Prevalence of genital campylobacteriosis and trichomonosis in crossbred breeding bulls kept on zero-grazed smallholder dairy farms in the Tanga region of Tanzania

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ABSTRACT

A survey to demonstrate the presence or absence of genital campylobacteriosis and trichomonosis in cross-bred breeding bulls kept under smallholding dairy farms in the Tanga region of Tanzania was carried out during the period of January–June 1996. Sheath washings, swabs and preputial scrapings were collected from 58 randomly selected bulls. *Campylobacter fetus* subsp. *venerealis* was demonstrated in 3/58 (5.1 %) and *Tritrichomonas foetus* in 0/58 (0 %) of all bulls tested. Bull-level variables of level of *taurine* genes (62.5 % *taurine* genes, F2; 75 % *taurine* genes, F3) and age were not significantly associated with campylobacteriosis ($P > 0.05$). The result of the study identifies *Campylobacter fetus* subsp. *venerelias* as the agent of enzootic infertility in smallholder herds and suggests that may be a significant problem.

Key words: breeding bulls, *Campylobacter fetus* subsp. *venerealis,* prevalence, smallholder, Tanzania, *Tritrichomonas foetus*.

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INTRODUCTION

Cattle production is the main component of livestock production in most farming systems in sub-Saharan Africa[10]. However, productivity of these cattle is low due to poor genetical potential, inadequate nutrition and animal health problems[9]. One of the factors causing low productivity is reproductive wastage characterised by infertility in both males and females. The causes of infertility are multifactorial ranging from specific infections to non-specific non-infectious causes[13,24]. Genital infections, characterised by multiple causes, are 1 example of the specific infections associated with infertility in both male and females[18]. The commonest genital diseases, which cause infertility in both males and females, include bovine virus diarrhea, brucellosis, genital campylobacteriosis, chlamydiosis, epididymitis-vaginitis (epivag), infectious pustular vulvovaginitis (IBR/IPV), leptospirosis, toxoplasmosis and trichomonosis. Both the intensive and semi-intensive management of stocking many cattle together results in the spread of these diseases. The sexually transmitted venereal diseases like genital campylobacteriosis and trichomonosis are also easily transmitted by communal bulls in management systems commonly found all over Africa[24]. Studies conducted in Egypt showed the prevalence of campylobacteriosis in bulls to be 4 %[7]. In Malawi, the range was 10–15 % in both intensive and abattoir samples[11]. The prevalence of *T. foetus* in Costa Rica ranged between 3.9 and 6.2 %[17], and in Nigeria it was 2.6 %[1]

Although infertility problems in livestock production is very significant economically and is well acknowledged as a serious reproductive performance problem worldwide, prevalence of infectious causes of infertility *i.e. Tritrichomonas* and *Campylobacter* spp. have not been investigated in Tanzania. This study was initiated and carried out firstly as a response towards reported cases of abortion from smallholders farms. Secondly as a part of a breeding bull health-screening routine and thirdly to generate information on the magnitude of these pathogens in crossbred breeding bulls kept on smallholder dairy farms in the Tanga region of Tanzania.

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MATERIALS AND METHODS

Study area

The study was conducted on 58 zero-grazed breeding bulls that were randomly selected from a sampling frame of 128 bulls in the Tanga region, northeastern Tanzania. The list of the breeding bulls was obtained from the Tanga dairy development programme (TDDP) database. Tanga region lies between longitude 36° and 38°E and latitude 4° and 6°S. The region has heterogeneous physical and climatic features varying from hot humid coastal lowlands in the east to the cool Usambara Mountains in the north and a semi-arid plain in the southwest. There are 2 rainy seasons, the long rains occurring between March and May and the short rains occurring between September and November. Daytime temperatures vary from 23–28.0 °C during the cool season (May to September) to 30–33.0 °C during the hot season (December to March).

