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Camel Trypanosomosis: A Review on Diagnostic Approaches and Immunological Consequences

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Abstract: *Trypanosoma evansi* causes a trypanosomosis known as 'surra'. It affects a large number of wild and domesticated animal species in the world. The principal host species varies geographically, but camels are particularly affected. It is an arthropod-borne disease; several species of haematophagous flies, including Tabanidsand Stomoxys, are implicated in transferring infection as mechanical vectors. In Brazil, vampire bats are also involved in a unique type of biological transmission. The general clinical signs of *T. evansi* infection are not sufficiently pathognomonic for diagnosis. Laboratory methods for detecting the parasite are required. In early infection, when the parasitaemia is high, examination of wet blood films, stained blood smears or lymph node materials can reveal the trypanosomes from blood or lymph samples. In more chronic cases, when the parasitaemia is low, the examination of thick blood smears, as well as inoculation of laboratory rodents is required. Several primer pairs targeting the subgenus or the species-specific (*T. evansi*) parasitic DNA sequences are available for diagnosis by polymerase chain reaction (PCR) and DNA probe. Serological tests using specific antibody responses and a variety of antibody detection tests have been introduced for laboratory and field uses. The most relevant are immunofluorescence test (IFAT), enzyme linked immunosorbent assays (ELISA) and card agglutination test (CATT/*T. evansi*). *T. evansi*, like other pathogenic trypanosomes induce a generalized immune-suppression of both humoral antibody response and T-cellmediated immune responses. As a result, in the long term, the host's immune responses fail and it succumbs to either the overwhelming parasite load or to secondary infection, consequently leading to occurrence of the *trypanosome*-induced immunopathology.

Key words: Immune suppression · Parasitological · Microbiological · Serology · *Trypanosoma evansi*

INTRODUCTION

Camels are vital domestic animal species that are best adapted to harsh environment and fluctuating nutritional condition of arid and extreme arid zones. These animals are endowed with extra ordinary feathers that enable them to survive and perform in such hard conditions [1].

Trypanosoma evansi, the protozoan parasitic cause of camel trypanosomosis (Surra), constitutes one of the major veterinary problems worldwide [2]. The disease is an important single cause of economic losses, causing morbidity of up to g30 % and mortality of around 3 % camels in Ethiopia [3, 4]. Ethiopia is placing the third position in camel rearing countries after Somalia and Sudan [5]. Camels suffer from trypanosomosis caused by *T. evansi* that is transmitted mechanically or non-cyclically, by haematophagous flies such as horseflies (*Tabanus* species) and stable flies (*Stomoxys* species) which are endemic in Africa, Asia and South America, although in America the vampire bat also acts as a vector as well as reservoir hosts [6].

Clinically, affected camels may have: reduced appetite and water intake, their hump disappears as the disease progresses; their hair coat is dull and rough with loss of hair at the tail. There is an oedema under the belly visible especially in the morning, pregnant females may abort and newborn calves of infected dams usually die. There is pallor of mucous membranes of the eyes, a fluctuating temperature with initial peaks of up to 41ºc and the urine usually has a characteristic smell [6, 7].There are no pathognomonic signs of the disease in the camels and any clinical examination is of little importance for a conclusive diagnosis, but the parasites can be detected in blood 13 to 16 days after a mechanical has had a meal [5].

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The diagnosis of trypanosomosis is basically divided into clinical, parasitological, molecular, chemical and serological. For research purposes, especially in epidemiological, sensitive and specific diagnostic methods, as well as their applicability in the field, are prerequisite. Diagnostic procedures vary according to the tools available and the purpose of the tests [5].

Parasitological methods used in the diagnosis of *T. evansi* in camels are considered easy, rapid and economic. However, they are not sufficient to detect all trypanosome infected animals, especially in case of low parasitaemia and also in the chronic form of the disease [8].The serological test such as the card agglutination test (CATT), ELISA and LAT are used for the detection of antibodies circulating in the serum of infected camels, the test could be used under both laboratory and field conditions [9].

