# Brucellosis and Tuberculosis in Arsi-Negele District, Ethiopia: Prevalence in Ruminants and People's Behaviour towards Zoonoses

K. Amenu<sup>11</sup>, E. Thys<sup>2,</sup>, A. Regassa<sup>1</sup> & T. Marcotty<sup>2, 3</sup>

Keywords: Zoonoses- Awareness- Livestock- Tuberculosis- Brucellosis- Ethiopia

## Summary

A study was carried out in Arsi-Negele District of Southern Ethiopia to estimate the prevalence of brucellosis and bovine tuberculosis in livestock and to identify risk behaviours that would facilitate the transmission of zoonoses to humans. The study involved testing some 400 cattle, 200 sheep and 170 goats for tuberculosis and brucellosis and interviewing 98 livestock keepers. Single comparative intradermal tuberculin test and Rose Bengal plate test were used for the diagnosis of tuberculosis and brucellosis, respectively. Tuberculosis was recorded in 27 cattle, 1 goat and 1 sheep. In cattle, the estimated individuallevel and herd-level tuberculosis prevalence was 5.9% and 35%, respectively. The individual-level and herdlevel brucellosis prevalence in cattle was 2.6% and 12%, respectively. The questionnaire survey showed that most respondents had no accurate knowledge about the transmission of zoonoses. It was also found that some of their behaviours would potentially facilitate the transmission of zoonotic pathogens to human, such as raw animal product consumption and backyard slaughtering. Even though the prevalence of the two diseases was relatively low, surveillance and prevention may be warranted taking into account possible animal genetic improvement programs, unrestricted animal movement in the area and low awareness of the community about zoonoses, which might result in an increased transmission to humans.

## Résumé

# La brucellose et la tuberculose dans le district d'Arsi Negele en Ethiopie: prévalence chez les ruminants et comportement des personnes envers les zoonoses

Une étude a été réalisée dans le district d'Arsi Negele en Ethiopie pour estimer la prévalence de la brucellose et de la tuberculose bovine chez le bétail et identifier les comportements à risque susceptibles de faciliter la transmission des zoonoses à l'homme. Quatre cents bovins, 200 moutons et 170 chèvres ont été testés et 98 éleveurs interviewés. Le test comparatif intradermique simple pour la tuberculose a été positif chez 27 bovins, une chèvre et un mouton. Chez les bovins, la prévalence de la tuberculose au niveau individuel et du troupeau était respectivement de 5,9% et de 35%. Pour la brucellose (test au Rose Bengal) ces valeurs étaient respectivement de 2,6% et 12%. La plupart des répondants n'avaient aucune connaissance précise de la transmission des zoonoses. Certains de leurs comportements, tels que la consommation de produits animaux crus et l'abattage des animaux dans l'arrière-cour, seraient propres à faciliter la transmission d'agents pathogènes zoonotiques à l'homme. Même si la prévalence des deux maladies était relativement faible, la surveillance et la prévention peut se justifier compte tenu d'éventuels programmes d'amélioration génétique, des mouvements incontrôlés des animaux dans la région et de la faible sensibilisation de la communauté aux zoonoses, qui pourraient aboutir à une transmission accrue à l'homme.

## Introduction

Bovine tuberculosis is an infectious disease caused by *Mycobacterium bovis* and characterized by progressive development of specific granulomatous lesions or tubercles in lung tissue, lymph nodes or other organs. Cattle are considered to be the primary hosts but the pathogen has a wide host range affecting many domestic and wild species, including man (13). Bovine tuberculosis has a negative impact on livestock production in developing countries through reduced production efficiency, carcass or organ condemnation and restriction of international trade (14). It has implications not only for the economy of the livestock farming communities but also for human health (9). In the absence of efficient control systems, an unknown but probably significant proportion of human tuberculosis cases are attributable to *M. bovis* (5).

Brucellosis is another infectious bacterial disease caused by members of the genus *Brucella*. Brucelloses caused by *Brucella melitensis* and *Brucella abortus* 

<sup>&</sup>lt;sup>1</sup>Department of Veterinary Medicine, Hawassa University, P.O. Box 5, Hawassa, Ethiopia.

<sup>&</sup>lt;sup>2</sup>Department of Animal Health, Institute of Tropical Medicine, Nationalestraat 155, B-2000, Antwerp, Belgium.

<sup>&</sup>lt;sup>3</sup>Department of Tropical Veterinary Diseases, Veterinary Faculty, University of Pretoria, South-Africa.

<sup>\*</sup> Corresponding author: Tel: +251-46-220-0230; Fax: +251-46-220-6517 e-mail: k.amenu@yahoo.com

Received on 06.05.10 and accepted for publication on 05.10.10.

