

Experimental transmission of *Besnoitia caprae* in goats

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ABSTRACT

Experimental transmission of *Besnoitia caprae* from naturally chronically-infected goats to susceptible ones was achieved by intra-nasal instillation and intra-conjunctival inoculation of cystozoite-containing suspensions, subcutaneous implantation of fascia containing cysts and alternate needle pricking between the infected and non-infected goats. Typical chronic symptoms developed in the fascia-infected does. Cystozoite inoculation into the eyes and mouth did not result in infection. Kids born of dams with acute and chronic besnoitiosis did not contract the infection *in utero*, suggesting that intra-uterine transmission may not occur. In contrast to does with acute besnoitiosis, which occasionally aborted, the does with chronic besnoitiosis gave birth to healthy kids. Kids below the age of 4 months (pre-weaned period) born of both infected and non-infected does were susceptible to besnoitiosis but appeared to be more resistant than adult goats.

Key words: *Besnoitia caprae*, goats, transmission.

Njenga MJ, Kang'ethe EK, Bwangamoi O, Munyua SJM, Mugeru GM, Mutiga ER **Experimental transmission of *Besnoitia caprae* in goats.** *Journal of the South African Veterinary Association* (1999) 70(4): 161–163 (En.). Faculty of Veterinary Medicine, University of Nairobi, Kabete, Kenya.

INTRODUCTION

While the epidemiology of *Besnoitia caprae* under field conditions is not yet elucidated, it has been shown that goats can develop clinical signs of besnoitiosis (*B. caprae* infection) when inoculated intravenously or subcutaneously with cystozoites harvested from chronic cases of caprine besnoitiosis or intravenously with endozoites in blood obtained from goats in the acute phase of the disease⁶. Attempts to reproduce the disease in cattle, sheep, rabbits, guinea pigs, rats and mice confirmed that only goats were susceptible to infection with *B. caprae*⁶.

During an outbreak of the disease in eastern Kenya, Bwangamoi *et al.*⁴ observed that goat kids developed besnoitiosis. However, it was unclear whether the kids were infected *in utero* or during the first 4 months of life (pre-weaning period).

Transmission of *B. besnoiti* from infected to healthy cattle has been accomplished by intravenous inoculation with cystozoites and endozoites, subcutaneous implantation of fascia containing cysts, alternate needle pricking between chronically infected and healthy cattle, intranasal and intra-ocular administration of cystozoite-containing suspension and

oral drenching with endozoite-containing material. Neither intranasal transmission using endozoites nor oral transmission using cystozoites was successful^{1,2}. This study was undertaken to investigate possible modes of transmission of *B. caprae* in goats. Goats were inoculated by various routes. Intraconjunctival inoculation was included to establish whether the presence of thorn remnants in the eyelids might play a role in mechanical transmission with subsequent development of infection. It was approved by the Faculty of Veterinary Medicine Research Committee, which also serves as the Ethics Committee.

MATERIALS AND METHODS

Experimental animals and infective material

Experimental animals were obtained and infective materials (cystozoites and endozoites) were prepared as previously described⁶ except that cystozoites were suspended in phosphate-buffered saline (PBS) instead of Hank's buffered salt solution and that only does and kids were used in this study. In addition, all the does were allowed to cohabit with intact bucks for a month before being recruited into the trials, except for those used for intra-uterine transmission trials, which were allowed to cohabit with the bucks for 3.5 months to increase the probability of conception.

The inoculum dose was standardised to contain $c. 1 \times 10^9$ cystozoites per ml PBS. However, in the experiments with endozoite-containing blood, fascia implantation, needle-pricking and *in utero* transmission, standardisation was not possible.

Four does were used for each of the experimental routes. All the does were monitored daily for temperature reactions or development of conjunctival cysts throughout the observation period, which varied from 24 days to 6 months. Establishment of the infection was confirmed histologically as described previously⁶.

Infection protocol

Subcutaneous implantation

Intact fascia containing *Besnoitia* cysts, obtained aseptically from a chronically-infected goat, was inserted in a subcutaneous pocket created aseptically on the lateral aspect of the neck of a recipient doe. The incision was closed with a nylon suture (No. 2.0, Johnson and Johnson, Kenya) in a single interrupted suture pattern. A tetracycline spray with a fly repellent (Alamycin[®], Norbrook, Kenya) was applied once a day until the lesion had healed.