With the introduction of molecular diagnostic techniques, several diagnostic assays based on the detection of trypanosomal DNA by PCR have been developed. Particularly in these cases of treatment success evaluation, DNA based techniques, as polymerase chain reactions (PCR) and DNA probes are useful [6]. Trypanosomes survive and multiply in the extracellular fluid of their mammalian hosts, especially in the blood. They are confronted with both innate and adaptive immune defenses. Selective pressure has enabled them to elaborate refined escape mechanism. Beside its direct pathogencity causes immune suppression [10].

Given this general back ground, the specific objectives of this seminar paper is:

- To review the diagnostic approaches and immunological consequences of camel trypanosomosis.

Camel Trypanosomosis (Surra)

Etiology: *Trypanosoma evansi*, the first pathogenic trypanosome to be identified in 1880 in India, belongs to the brucei group (subgenus *Trypanozoon*) but is not capable of cyclical development in tsetse *Glossina* species. In blood smears, *T. evansi* is morphologically indistinguishable from *T. brucei*, but at the molecular level, the structure of the kinetoplast DNA of *T. evansi* is different [11].

Epidemiology

Distribution: *Trypanosoma evansi* occurs not only in Africa, but also in Central and South America, the Middle East and Asia. The parasite has a wide host spectrum, the main host species varies with the geographical region. In Africa, beyond the northern most limits of the tsetse fly belt and in parts of East Africa, camels are the most important host, whilst in Central and South America the horse is principally affected [12].

Transmission: *Trypanosoma evansi* is transmitted in several ways, via blood or lymph sucking insects and vampire bats. Transmission can also be vertical, horizontal, iatrogenic and per-oral, with various epidemiological significances, depending on the season, location and host species [13].

Host Factor: The disease is most severe in horses, donkeys, mules, camels, dogs and cats. Camels, horses, dogs and Asian elephant are more susceptible than sheep and goat, which are more susceptible than bovines and pigs. Rats and mice are highly susceptible as experimental hosts for detecting subclinical (non-patent) infection [14].

Pathogenesis, Pathology and Clinical Findings: *Trypanosoma evansi* can infect a variety of hosts and causes a species-specific pathology. In camels, the disease is manifested by elevation of body temperature which is directly associated with parasitaemia. Infected animals show progressive anaemia, marked depression, dullness, loss of condition and often rapid death. Anaemia was observed to be a major clinical finding in camel trypanosomosis in Morocco [15].

Anaemia is a major component of the pathology of surra and of African trypanosomosis generally. Anaemia, in *T. evansi* infections of camels, is reportedly macrocytic and hypochromic, however; in the early phases of infection the anaemia is haemolytic and haemophagocytic. The mechanism(s) responsible for this increased erythrophagocytic activity are not fully understood [16].

Treatment: Suramin and quinapyraminesulphate are the two drugs available for the treatment of *T. evansi* infections in camels. Suramin is administered at a dosage rate of 12mg/kg body weight intravenously for curative and prophylactic activity. Quinapyramine, when administered as methyl sulphate at a dosage rate of 3-5 mg/kg body weight subcutaneously is for curative purposes but as a pro-salt chloride/methyl sulphate mixture at 5-8.3 mg/kg body weight is for curative and prophylactic activity. Most drugs are either not curative such as homidium bromide, or are too toxic for camels such as diminazeneaceturate [17].

Control and Prevention: The prevention and control of trypanosomosisis mainly depend on the proper usage of the few available trypanocides, especially the strategic deployment of the sanative drugs in order to reduce development of drug resistance plus the continued use of environment-friendly vector control programs [5, 18]. Trypanosomosis control has been carried out in endemic countries by use of three approaches coupled with modified management: vector population control; chemoprophylaxis; and use of Trypanosomatolerant animals [18].

Diagnostic Approaches of Camel Trypanosomiasis (Surra): There are no pathognomonic signs of surra; therefore, laboratory diagnosis has been to be carried out to confirm infection. This involves parasitological, molecular and serological diagnosis. Parasitological diagnosis is mainly carried out by the direct microscopic examination of blood or buffy coats and/or sub-inoculation of camel blood into rodents such as mice or rats. However, this test has a poor sensitivity, often less than 50% [19]. The implication of this is that in most situations *T. evansi* is under-diagnosed and the level of infection is greater than frequently reported. On the other hand, serological techniques, such as immunofluorescent antibody test (IFAT), Enzyme Linked Immunosorbent Assay (ELISA) and the Card Agglutination Test for trypanosomosis (CATT), although sensitive, cannot distinguish current from cured infections [16].

