play a role in transmission of nagana the animal form of sleeping sickness and the human disease form. It is produced by the trypanosomal parasite in sleeping sickness. Typical trypomastigote stages the only stages found in patients , with a posterior kinetoplast, a centrally located nucleus, an undulating membrane, and an anterior flagellum. The two Trypanosoma brucei subspecies that cause human trypanosomiasis , T. CDC The gold standard for diagnosis is identification of trypanosomes in a patient sample by microscopic examination. Patient samples that can be used for diagnosis include chancre fluid, lymph node aspirates, blood, bone marrow , and, during the neurological stage, cerebrospinal fluid. Detection of trypanosome-specific antibodies can be used for diagnosis, but the sensitivity and specificity of these methods are too variable to be used alone for clinical diagnosis. Further, seroconversion occurs after the onset of clinical symptoms during a T. A wet preparation can be used to look for the motile trypanosomes. Often, the parasite is in relatively low abundance in the sample, so techniques to concentrate the parasites can be used prior to microscopic examination. For other samples, such as spinal fluid, concentration techniques include centrifugation followed by examination of the sediment. The first uses dried blood, while the other two use

whole blood samples. Although the risk of infection from a tsetse fly bite is minor estimated at less than 0. Systematic screening of at-risk communities is the best approach, because case-by-case screening is not practical in endemic regions. Systematic screening may be in the form of mobile clinics or fixed screening centres where teams travel daily to areas of high infection rates. Such screening efforts are important because early symptoms are not evident or serious enough to warrant patients with gambiense disease to seek medical attention, particularly in very remote areas. Also, diagnosis of the disease is difficult and health workers may not associate such general symptoms with trypanosomiasis. Systematic screening allows early-stage disease to be detected and treated before the disease progresses, and removes the potential human reservoir. It occurs regularly in southeast Uganda and western Kenya, and killed more than 48, Africans in At this rate, sleeping sickness elimination is a possibility. The World Health Organization plans to eradicate sleeping sickness by the year

Chapter 4 : Methods for diagnosis of trypanosomiasis in livestock

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Conceived and designed the experiments: Supervision of lab and animal work in Uganda: Received Sep 27; Accepted Mar 9. This article has been cited by other articles in PMC. Abstract Patterns of gene expression in cultured Trypanosoma brucei bloodstream and procyclic forms have been extensively characterized, and some comparisons have been made with trypanosomes grown to high parasitaemias in laboratory rodents. We do not know, however, to what extent these transcriptomes resemble those in infected Tsetse flies - or in humans or cattle, where parasitaemias are substantially lower. For clinical and field samples it is difficult to characterize parasite gene expression because of the large excess of host cell RNA. We have here examined two potential solutions to this problem for bloodstream form trypanosomes, assaying transcriptomes by high throughput cDNA sequencing RNASeq. We first purified the parasites from blood of infected rats. We found that a red blood cell lysis procedure affected the transcriptome substantially more than purification using a DEAE cellulose column, but that too introduced significant distortions and variability. As an alternative, we specifically amplified parasite sequences from a mixture containing a fold excess of human RNA. Finally, the cDNA was amplified using nested primers. Synthesis of the second cDNA strand with a spliced leader primer, followed by amplification, is sufficiently reproducible to allow comparison of different samples so long as they are all treated in the same way. The amplification method might be suitable for clinical samples with low parasitaemias, and could also be adapted for other Kinetoplastids and to samples from infected vectors. Author Summary Most experiments on African trypanosomes - including those designed to look for new drugs - have studied parasites either from culture, or from laboratory rodents. We are interested in comparing these parasites that grow in man and domestic animals, where the parasites generally have different nutrient concentrations and much lower parasitaemias than in experimental models. The most accessible way to make the comparison is to measure the amounts of mRNAs. In this paper we describe how methods that are used to purify the parasites from human cells can change the relative amounts of mRNA. We also describe a method to examine RNA from relatively small numbers of parasites that are mixed with host cells. Introduction African trypanosomes live in several niches - in the blood, tissue fluids and brain of patients with second stage sleeping sickness, and in various parts of the Tsetse fly digestive tract. Nearly all experiments on African trypanosomes, however - including those designed to look for new drugs - have studied parasites either from culture, or from laboratory rodents with high parasitaemias. Drugs have to kill parasites that are living in humans or ruminants. These parasites are very difficult to study because parasitaemias are low, so we have no idea whether their metabolism is really the same as that of parasites in culture. The ideal way to assess differences between these parasites and the standard lab models would be to characterise their proteomes or metabolomes, but the numbers of parasites are insufficient. The amounts of RNA are however sufficient, for transcriptome analysis, especially since amplification techniques are available. The most sensitive method to characterise transcriptomes is to make cDNA by reverse transcription, followed by second strand synthesis and analysis of the products by high throughput sequencing [1]. To create a good library for sequencing, 50â€” ng of mRNA is generally needed. When less is available, the cDNA can be amplified. One approach is to include tags at the ends of the cDNA, and use those sequences for polymerase chain reaction amplification or linear amplification [3] , [4] , [5]. An alternative is to include a promoter for a bacteriophage polymerase in the cDNA primer [3] , [5] , [6]. Once double-stranded cDNA is made, it has the promoter at one end, and this can be used as a template for further RNA synthesis by the bacteriophage polymerase. The various amplification techniques can be qualitatively very good for transcript detection, but when tested quantitatively, they were found to cause considerable distortions in measured transcript levels [3] , [5]. Several groups have obtained trypanosome transcriptomes using RNASeq [7] , including quantitation of full-length developmentally regulated mRNAs [8]. To map the splice sites, the spliced leader sequence can be used as a primer for second strand synthesis on cDNA. In addition, it has been used to assess the effects of a gene knock-out in