Cattle breeding system and type of bulls

Natural mating is the most commonly used breeding system in most of the small-scale dairy farms (defined as those having less than 10 dairy cattle of both sexes) . Artificial insemination (AI), often practiced by less than 5 % of all smallholder farms in the region, is limited to urban (occurring within towns) and periurban (those peripheral to towns but within a 15 km of the town centre) located farms. AI is not practiced in rural (those occurring at 15 km or more from a town centre) located farms due to logistics and economy of scale reasons. The type of breeding bulls kept include taurine breeds (Friesian, Aryshire, Simmental) and crosses of these breeds with *Bos indicus* (Tanzania short-horn zebu, boran and Sahiwal). The level of *taurine* genes varied from 62.5 % for F2 (second filial generation) and up to 75 % for F3 (third filial generation). The majority (>80 %) of the breeding bulls used are crosses of Holstein Friesian and Tanzanian short-horn zebu. Identification of the future breeding bulls is done through TDDP.

Artificial insemination born bulls from pure taurine sire and best performing first filial generation (50 % level of *taurine* genes for F1) dams are bought at the age of 3–6 months. Apart from breeding soundness examination, screening for any genital infection is not performed for these bulls. These bulls are eventually sold or supplied to peri-urban and rural farmers at a subsidised price or on loan (so that each was not used for too long in a single herd). Sampling of bulls was carried out during the period January–June 1996.

Study design

All recorded and 'approved' bulls (defined as a bull born from F1 dam and pure taurine sire, F1 dam producing >10 ℓ/day during first lactation) were eligible for the study[23]. *A priori* prevalence (P) of 7.5 % for both *Tritrichomonas* and *Campylobacter* spp. was used in computing the sample size required, based on the results from the previous study in the tropics[6,8,11,16]. There are a total (N) of 128 eligible 'approved' breeding bulls listed in the proposed study area. The sample size needed was determined as[15]:

$$n = (Z^2_{\alpha(2)} \times PQ)/L^2,$$

where $Z = 1.96$, P = the disease risk, $Q = 1 - P$ = disease-free risk, L = desired absolute precision level set at 5 %, $n = 1.96^2 \times 0.075 \times 0.925/0.05^2 = 106.6$ bulls. Since sampling fraction (f): ($f = n/N$) was larger than 5 % of the total population size, a correction factor arrived at as follows was adopted: $n(c) = n/(1 + f)$. The number of bulls to be sampled was given as: $n(c) = 106.6/(1 + 0.831) = 58$.

Collection of samples

Campylobacter fetus subsp. *venerealis*

Swabbing. Bulls to be sampled were restrained in a bull pen crush and preputial hairs washed with phosphate buffered saline (PBS). The preputial orifice was parted with a gloved hand and a sterile guarded swab (Sterlin, UK) was pushed about 4–6 cm into the preputial cavity. The swab was removed and placed into a charcoal transport medium (Biotrading, T/S 5–6) and transported to the local laboratory within 8 h[14]. Inoculation was performed immediately upon arrival from field on a selective *Campylobacter* medium (Campylobacter Blood free selective medium, Biotrading). The isolation medium contained peptone 25g/ℓ, charcoal 4 g/ℓ; sodium chloride 3 g/ℓ, sodium-desoxychocolate 1 g/ℓ, ferrisulphate 0.25 g/ℓ, sodium pyruvate 0.25 g/ℓ and agar 12 g/ℓ. The medium was enriched with cefoperazon 32 mg/ℓ and amphoteracin 100 mg/ℓ. Media were incubated at ± 37 °C for 72 h under micro-aerophilic conditions in anaerobic jars with Compypac Plus micro-aerophilic envelope with Catalyst (Becton Dickinson).

Washings. Preputial washings were harvested through the use of an artificial insemination pipette. Sixty mℓ of sterile PBS, pH 7.2, was introduced into the prepuce and massaged vigorously for 3 min. Collected fluid was transported to the local laboratory within 8 h (under cooled condition, 2–10 °C). Collected washings were centrifuged (3000 *g* for 20 min). The sediment was inoculated on a selective *Campylobacter* medium (as above) and incubated at 37 °C for 72 h under micro-aerophilic conditions (see above).