Parasitological Examination: Parasitological methods include: microscopic examination of blood; parasite concentration techniques; and animal inoculation. The easiest and most frequently used of the three techniques is direct microscopic examination of blood, either by wet blood film method, or as stained thick and thin smears [5]. In the wet film smear, the trypanosomes are seen either directly between blood cells or indirectly as they cause blood cells to move. Fresh lymph preparations and thin smears of lymph may also be used. These smears (thin blood and lymph) are useful for morphological identification of different trypanosomes under the light microscope [20].

The evaluation of some of these techniques under experimental conditions has given an indication of their detection limits in relation to the numbers of different species of trypanosomes in a blood sample. In order of decreasing sensitivity, the results were as follows: dark ground buffy coat technique (DG)>heamatocrit centrifuge technique (HCT>thick film>thin film>wet film [21].

Blood Examination: The easiest technique for detection of trypanosomes in peripheral blood is by direct microscopic examination of blood, either by the wet film method to detect motile trypanosomes or, as stained thick and thin smears, when parasites are identified on the basis of their morphology by light microscopy. Examination of wet blood films is quick and the method is suitable for screening large numbers of animals. This method, however, is insensitive as half of the infected animals may be missed. The basic technique, i.e. examination of fresh or stained blood films has been modified to improve diagnostic sensitivity by concentrating the blood through centrifugation in a haematocrit technique, wet film method to detect motile trypanosomes or, as stained thick and thin smears, when parasites are identified by the basis of their morphology by light microscopy [22].

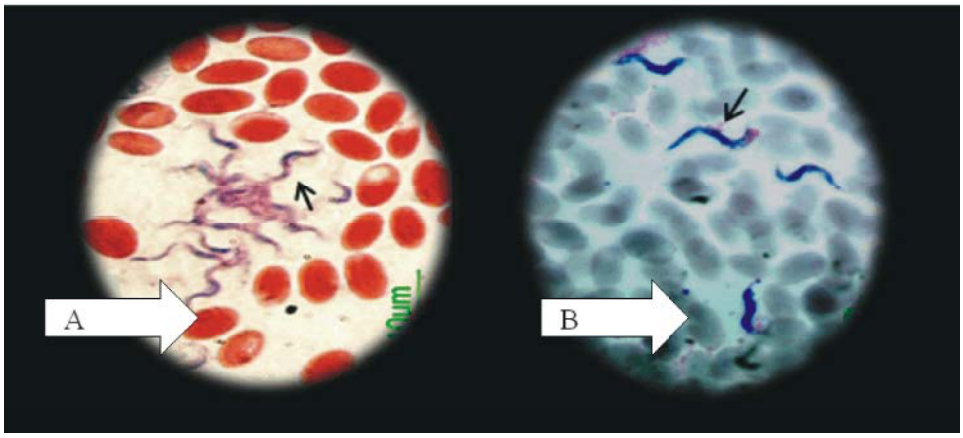

Fig. 1: Microscopical examination of the stained blood smear from a naturally camel infected with *T. evansi* (arrow) (1000×). A. Hematoxylin and eosin-stained blood smear; B. Giemsa-stained blood smear (Source:[23]).

Buffy Coat Technique: Collect blood (70 µl) into two heparinized capillary tubes (75 × 1.5 mm). Close the wet end with plasticine and centrifuge at 3000 g for 5 minutes (generally 12,000 rpm in a haematocrit centrifuge machine). The capillary tube is examined and improve diagnostic sensitivity by concentrating the blood through centrifugation in a haematocrit tube, namely the haematocrit centrifuge technique (HCT) or the dark ground buffy coat technique (DG) [24].

Animal inoculation (also called as Xenodiagnosis): Inoculation of infected camel blood in to laboratory rodents is valuable for detecting sub patent *evansi* infections in camels. This had been confirmed by Pegram and Scott in 1929, who considered the inoculation of camel blood in to laboratory rodents are to be the best direct diagnostic method [25].

Due to the increasing concern to eliminate the use of animals for biological testing, animal inoculation should be limited as far as possible and only used if fully justified. Laboratory animals may be used to reveal subclinical (non-patent) infections in domesticated animals. *Trypanosoma evansi* has a broad spectrum of infectivity for small rodents and so rats and mice are often used [19].