Trypanosoma cruzi [12]. The study of the transcriptomes of trypanosomes from natural infections is made difficult not only by the low parasite numbers, but also by the fact that the samples contain such an excess of host cells. For all but very high parasitaemias, a purification step will be necessary in order to obtain sufficient parasite sequence reads. One option is to purify the trypanosomes away from the host cells before RNA is prepared. The alternative is to select trypanosome sequences from the RNA mixture before starting the high-throughput sequencing procedure: We here describe an optimized spliced leader priming protocol, and compare the effects of both trypanosome purification and spliced leader priming on the final transcriptome data. Uganda has no law governing experimentation on animals, so the Makerere committee follows EU guidelines. Trypanosomes To test purification methods, approximately T. EDTA acts as an anticoagulant. For column chromatography [13] 20 ml packed slurry of DEAE cellulose resin Sigma pre-equilibrated in phosphate saline glucose PSG buffer, pH 8, was set up in a 30 ml syringe. The column was equilibrated with 3 column volumes of PSG and thereafter loaded with the anti-coagulated rat blood approximately 5 ml at room temperature. The procedure took approximately 30â€”40 min and this would be similar for standard 5 ml samples of human blood. Trypanosomes in infected rat blood were also purified by hemolysis followed by centrifugation. The content of the Qiagen solution is not known but most common procedures involve the use of isotonic ammonium chloride. In each case, the trypanosome pellet was immediately resuspended in Trifast reagent and then frozen. The total RNA concentration was then determined using the Qubit 2. The RNA was ethanol precipitated and resuspended in water. The second strand cDNA was synthesised using 0. All the samples were multiplexed. The sequence reads were aligned to the reference T. We extracted all reads that mapped to the annotated mature RNAs for the amplification experiment or to coding regions for the purification experiments. Annotated mature mRNAs in the database do not always include both untranslated regions. For those that did not align, we extracted reads containing sequences of the spliced leader SL tags or poly A and the T3 promoter, trimmed them, and assigned them to an open reading frame based on their positioning within annotated gene coordinates [15]. The minimum sequence length trimmed was 5 nt. The processing and sorting of the aligned reads was carried out using SAMtools [16] and the read alignment to the genome visualized by Artemis [17] , [18]. DESeq was used to identify differentially expressed genes, with a cutoff p-adjusted value of 0. Functional category enrichment was carried out using Fisher exact test in R. The heat map was generated in R. Reads per million were calculated using the unique gene list of Siegel et al [8] , which excludes all but one gene copy of multigene families. Coding sequence lengths were extracted from TritrypDB. PERL scripts were written to extract reads aligning to a window of 0. Routine calculations were done using Microsoft Excel. Before the calculation of correlation coefficients, the data were log2 transformed. If this is not done, a few very abundant mRNAs excessively influence the result. Results Purification of trypanosomes from blood To test the effects of trypanosome purification from blood, we compared the two available methods - DEAE cellulose column purification [13] and red cell lysis - with simple centrifugation followed by taking the buffy coat Table 1. The red cell lysis method does not remove lymphocytes, so would not be suitable at low parasitaemias. The DEAE column does remove a large proportion of the lymphocytes [13] , but it takes longer than erythrocyte lysis. Both methods give up to ten times higher trypanosome yields than taking the buffy coat, since many trypanosomes are in the erythrocyte layer after blood centrifugation. However, the buffy coat method does not involve harsh treatment of the parasite, and thus we used it as a reference for the effect of purification methods on the transcriptome. Table 1 Approaches used to analyse trypanosome transcriptomes.