After 72 h the media were inspected for any colony growth. All 'suspicious' colonies growth was sub-cultured for bacterial isolation using standard procedures[3,12]. Bacterial isolates were identified by Gram stain and biochemical reaction characteristics. Identification of *C. fetus* isolate was carried out using cytochrome oxidase with a test strip (Bactident®, Merck) and catalase test (carried out with 3 % H_2O_2). Confirmatory tests to differentiate *C. fetus* subsp. *venerealis* from other *Campylobacte*r species were carried out at ID-DLO, Lelystad, the Netherlands. Samples were dispatched in a special incubation bags (Anaerocult®, C mini, Merck). Isolates showing a growth at 25 °C in the presence of 1 % glycine and producing H_2S in a cysteine-medium measured with a lead acetate-impregnated strip were considered as *Campylobacter fetus* subsp. *venerealis.*

Tritrichomonas foetus. To investigate the presence of *T. foetus,* the In pouch™ TF test (Bio Med Diagnostic, USA) was used. The test is known to a have a sensitivity of 88 %[22]. The test is flexible plastic pouch with an upper and a lower chamber. These chambers together contained about 4 mℓ of an enriched protease peptone medium[20,25]. The inoculation is done into the upper chamber. After that, the pouch is folded down and the contents of the upper chamber are forced into the lower chamber as described by Borchardt *et al.*[2]. Preputial scrapings collected near the fornix, by AI pipette while applying suction with a 20 mℓ syringe were immediately inoculated into the In pouch™ TF test. The pouches were examined for the presence of *Tritrichomonas foetus* with a microscope at a magnification of ×10 or ×100 after 48, 72 and 120 h of incubation at ±37 °C.

Data analysis

Data files for the studied parameters were edited, developed and analysed using Epi-Info[5]. Descriptive statistics were computed for different variables. The proportion of categorical or independent variables (*i.e.* age, level of *taurine* genes) were computed and compared for statistical significance by chi-square (χ^2) tests at a critical probability of $P = 0.05$. Confidence limits for the exact binomial proportions [5] for each variable were generated for all prevalences. Only *C. fetus* subsp. *venerealis* results were analysed statistically, as there was no *T. foetus* isolate recorded in our study.

RESULTS

Descriptive statistics

All selected bulls were sampled during the period January to June 1996. The proportion of bulls in each category of each variable investigated during the study is shown in Table 1.

The average age of study bulls was 3 years (range 1.5 to 7 years). Comparative analysis of prevalence between F2 and F3 crossbred bulls indicates that the prevalence of *C. fetus* subsp. *venerealis* was not affected markedly by the level of *taurine* blood genes ($P > 0.05$). Stratification of bull by age group, reveals that bulls of 1.5 to 3 years were more susceptible to *C. fetus* subsp. *venerealis* ($P > 0.05$) than above this age group. The overall prevalence of *C. fetus* subsp. *venerealis* (confirmed by Gram stain and biochemical tests) and

Table 1: **Proportion and prevalence of *Campylobacter fetus* subsp. *venerealis* for each category investigated.**

Variable	Category	No. of bulls (%)	Prevalence (%)	95 % CI of prevalence
Age*	1.5 to 3 yr	39 (67.23)	2/39 = 5.10	0.59–16.5
	>3 to 5 yr	17 (29.3)	0/17 = 0	0.00–19.5
	>5 yr	2 (3.4)	1/2 = 50	1.25–98.7
Level of *taurine* blood gene*	F2	48 (82.7)	1/48 = 2.08	0.05–11.36
	F3	10 (17.3)	2/12 = 16.60	2.05–48.4
Overall		58 (100)	3/58 = 5.10	1.04–13.93

Differences with asterisk (*) are not significant at $P > 0.05$.

T. foetus was estimated to be 5.1 % (95 % confidence interval (CI =1.04–13.92) and 0 % (95 % CI = 0.00–6.16), respectively.

Culture and biochemical tests

Preliminary culture results (swab and preputial washing) of *Campylobacter fetus* subsp. *venerealis* are shown in Table 2. For *Campylobacter fetus* subsp. *venerealis,* 15 (25.8 %) swabs and 13 (22.4 %) washing samples were 'suspicious'. All preputial scraping were negative for *Tritrichomonas foetus* after 48, 72 and 120 h.