Rodent inoculation is not 100% sensitive [19] but further improvement in its efficacy can be obtained by the use of buffy coat material. This procedure is able to detect as few as 1.25 percent of *T. evansi*/ml blood [14]. This technique is suitable when highly sensitive detection is required and it can be done by inoculating heparinized blood intraperitoneally into rats (1–2 ml) or mice (0.25–0.5 ml). Then bleed animals from the tail after every 48 h to detect parasitaemia. The incubation period before appearance of the parasites and their virulence depends on the strain of trypanosomes, their concentration in the inoculum and the strain of laboratory animal used, however; in most cases it is very short (5 ± 2 days), but can extend to 2 weeks in rare cases [14].

Chemical Examination: Only clinical study or microscopic examination of blood is not sufficient for the diagnosis of *T. evansi* infection in all its stage. Therefore, certain non-specific biochemical tests are applicable. Which indicates increased serum protein levels as mercuric chloride test, formol gel test, stilbamidine test and Jon's nitric acid test have been used [27].

Mercuric Chloride Test: This is an indirect mass screening of dromedary herds [28] which is a solid liquid weak solution in the detection of camel surra. One milliliter of mercuric chloride solution is taken in to a test tube then adds 1-2 drops of suspected serum and gently mixed with the solution. After 15 minutes white precipitate will be formed [29].

Stilbamidine Test: Stilbamidine aqueous solution is taken the amount of 0.5-200 ml of 10 %in the test tube. Then add 1-2 drops of suspected serum and overlayed the solution. In the case coagulation occurs and it sinks and gets dissolved in the solution test within 5-10 minutes. The stilbamidine provides the possibilities not only being used for surra [28], but also to serve for differentiation of various stages of the infection.

Jon's Nitric Acid Test: Take 1ml of 1.8% of nitric acid in the test tube then add one drop of suspected serum. If the case is positive turbidity appears after one hour [28].

Formol Gel Test: For mass screening of dromedary herds there are numerous indirect tests that demonstrate the presence of the parasite. The procedure has been performed by adding 2 drops of 40 % formaldehyde in to 1ml of suspected serum. There occurs a formation of gel after 1 hour if the case is positive [30].

Serological Examination: Specific serological tests such as: the capillary agglutination tests, the passive haemagglutinatination test [31], the immune fluorescent antibody test and the enzyme-linked immunosorbent assay (ELISA) using simplified ELISA for camel trypanosomosis which is commercially available protein A peroxidase conjugate have been used to diagnose the trypanosome infection in camels. Recently, however, card agglutination test has been introduced for the diagnosis of Gambian sleeping sickness ("Testryp CATT", Smith & Kline).This card test has been successfully adapted for the serodiagnosis of evansi infection in camels [32].

Antibody Detection: A type of serological test which is used to detect specific antibodies (which are blood proteins belonging to the immunoglobulins), developed by the host against the infection. Antibody techniques include complement fixation test (CFT) that has been used in the diagnosis of *T. equiperdum* in equines and *T. evansi* [33]. Enzyme-linked immunosorbent assays (ELISA) and indirect fluorescent antibody tests (IFAT) have been used in herd diagnosis of trypanosomes [34] (Stephen, 2003). Card agglutination test for trypanosomosis (CATT), the simplest test for *T. evansi*, has also been used [35].

Antigen Detection: Trypanosome-antigen detection in blood or serum is more reliable and has shown a high correlation with patent or sub-patent disease in camels [36]. Enzyme immunoassays have been developed for the detection of antigens rather than antibodies as a means of diagnosis [37]. These assays detect the circulating antigens of *T. congolense*, *T. vivax* and *T. brucei* in blood of infected animals. Latex agglutination test (LAT) has also been used specifically for *T. evansi* [38]. The demonstration of trypanosome antigens is equivalent to parasitological diagnosis and thus an indicator of current infection if an animal has not been recently treated for the disease [39].

The monoclonal antibody used in antigen ELISA is directed at an internal or somatic unsecreted antigen that is only released after trypanosome lysis. Thus, in early infection, before the first parasitaemic peak, the test can give negative results due to absence or low levels of antigens in blood. It is, therefore, important to combine antigen detection ELISA with the parasitological techniques for effective diagnosis of trypanosomosis[40].