Chapter 5 : CDC - African Trypanosomiasis - Diagnosis

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These concentrations were the same for both primer sets. A DNA-positive control, as well as a negative control, was used every time a set field samples was tested. This was poured into a 30 x 20 cm mould. The gel was stained with Ethidium bromide and the samples were examined under UV light. Spearman correlation coefficient SAS, version was used to examine the relationship between the eye scores, PCV and the trypanosomes. Arithmetic means and standard errors were also used to determine the relationship between trypanosome infected animals and their PCV. The overall prevalence rate obtained was 9 1. The haematocrit centrifugation technique detected more trypanosome species than others in the parasitological analysis Table 2. The White fulani breed harboured most of the trypanosomes above other breeds encountered in the course of this study Table 3. The FAMACHA eye score chart rated as ordinal ranking scale Thrusfield, indicating scores 1, 2 and 3 as non anaemic while 4 and 5 scores are anaemic. The haematological parameters obtained are as shown in Table 5 and there are significant variations in the normal values and the obtained values for infected and uninfected cattle. Two of the rats that were inoculated with infected blood died four days later and the histopathology of the kidney revealed fatty degeneration, coagulative necrosis and liquefactive necrosis. Other haemoparasites encountered through parasitological analysis include Babesia bovis, Table 2: In parentheses are the percentages Table 3: In parentheses are the percentages Table 4: Out of the blood samples examined for the presence of T. The two positive samples from STDM were also positive at this level. Also the animal rearing activities in the study area is basically on extensive method where there is little restriction to the movement of animals. All these make it difficult to ascertain the origin of the animals presented at the abattoir for slaughter, thus inability to trace back the infection Thrusfield, The microhaematocrit centrifugation technique detected most of the infection than others and this is in agreement with Omotainse et al. Buffy coat and haematocrit centrifugation are still regarded as the best parasitological techniques for quick detection of the parasite as they concentrate the trypanosomes allowing for quick and clear visualization Chappuis et al. The obtained prevalence rate of 1. This declaration might have been borne out of the fact that the study area is in the extreme northwest of the country and always experience high environmental temperature and low humidity all year round which is unfavourable for the development of tsetse flies the major vector of the infection Ahmed et al. It has been found out that the Morsitans and Palpalis groups are most prevalent in the Savannah zones covering mainly the northern parts of Nigeria and are found along the drainage lines and riverine vegetation Bourn et al. The study area is drain mainly by River Sokoto and River Rima and there is the tendency of tsetse flies that incure into this microclimate to survive and become focal source of infection. Biting flies such as Tabanids and Stomoxys have been incriminated also as mechanical vectors Ahmed et al. Most of the cattle reared within the study area are kept semi-intensive and extensively and there is seasonal migration of the Fulani herdsmen who are in possession of a large number of cattle herds during dry season towards the middle belt in search of greener pasture and water. The middle belt region is known to be endemic for trypanosomosis as there is abundance of tsetse flies. The exposed cattle at the beginning of rainy season in the northwest returned possibly with the infection and this can be spread by mechanical vectors on their arrival. Transhumant activities of the herdsmen encourages the long range flying tsetse to follow the animals along the cattle route thus spreading the infection among the sedentary herds closer to routes Onyiah, Gibson and Brady reported that wild male and female tsetse are known to have the ability to travel at ground speed of Trypanosoma vivax was found to occur mostly in this study and they are known to be dominant species in ruminants in most parts of northern Nigeria Omotainse et al. This parasite is haematinin which are found mainly in the plasma. Its infestation leads to rapid destruction of erythrocytes which leads to anaemia and rapid weight loss thus reduction in market value of the animals Losos and Ikede, In Nigeria an estimated Death of two mice at the fourth day of inoculation with infected blood is suggestive of the presence of T. The vascular tissue destruction at histopathology also lends credence to their presence.