Needle-pricking

A chronically-infected goat served as the source of the infective material. The donor goat and 4 healthy recipients were sedated with 0.3 ml xylazine hydrochloride (Rompun[®], Bayer, EA). The plantar surfaces of the forelimbs from the carpal to the metacarpal joint were shaved and the skin desensitised with 2% lignocaine hydrochloride (Dawa Pharmaceutical) at the carpus. A roughened sterile 25 g hypodermic needle was then used to make 150 subcutaneous alternate needle-pricks between the sick goat and the healthy ones.

Intra-uterine inoculation

Eight does (4 healthy and 4 with chronic besnoitiosis) were used for the intra-uterine transmission trials. They were allowed to cohabit with intact bucks for 3.5 months to increase the chances of conception. The 4 healthy does were each inoculated with $c. 10 \text{ ml } 1 \times 10^9$

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Received: April 1999. Accepted: September 1999.

cystozoites slowly intravenously. On day 24 post-inoculation 2 pregnant does, one artificially infected and the other naturally infected, were euthanased and, together with their foetuses, fully necropsied. Tissues, including the skin, ears, conjunctiva and the placenta, were histologically checked for the presence of *Besnoitia* cysts. The other 6 does were allowed to carry their pregnancies to term.

Intra-nasal instillation

The 4 does used for this part of the study were each treated by slow intranasal instillation of 4 ml PBS containing $c. 1 \times 10^9$ cystozoites per ml into the nasal cavity. The head was held high during inoculation and held in the same position for a further 3 minutes thereafter to avoid loss of the inoculum through the nostrils.

Intra-conjunctival inoculation

Four ml PBS containing $c. 1 \times 10^9$ cystozoites/ml were concentrated by centrifuging and decanting the supernatant to leave a volume of 1 ml into which the packed cystozoites were resuspended. Each of the 4 does was intraconjunctivally injected in both eyes with 1 ml of the inoculating material.

Ocular instillation

Two ml of PBS containing $c. 1 \times 10^9$ cystozoites/ml were concentrated as above, and 0.5 ml of the suspension very slowly instilled as droplets into both eyes of the 4 goats.

Oral instillation

Forty ml PBS containing an estimated 1×10^9 cystozoites/ml were dosed orally to each of the 4 goats.

Inoculation of unweaned kids (aged less than 4 months)

Two groups, each consisting of 4 unweaned kids aged between 2 and 3 months, were inoculated with 5 ml PBS containing 1×10^9 cystozoites/ml by the intravenous and subcutaneous routes respectively.

All experimental animals were clinically examined every morning, while blood smears were prepared on alternate days. The smears were subsequently stained with Giemsa and microscopically examined for endozoites. Placentas from ewes that aborted, kiddens normally, and were euthanased, were all examined for cysts.

RESULTS

Sub-cutaneous implantation of fascia

On the 3rd day post-inoculation the prescapular lymph nodes on the side

where the implant was placed of all 4 does started swelling and remained swollen for about a month. Lymph node aspirate smears did not reveal the presence of endozoites or cystozoites. On day 24 post-inoculation the does developed a large number of minute dew-drop-like transparent immature cysts in their scleral conjunctiva, which transformed into typical sand-grain-like cysts as they matured. The presence of cysts in the conjunctiva of the does was confirmed histopathologically after euthanasia. One doe gave birth to a kid that did not have or develop cysts in the eyes or skin before the end of the observation period at weaning. The other does did not conceive.

Within 6 months the does had all developed symptoms of chronic besnoitiosis characterised by alopecia and hyperkeratosis of the skin, especially over the carpal, metacarpal, hock and metatarsal joints, the muzzle and the dorsum. The does had a bright demeanour throughout the observation period.

At necropsy a large number of subcutaneous *Besnoitia* cysts were observed in the limbs, muzzle, ears, nasal cavity, trachea and dorsum. The uterine horns and body, oviducts and the broad ligament were free of cysts. In each case the cervix had occasional cysts and the density of cysts increased progressively through the vagina to the vulva and the skin around the perineum.

Needle-pricking

The 4 does recruited for this trial showed inappetence, dullness and were reluctant to move for the first 3 days post-pricking due to pain in the legs. From the 4th day they started to regain their appetite and their demeanour brightened. *Besnoitia* cysts appeared in the scleral conjunctiva of the does on day 37 post-inoculation. This was confirmed by histopathology after euthanasia of 2 of the does. The other 2 does gave birth to healthy kids.