Molecular Examination: Molecular techniques are suitable for detecting parasites in the mammalian host and in the insect vector and currently they are the main research tools. The principle of molecular tests is the expression of the occurrence of nucleotides, which are specific for a trypanosome subgenus, species or even a type or strain. Two main methods are used: DNA-probes and polymerase chain reaction (PCR) [5].

DNA Probes (Nucleic Acid Probes): In DNA-probes, the sample to be examined is heated to separate the two strands of DNA, which are then fixed to the membrane to avoid recombining after cooling. A probe, which is a linear sequence of nucleotides prepared to correspond with a similar sequence of the parasite in the sample, is added. Then, the probe will link (hybridize) with that part of parasite DNA and this will be detected when the probes are labeled with radioactive isotopes or enzymes for use in ELISA [41].

Polymerase Chain Reaction (PCR): The PCR is applicable based on the use of enzyme DNA polymerase that will amplify the sequences of DNA bases, until sufficient DNA material is produced to detectable levels. The parasite DNA (e.g RoT1.2VSG) is denatured by heat and two primers are used that are short sequences of nucleotides (one for each DNA 18 strand) complementary to a specific site on one of the two single parasite DNA strands. The primers attach to the complementary sites and the DNA polymerase then starts to reproduce the rest of each complementary sequence, which follows from that primer. Thus, the polymerase amplifies minute DNA bases when the cycle is repeated. Small amount or a specific region of DNA to be amplified, using precise temperature conditions and ingredients like primers, which read the specific region of DNA polymerase, which can synthesise a copy of the DNA region and deoxynucleotide triphosphates (dNTPs), which build up the new DNA copy [42].

In order to detect trypanosomes and avoid false positive results, it is possible to combine PCR and the DNA probes technology [38]. Small amount or a specific region of DNA to be amplified, using precise temperature conditions and ingredients like primers, which read the specific region, a DNA polymerase, which can synthesise a copy of the DNA regon and deoxynucleotide triphosphates (dNTPs), which build up the new DNA copy [42].

Immunological Consequences: Pronounced immune consequences occur in camel trypanosomosis. An increase in gamma-globulin (IgM) during both acute and chronic *T. evansi* infections in camels has been reported [43], but this is not protective, as the majority of the antibodies are auto antibodies. Leucocytosis, neutrophilia and eosinophilia have been reported in *T. evansi* infections of camels [44].

The mononuclear phagocytic cells are expected to accumulate in tissue in response to tissue injury. In the acute phase of the disease, lymph nodes and spleen are remarkably reactive, with plasma cells predominating. This may account for the generalized lymphoid tissue hyperplasia characteristic of *T. evansi* infections, while in the late stages the immune system becomes depleted of lymphoid cells [45].

Immune Responses: Trypanosomosis is a disease affecting the immune system of the host animal. Although the immune system is designed to protect from pathogens, it can sometimes be overwhelmed, respond inappropriately or result in immune mediated disease with clinical signs [46]. Circulating trypanosomes are rare this may be because the immune response is directed against both parasites and self antigens. The parasites might achieve this through molecular mimicry or inflammation and tissue damage leading to the release of tissue proteins which stimulates formation of self antigens [47].

Trypanosomaevansi is purely extracellular parasites survives, multiply and differentiates in extracellular fluids of the mammalian host including the aggressive vascular environment. Thus, these parasites are permanently confronted with the multiple components of the host's immune system ranging from innate to adaptive immune responses. Among many molecules, the trypanosomal DNA and the GPL anchor of the VSG that might be released from the dead trypanosomes has been shown to activate macrophages to secrete pro-inflammatory molecules like TNF, IL-6, IL-1, IL-10 and NO as the first response of the host immune system that are involved in the control of the first peak of parasitaemia by the toxic nature of TNF and NO for both the host cell and the parasite [46].

An increase in gammaglobulin (IgM) during both acute and chronic *T. evansi* infections in camels has been reported [48] but this is not protective, as the majority of the antibodies are auto antibodies. In the acute phase of the disease, lymph nodes and spleen are remarkably reactive. This may be accounted for the generalized lymphoid tissue hyperplasia characteristic of *T. evansi* infections, while in the late stages the immune system becomes depleted of lymphoid cells [49].