This may be due to destruction of red blood cells by the hematinin trypanosomes in infected ones Anosa, and may also be attributed to the inhibition of erythrocyte formation in the bone marrow or their lysis by endotoxin liberated by trypanosomes. High environmental temperature as obtainable in the study area may equally altered the biochemical and haematological values alongside the presence of the parasites Azab and Abdel-Maksoud, ; Anosa, The total WBC counts in all the infected animals were higher than the uninfected ones, this is as a result of the response of the affected animals to the infection Anosa, There were no appreciable changes in monocyte and basophil counts between the two groups and this is in agreement with Rajkhowa et al. Lanes 5,6,9 show clear bands at bp as in the positive control Anaemia is a major hallmark for trypanosome infections and the use of FAMACHA eye chart in the study compliment what was obtained through the PCV values Grace et al. The high percentage of animals recorded with the anaemic condition may be as a result of extraneous factors such as the state of nutrition of the host, the presence of other inter-current infections of infestations and stresses caused by work, thirst, parturition, lactation and trekking Bourne et al. Other haemoparasites encountered include.

Chapter 6 : Prof WH Russell Lumsden - The Scotsman

- Trypanosomes also enter the lymphatics resulting in lymph node enlargement and the lysis of trypanosomes release toxic materials that stimulate macrophages to release tumor necrosis factor - This results in cyclic or relapsing fever with aprox cycle of days.

Tsetse control The earliest methods of tsetse control included widespread bush clearing to destroy tsetse breeding habitats, supported by shooting of game wildlife or installation of game fences to prevent the game hosts from carrying flies into the tsetse-free area, and clearing wide forest corridors to prevent re-invasion of tsetse. Insecticide-based control techniques, i. In view of environmental concerns and public outcry regarding the use of chemicals and the destruction of forests in the s, these methods gave way to other less-intrusive techniques including trapping, the use of odour-baited targets Jordan, and the treatment of animals with insecticide Bauer et al. The application of the sterile insect technique SIT has also had a limited impact. The major shortcomings of these methods lie in the limited size of area for which they can be economically deployed relative to the total size of the tsetse-affected area and the continual costs associated with preventing re-invasion. There are also concerns about non-tsetse targets, for example ticks, becoming resistant to pyrethroids used as insecticides on livestock. Yet in theory, tsetse control appears to offer the greatest likelihood of success in ridding the African continent of the tsetse-trypanosomiasis problem. As a control measure, drug therapy strategies are currently protecting more cattle approximately 25 percent of all affected cattle against the disease than any other method Budd, Not only are individual cases recognized Peregrine, , but regional distribution is increasingly being reported in East and West Africa. Furthermore, the much anticipated breakthrough in the development of vaccines to control trypanosomiasis appears unattainable in the near future owing to the antigenic variation of trypanosomes and the complexity of their antigenic repertoire. New strategies that integrate several options in controlling trypanosomiasis are being developed to extend the period during which the currently used drugs remain effective FAO, ; Holmes, Exploitation of trypanotolerant livestock as an option for trypanosomiasis control Trypanotolerant livestock play a significant role in moderating the problem of tsetse-trypanosomiasis in West and Central Africa, primarily through their use for food, traction and as a source of cash income in areas where livestock agriculture would otherwise not be possible. The wide geographical distribution of these animals especially small ruminants , scattered from the southern limits of the semi-arid zone to the coastal humid zones and found in almost all the countries in the region, suggests that livelihoods are being supported in significant ways by the presence of these stock in the affected areas. In addition to the use of trypanotolerant livestock in directly minimizing the tsetse-trypanosomiasis problem, there is some suggestion that because of their capacity to rid themselves of trypanosome parasites and maintain low parasitaemia, once infected, they indirectly reduce the trypanosome parasite load associated with any given location. While the mechanisms that lead to the maintenance of lower parasite loads in trypanotolerant breeds compared with susceptible breeds are not clearly understood, results from laboratory experiments indicate that the killing of trypanosomes in host animals results from inhibition of the trypanosome glycolytic pathway and of adenosine triphosphate ATP production Muranjan et al. There is also the suggestion that animals infected with trypanosomes tend to attract more tsetse flies than uninfected animals and that the feeding success on animals is substantially greater in infected than in uninfected animals Baylis and Mbwabi, Thus, it can be argued that, given two areas with similar initial trypanosome prevalence and the same number of either trypanotolerant or trypanosusceptible livestock, the rate of infection will be lower among the trypanotolerant stock. A logical extension of this hypothesis is that where the population of trypanotolerant livestock is high relative to trypanosusceptible livestock maintained under chemotherapy or chemoprophylaxis , or where there are only trypanotolerant livestock, the rate of transmission of the disease will be much lower. Thus, the investment made to control the tsetse-trypanosomiasis problem by drug-based methods chemoprophylaxis, chemotherapy, etc. These postulations are supported by the observations of Leak et al. Multidisease integrated approach In terms of cost and simplicity, it can be considered desirable to be able to control several diseases simultaneously with one or a few packaged treatments or control options. Such an