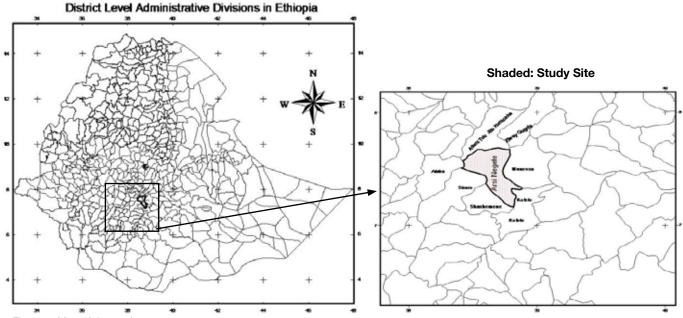


Figure 1: Map of the study area.

belong to the world's major zoonoses (15), causing great economic losses in the ruminant production systems and representing a serious health issue for the farming community. In livestock, they cause abortion, late first calving age, long calving interval time, low herd fertility and comparatively low milk production (3). Carpal hygroma is also a common clinical manifestation in cattle (15). Brucellosis is a true zoonosis in that all human cases are acquired from animals and, more specifically, from domestic ruminants as far as *B. abortus* and *B. melitensis* are concerned.

In rural parts of Ethiopia, people are mainly dependent on animals and their relationship with them is very close. Moreover, people often consume raw animal products (2). This may predispose them to zoonotic diseases such as *Mycobacterium bovis* and *Brucella* infections.

Under Ethiopian context, livestock of different species usually share pastures and dwellings. This may play a role in maintenance and transmission of endemic diseases such as tuberculosis and brucellosis.

The control of brucellosis and bovine tuberculosis in rural sub-Saharan Africa should be based on the burden of the diseases on animal production and on the actual zoonotic risk caused by these diseases (8). The zoonotic risk should ideally be measured in humans. Yet, random selection of human samples and access to biological material is often restricted by ethical and practical considerations. Alternatively, the risk of zoonotic transmission of bovine tuberculosis and brucellosis to people could be evaluated through comprehensive assessments of the pathogen occurrence in livestock and risk behaviours performed by the communities (5). Therefore, the present study was conducted to estimate, in the study area, the prevalence of tuberculosis and brucellosis in cattle, goats and sheep and to evaluate the occurrence of the main risk behaviours related to the transmission of zoonotic diseases.

## **Material and methods**

#### Study area and animal husbandry system

The study was carried out in Arsi-Negele District, Oromia Regional State (Figure 1), end of 2007. The eponymic district capital is located at 7°21°N latitude and 38°42°E longitude. Agricultural production system is of mixed crop and livestock production. Dairy farming using improved breeds is a common practice in urban and peri-urban areas. In rural areas, mainly local breeds are found, grazing on communal land. The area was selected on the basis of livestock production potential and the presence of different livestock species and breeds. The selected area represents typical crop-livestock production system of the Rift Valley area of Ethiopia. The minimum and maximum annual temperatures are 10° and 25 °C, respectively. Generally, the climate of the area is divided into subhumid (32%), semi-arid (42%) and arid (26%) zones with an average annual rainfall ranging from 500 mm to 1150 mm.

## Sampling scheme and diagnosis

Arsi-Negele district includes one town and 43 villages. The selection of animals was based on a stratified sampling method. Urban and rural environments were considered as strata. The town and six villages were selected and 98 households were randomly selected among them. In each household, all ruminants more than 6 months old were recruited. A total of 425 cattle, 170 goats and 203 sheep were tested for tuberculosis. Assuming a prevalence of 10% in cattle, 384 animals would be required to estimate the prevalence with a precision of 3% (alpha= 5%). The prevalence in

small ruminants was expected to be low. At least 149 animals must be tested to detect at least one positive if the prevalence is 2% (alpha= 5%) (17). Information pertaining to individual animals such as age, sex and breed was recorded. Body condition scoring was made based guideline described by Nicholson and Butterworth (11) and latter aggregated into three groups (good, medium and poor). The single comparative intradermal tuberculin (SCIT) test was used for the diagnosis of tuberculosis (12). In cattle, two sites 12 cm apart on the mid-neck were used for purified protein derivative (PPD) injection. Aliquots of 0.1 ml of 20,000 IU/ml bovine purified protein derivative (PPD) (Bovituber; Rhône Mérieux, France) and 0.1 ml of 25,000 IU/ml avian PPD (Avituber; Rhône, Mérieux) was injected into the dermis of the sites. In small ruminants, the PPD was injected on the left and right neck due to space limitation. For brucellosis, the same animals were sampled, with the exception of 17 cattle in which blood collection failed. Rose Bengal Plate (RBP) test (Institute Pourquir, rue de la Galera 34097 Montpellier, France) was used, to detect Brucella spp. specific antibodies (12).

## Questionnaire

Verbal consent was obtained from the respondents and the objective of the survey explained to them before start of the interview. The interviews were conducted by two of the authors (KA & AR) in local languages (Oromo or Amharic). The questionnaire focused on demographic characteristic of the interviewee, animal feeding and housing practices, knowledge about zoonotic diseases, habit of animal product consumption and handling, and dead-animal/ aborted foeti disposal practices. In total, 98 persons (46 from urban or peri-urban and 52 from rural areas), from which their animals tested for brucellosis and tuberculosis, were interviewed.