Biochemical reaction and Gram stain for suspicious *C. fetus* subsp. *venerealis* culture growth revealed that 3 bulls out of 13 that were (washings) culture growth suspicious continued showing positive *C. fetus* subsp. *venerelias* reaction following biochemical tests and Gram staining procedures. All suspicious swab culture were negative for Gram staining and biochemical tests. The result of the final bacterial identification and biochemical tests (done in the Netherlands) of the *Campylobacter* isolate in 3 bulls revealed that, all showed growth at 25 °C and in the presence of 1 % glycine. None of them showed growth at 43 °C and also none produced H_2S with a lead acetate-impregnated strings. All were identified and confirmed as *Campylobacter fetus* subsp. *venerealis.*

Table 2: **Preliminary** (suspicious) **culture results for *Campylobacter fetus* subsp. *venerealis* after inoculation in selective media.**

Age category (years)	Type of sample	
	Swab	Washing
1.5–3	10/40 = 25	9/40 = 22.5
>3–5	5/16 = 31.2	2/16 = 12.5
>5	0/2 = 0	2/2 = 100
Overall	15/58 = 25.8	13/58 = 22.4

DISCUSSION

In this study, there was evidence that breeding bulls had been exposed to *C. fetus* subsp. *venerealis.* The estimated prevalence of 5 % was comparable to the findings of other studies in Central and southern African countries[19,21]. The observed prevalence was slightly lower than the *a priori* estimate of 7.5 %. The possible explanation could be due to the zero-grazing system of bull-keeping employed coupled with few breedable females per farm (range 1 to 3), thus limiting wide-scale contact between bulls and cows[18]. The reason for the increased number of cases of genital campylobacteriosis in bulls of a young age group (1.5–3 years) was not very clear. Differences in sample size may account for the relative susceptibility of the crossbred bulls in this small-scale farming system. The higher prevalence amongst this age group indicates that *C. fetus* subsp. *venerealis* infection was common among young bulls. The young bulls may be a potential source for spread of *C. fetus* subsp. *venerealis* in this region. This observation is in disagreement with other reports [4,9] which singled out young age group as less susceptible to *C. fetus* subsp. *venerealis* and therefore a less competent reservoir of pathogen because of decreased size and smaller number of crypts in the penis epithelium.

As *C. fetus* subsp. *venerealis* is not readily laboratory detectable, 2 methods of sampling (swab and washing) were used in this study. There was a degree of agreement between the results for swabs and washing. All swab 'positive suspicious' also showed 'positive suspicious' results for preputial washings. The preputial washings appeared to be more sensitive than swab in this study suggesting that optimum isolation of *C. fetus* subsp. *venerealis* can be made *via* samples derived from preputial washings. This procedure may therefore be recommended (in the present area of study) for routine genital sample collection. The reason for using 2 methods was to provide a wide range or probability to detect the pathogen. The use of blood-free selective medium contrary to the blood agar was purposely made due to the advantage of long shelf life (84 days) and less environmental susceptibility[12,14,22]. The disadvantage of the blood-containing media is the short shelf life (21 days) and the higher susceptibility to changes in the environment.

Tritrichomonas foetus could not be detected in this study. The reason was less clear, possibly due to the small number of breeding bulls sampled. However, further surveillance and investigation is required. Although *T. foetus* could not be detected in this study, preputial scraping method for the detection of *T. foetus* proved to be handy and useful under field conditions.

Consistent with the views of the present authors, the role of level of *taurine* genes (F2 and F3) in maintaining the *C. fetus* subsp. *venerealis* remain uncertain in this study. This is because, since there is no reference available on genital campylobacteriosis in smallholder units (evolved during mid 1980s) in Tanzania, no definite opinion can be expressed on the role of the level of *taurine* genes in genital campylobacteriosis. Nevertheless, the small sample size of F3 recorded could have contributed to the lack of association in this study. As far as we are aware, this is the first structured study to have identified and quantify the 2 genital infections in smallholder dairy herds kept in urban, peri-urban and rural areas of Tanzania.

One practical limitation of cross-sectional studies like this one is the inability to detect disease of low prevalence rates (*i.e.* most of genital related infections)[15]. Larger prospective, longitudinal studies would be required for a comprehensive and specific investigation of potential causes of sub-fertility and economic impact in this farming system.

In conclusion, *C. fetus* subsp. *venerealis* proved to be present in the breeding bulls and could be one of the causes of infertility in smallholder herds. The use of AI and regular vaccination of all cows and bulls including newly introduced animals could be a potential strategy. Regular screening of all breeding bulls and treating or culling of all positive carrier as a policy required to be developed, implemented and monitored.

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