approach to controlling several diseases with one treatment or option in an integrated manner may be referred to as a multidisease integrated approach. Thus, the application of one drug or chemical compound to control agents or vectors of one disease and that might simultaneously control vectors of other diseases should be considered as a superior approach to alternatives that involve attacking the vectors with several chemicals. For example, the use of trypanocidal pour-ons on livestock to control tsetse flies and nuisance flies may lead to the control of ticks, and hence tick-transmitted diseases. Another example of such an approach may be the theory that genes conferring trypanotolerance in cattle breeds might be linked with those controlling dermatophilosis. The use of trypanotolerant livestock in areas endemic for tsetse-transmitted trypanosomiasis and tick-borne diseases associated with dermatophilosis might provide an integrated approach to dealing with multiple diseases of trypanosomiasis and various tick-borne diseases. Given the range of diseases that trypanotolerant livestock are credited to be resistant, tolerant or resilient to, it can be argued that their utilization in several ecosystems is feasible and could thus play a significant role in multidisease integrated control approaches and methods. However, a combination of two or more control measures may be more effective in overcoming a particular disease that is difficult to treat with one control measure. For example, Gibson noted that, in most instances, utilization of genetic resistance will be one component of an integrated approach to disease control and argued that, with the use of purebred indigenous livestock, resistance is not always complete and that a proportion of animals often suffer some degree of production loss as a result of the disease. Furthermore, in cross-breeding and other systems that exploit resistance, the level of resistance will usually be incomplete. Consequently, Gibson concluded that the design of cost-effective, disease-control strategies requires knowledge of the degree and nature of the resistance of the breed s used in the production system. Based on this reasoning, Gibson hypothesized that sheep that are partially resistant to helminthosis can exhibit lower susceptibility to infection and have lower faecal egg counts when infected. Where stringent management is difficult to ensure, this may mean that rotational grazing will be more effective with the use of partially resistant animals than with the use of susceptible breeds. However, the increasing drug resistance in trypanosusceptible populations and the difficulties of sustaining tsetse fly control increase the need for enhancing trypanotolerance through selective breeding either within breeds or through crossbreeding. These observations support the analysis by Holmes and Geerts and Holmes FAO, that more integrated strategies need to be developed.

Chapter 7 : African trypanosomiasis - Wikipedia

techniques were recorded through the introduction of the indirect haemagglutination test (10, 11)Th. e test was applied to the diagnosis of trypanosomiasis in camel(16, s 39) and other ruminant(43)s Th. e basic problems with this technique were that the.