Intra-uterine inoculation

Besnoitia cysts were observed in the skin of the 2 does euthanased on day 24. The foetuses removed from the uteri, after euthanasia, were found to be normal. Six kids born from the other 6 does, which were observed for 6 months, were normal. The placentas in all cases were free of cysts.

Intra-nasal installation

The 4 does used for intranasal inoculation remained bright throughout the observation period but developed cysts on days 32 and 40 post-inoculation. One

infected doe gave birth to a healthy unaffected kid, while the other 3 did not conceive.

Intra-conjunctival inoculation

One of the does developed a high fever of 41 °C on day 16 post-inoculation, which persisted for 6 days before returning to normal. On the 2nd day of fever, the doe developed oedema of the skin, limbs and head and she aborted on the 4th day of fever. No *Besnoitia* cysts were observed in any of the tissues obtained from the aborted foetus. The placenta was oedematous but no *Besnoitia* cysts could be found. Endozoites were found in blood smears during the period of fever and for 3 days thereafter. The other 3 does were not pregnant but developed conjunctival cysts on day 25 post-inoculation

Ocular instillation

The does showed increased lachrymation for 2 hours after administration of the inoculum but remained bright and unaffected throughout the 6-month observation period. Repetition of this experiment yielded the same results. The does used in this trial did not conceive.

Oral drenching

The does used in the oral transmission trial remained normal throughout the observation period. One doe gave birth to a normal kid, while the others did not conceive.

Transmission to pre-weaned kids.

Three of the 8 kids (37.5 %) inoculated became infected. Two of the 3 kids had been inoculated intravenously and the other subcutaneously. All the affected kids developed *Besnoitia* cysts 32 days post-inoculation. The other 5 had no evidence of infection.

DISCUSSION

The findings reported in cattle^{1,2} were confirmed in goats in the present study using *B. caprae*. In addition, it was observed that oral and ocular transmission were not possible using cystozoites. During an earlier study⁶, we confirmed that transmission of *B. caprae* by intravenous and subcutaneous inoculation with cystozoites and intravenous inoculation with endozoites was possible. Based on the findings of the present study, it is postulated that infectivity of endozoites and cystozoites differs. Further studies are necessary to determine their role in the pathogenesis of the disease and their apparent differences in infectivity.

Many necropsies performed on naturally-infected goats revealed the presence of remnants of thorns in the subcutis of

the extremities, including ears, eyelids and the fore limbs. This finding, together with the preference of goats to browse on thorny *Acacia* bushes and the successful transmission by needle punctures, indicates that the possible role of thorns in mechanical transmission of *B. caprae* should be studied.

In the present and earlier studies³, pneumonia was frequently the immediate cause of death in cases of caprine besnoitiosis. Histopathological examination of the upper respiratory tract of naturally and experimentally infected goats revealed a large number of cysts. This finding further indicated that the cysts could rupture spontaneously to release cystozoites. Close contact between animals in overnight enclosures and the presence of many pneumonic coughing goats with chronic besnoitiosis could facilitate transmission through inhalation. Successful intranasal transmission in the present study further supports this hypothesis.

It is not clear why ocular transmission was not successful, since the lachrymal ducts open into the nasal cavity. The role of the excess tears in physically clearing the inoculant and the possible role played by humoral factors in the tears need further investigation.

In utero transmission was unsuccessful despite repeated trials. The absence of *Besnoitia* cysts in histological preparation of tissues of the uterus, placenta, foetuses and kids, and the ability of chronically sick does to give birth to healthy viable kids, makes this an unlikely route of transmission.

Abortions, when they occurred, were always associated with accompanying fever and oedema of the does. This may partly explain the abortions reported by Wilsmore⁷ in the Embu, Meru and Isiolo (EMI) goat breeding station of Marimanti in Meru district.

Bigalke² reported that the incidence of bovine besnoitiosis was lower in calves than adult cattle. The low clinical experimental infection rate in kids observed during this study and the field findings of low incidence, may indicate that the kids are more resistant to besnoitiosis than adult goats. The immunological or physiological basis of this resistance should be investigated, since young animals are more susceptible than adults to toxoplasmosis, which is closely related to besnoitiosis⁵.

ACKNOWLEDGEMENTS

We are grateful to Karira Ngigi for taking care of the experimental animals, Edward Kiarie for technical assistance

and Manuel E. Rodriguez of St Carlos University for reading the manuscript. Funds for this study were provided by DAAD, Germany.

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