Immune Evasion and Antigenic Variation: The blood stream form of African trypanosomes are entirely covered by 5×10^6 dimers of variable surface glycoproteins (VSG), which is the most abundant surface protein in the blood stream form of the trypanosomes. It forms a dense surface coat of 12 to 15 nm over the entire surface of the trypanosome and accounts for about 15 to 20 % of the total protein content of the bloodstream form of the parasite [50]. This surface coat is attached to the outer membrane of the trypanosomes by glycosylphosphatidlinositol (GPL) anchors, which make the variable surface antigen water insoluble and may contribute to the host's immune response to trypanosome infection [51].

The VSG repertoire of *T.evansi* is smaller than that of trypanosomes with a tsetse fly intermediate host because exchange of genetic information and rearrangement of VSG repertoires occurs in this vector [52]. During the ascending of the parasitaemia, the majority of parasites are the same antigenic type (called homotype). The host immune system recognizes this homotype and makes antibodies against it. As the parasites of the major variable antigenic type (VAT) are eliminated the parasitaemia goes in descending phase but at the same time, the parasites expressing the heterotype or the minor VATs are multiplying and one of them overgrows others. As a result, this one becomes the new homotype, leading to a new wave of parasitaemia and resulting in a long-lasting chronic infection. So expression of the VSG is central in the antigenic variation process and eventually for exhausting the host immune system in the benefit of the parasite [53].

Immune Suppression: Pathogenic trypanosomes induce a generalized immunosuppression of both humoral antibody response and T-cellmediated immune responses. As a result, in the long term, the host's immune responses fail and it succumbs to either the overwhelming parasite load or to secondary infection, consequently leading to occurrence of the trypanosome-induced immunopathology. Various studies have shown that polyclonal B-cell activation, generation of suppressor T-cells and macrophages and altered antigen handling and presentation are all mechanisms that could be involved in trypanosome mediated immunosuppression [32].

Macrophages are central to immunosuppression and that up on activation of these cells a variety of factors and cytokines are released which cause a range of effects such as B-cell activation and T-cell suppression. During trypanosome infections, TNF which are secreted by classically activated macrophages are involved both in parasitaemia control and infection associated pathology like anemia, organ lesion and fever. Trypanosome induced immunosuppression is also appeared to be due to the action of trypanosome enzymes. Trypanosome enzymes, such as phospholipase, neuraminidases and proteases have all been implicated in membrane fluidity and cellular damage [54].

CONCLUSION AND RECOMMENDATIONS

Trypanosomosis in camel caused by *T. evansi* is still a serious problem in camel husbandry and causes considerable economic losses in many camel-rearing regions of the world. There are different diagnostic techniques have been developed for diagnosing trypanosome infections. These include parasitological demonstration using Giemsa stained blood smear, animal inoculation and haematocrit centrifugation technique,serological tests by detecting anti-trypanosomal antibodies and antigens by card agglutination test for trypanosomes (CATT), chemically identification of protein level and for DNAamplification, by Polymerasechain reaction (PCR), with primers yielding

for the specific detection of Trypanozoan.*T. evansi* as purely extracellular parasites are permanently confronted with the multiple components of the host‘s immune system ranging from innate to adaptive immune defences. Among many molecules, the trypanosomal DNA and the GPL anchor of the VSG that might be released from the dead trypanosomes has been shown to activate macrophages to secrete proinflammatory molecules as the first response of the host immune system. However as a prototype of extracellular parasites, these pathogens defend humoral immunity through a subtle mechanism of antigenic variation.

Thus, based on the above conclusion, the following recommendations are forwarded:

- Because of the negative effect of *surra* in the world there is a need for operational research to compare, using field trials, the feasibility and sustainability of alternate *surra* diagnostic and control programmes.
- Veterinarians should take precaution during diagnosis since the parasite is mostly appearing in chronic form.
- Biology of the parasite as well as the host-pathogen interaction needs to be studied for each specific geographical area as there might be variations in the strains of the parasites and the responses of camels to the disease.
- The dynamics of mechanical transmission of camel trypanosomosis in endemic areas has to be thoroughly studied by including those factors contributing to occasional outbreak.

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