Immunodiagnostic techniques Although direct demonstration of trypanosomes in the infected animal gives conclusive proof of infection, the limitations of parasitological diagnosis has been the driving force for a great deal of research into alternative techniques that provide indirect evidence of infection, namely immunodiagnostic techniques. There are many reports of the use of immunodiagnostic techniques for diagnosis but, invariably, most of them have been retrospective surveys, intended to add further information rather than play an integral part in a control programme. The one exception to this generalization is in the application of the complement fixation CF test to the diagnosis of T. Serology has always played a major role in diagnosis of this disease since trypanosomes are rarely found in blood or other body fluids. The CF test was used successfully in the control and eradication of dourine in North America Watson, and was also used in the diagnosis of surra in buffalo in the Philippines Randall and Schwartz, This assay, little changed, is still in use today in testing sera before the import and export of horses between different countries. The test has not been used extensively for the other animal trypanosomiases because of problems with antigen preparation, standardization of the assay and interference by anticomplementary activity in sera from several animal species. Problems in the control and standardization of another sensitive test, the indirect haemagglutination IHA test, have precluded its general use although it was used in the diagnosis of T. In tests with T. The breakthrough in immunological diagnosis came with the introduction of primary binding assays for the detection of trypanosomal antibodies. These tests directly measure the interaction between antigen and antibody rather than relying on a secondary reaction consequent upon the initial binding. The indirect fluorescent antibody test IFAT has been used extensively in the detection of trypanosomal antibodies in animals and humans. Antigens are usually prepared from blood smears which are fixed in acetone and then stored at a low temperature. The IFAT has proven to be both specific and sensitive in detecting trypanosomal antibodies in infected cattle Wilson, ; Luckins and Mehlitz, and camels Lucking et al. However, cross-reactions between different trypanosome species do occur. Ashkar and Ochilo found that more than 85 percent of cattle infected with T. When sera were tested against all three pathogenic trypanosome species, between 45 and 66 percent of sera reacted in the assay, and only by combining all the results did the test detect 94 percent of infected animals Table 3. Hence, although there is considerable cross-reactivity, these results indicate a degree of species specificity that requires the use of all three antigens in order to obtain maximum efficiency. Modifications in the preparation of antigens involving fixation of the parasites in acetone and formalin Katende et al. The major drawback of the IFAT - apart from its requiring sophisticated microscopy - is its subjectivity, which can make comparison of results quite difficult. Undoubtedly, the introduction of enzyme-linked immunosorbent assays ELISA for use as diagnostic tests for animal trypanosomiases Lucking and Mehlitz, ; Luckins et al. The tests are carried out in well polystyrene micro-ELISA plates on which trypanosomal antigen is adsorbed. The test is visualized by the addition of enzyme substrate and chromogen, with the resulting colour change allowing a photometric interpretation. However, where tsetse-transmitted trypanosomiases occurred, cross-reactions were a problem Table 3. As with the IFAT, to ensure that a high proportion of infected animals were diagnosed, sera had to be screened against all trypanosome antigens in order to obtain the highest diagnostic sensitivity. Fractionation of the crude trypanosomal antigen extracts has identified antigens that are species specific, and this method should enable discrimination between T. In addition, species-specific monoclonal antibodies developed against T. The test, which is simple to perform, has been used for diagnosis of T. A modification of the ELISA, currently of great interest, is based on an antigen capture assay which enables detection of circulating trypanosomal antigen in the blood of infected animals. Antibody against trypanosomal antigen is used to coat ELISA plates and any antigen present in test sera binds. The complex so formed is then incubated with the same antibody, conjugated with enzyme and

visualized with a suitable substrate. Early assays using polyclonal antibodies raised against crude trypanosomal antigen preparations were found to detect antigen in animals infected with T. Later, the species specificity of the assay was improved following the development of monoclonal antibodies as capture antibodies that recognized antigens present in T. Specific circulating antigens could be detected in cattle from eight to 14 days after infection, but within 14 days of treatment they were no longer detectable Nantulya and Lindqvist, The figure shows the development of antigenaemia and antibodies in rabbits infected with T. Although antigen levels fall rapidly after treatment, antibody levels continue to rise and are still high some time after treatment. The successful treatment of the animals is confirmed by the rapid disappearance of antigen from the circulation. Antigen ELISA was shown to have a high diagnostic sensitivity; more than 90 percent and 95 percent, respectively, in cattle and camels Nantulya et al. A simplified modification of the assay in polystyrene tubes was found to give similar results to the plate assay Nantulya et al. In goats and cattle, experimentally infected with T. The false negative results occurred during the early stages of infection, possibly when antigen levels were below the detection limits of the assay.

Chapter 8 : Techniques with trypanosomes.

Patterns of gene expression in cultured Trypanosoma brucei bloodstream and procyclic forms have been extensively characterized, and some comparisons have been made with trypanosomes grown to high parasitaemias in laboratory rodents.

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This relies on conventional techniques such as lymph node puncture, blood film examination, or various more elaborate techniques to concentrate parasites in the blood. 8 As the concentration of trypanosomes in the blood undulates, often decreasing below detection levels in west African trypanosomiasis, examinations may have to be repeated daily.