#### Data analysis

Infection prevalence data were analysed in a complex survey data model in Stata 10 (16) using area (rural or urban) as strata, villages and households as primary and secondary sampling units, respectively. The data was given a weight corresponding to the inverted products of the sampling fractions at each stage. The proportions of positive SCIT and RBP tests were analysed in multivariate logistic regression models, using the area type (rural or urban), breed, sex, age class and the body condition (for SCIT only) of the animals as discrete explanatory variables. Doubtful SCIT test results were considered negative for the statistical analysis. Three age classes ( $\leq$ 5, 5-10, >10) and 3 body condition score classes (good, medium, poor) were considered. The herd prevalences were analysed in similar models. Positive herds were those herds with at least one positive animal. Here, only the area type was tested as explanatory variable.

# Results

## Results of tuberculosis and brucellosis test

Positive skin reaction to bovine PPD was recorded in 27 cattle, 1 goat and 1 sheep (Table 1).

The sheep had a skin thickness of 14 mm three days following bovine PPD inoculation while the skin thickness before injection was 2 mm and the avian PPD only caused a skin thickness of 4 mm. The animal was a 1 year old female living in an urban area. For the 2.5 year old male positive goat, bovine PPD caused an increased thickness from 3 to 8 mm whereas avian PPD only caused an increase from 3 to 4 mm. In cattle, most skin swellings were mild, with the largest increase of 13 mm after injecting bovine PPD. A large number of inconclusive tests were recorded. The statistical analysis of positive bovine PPD reactions in cattle gave an estimated prevalence of 6.7% (95% CI: 4.4 – 10.1%) in town and 5.8% (95% CI: 3.6% - 9.4%) in rural areas, without statistically significant differences between them. No significant difference was found in bovine PPD reactivity of cattle of different age, sex, breed and body condition). The overall individual cattle level tuberculosis prevalence in Arsi-Negele was estimated to be 5.9% (95% CI: 3.7-9.3%) whereas the herd prevalence was 35% (95% CI: 20 - 55%).

		Number of positive results		Number of doubtful results*	
Species	Number tested	Mycobacterium bovis**	Environmental Mycobacteria***	Mycobacterium bovis**	doubtful results* Environmental Mycobacteria*** 12 3 1
Cattle	425	27	21	33	12
Goat	170	1	1	2	3
Sheep	203	1	1	1	1
Total	798	29	23	36	16

Table 1
Tuberculin reactivity of cattle, sheep and goats in Arsi-Negele District, Ethiopia

\* Not inclusive of positive results

\*\*Results are positive or doubtful if the skin thickness difference is at least 4 or 2 mm, respectively, more pronounced with bovine PPD than with Avian PPD [(B2-B1)-(A2-A1)  $\ge$  4 or 2 mm]

\*\*\* Results are positive or doubtful if the skin thickness difference caused by avian PPD is at least 4 or 2 mm, respectively, while the result is negative for *Mycobacterium bovis* [(A2-A1  $\ge$  4 or 2 mm) & ((B2-B1)-(A2-A1) < 2 mm)]

Table 2
Socio-demographic characteristics of respondents involved
in the survey on zoonotic disease awareness in Arsi-Negele
District, Ethiopia

Variables	Category	Frequency	Percent
Gender	female	40	40.8
	male	58	59.2
Age group (years)	≤18	3	3.1
	19-30	38	38.8
	31-45	33	33.7
	≥46	24	24.5
Occupation	farmer	42	42.9
	housewife	35	35.7
	mechanic	1	1.0
	merchant	8	8.2
	retired	3	3.0
	student	9	9.2
Ethnicity	Amhara	17	17.4
	Gurage	3	3.1
	Kembata	11	11.2
	Oromo	66	67.3
	Tigre	1	1.0
Religion	Christian	55	56.1
	Muslim	43	43.9
Educational level	no formal education	38	38.8
	primary school	47	47.9
	secondary school	13	13.3

RBP test positivity was detected in 9 cattle, 1 sheep and 1 goat. All positive animals were between 4 and 13 years old. The positive sheep was a 2 year old female living in town whereas the positive goat was a two year old male living in rural area. The estimated *Brucella* spp. seroprevalence in cattle was 2.6% (95% Cl: 0.8 - 7.4%). No effect of areas, breed or age could be detected. Sex had a statistically borderline effect (p= 0.03) with males being more infected than females. The estimated herd prevalence was 12% (95% CI: 4 – 32%), without statistical differences between rural and urban areas.

#### **Results of questionnaire survey**

Table 2 depicts the socio-demographic characteristics of the respondents. Many respondents (80/98= 81.6%) agreed with the idea that some diseases can be transmitted from animals to humans.

A relatively high proportion of the respondents acquired the knowledge about zoonotic diseases from elders (34.7%) and from their personal observation (32.7%). A large proportion of respondents (96.3%) indicated meat as a vehicle for disease transmission to humans. Only a few people responded that zoonotic pathogens could be acquired through direct contact and inhalation (Table 3).

About one- third of the respondents cook meat and boil milk to minimize disease transmission. About three-quarters of the interviewed household members in the study population reported practicing at least one activity considered at risk for transmission of zoonotic diseases. These activities were mainly related to handling animals and their products (milking, slaughtering, skinning and chopping meat) and consumption of raw animal products (Table 4). Participants were asked about their practice of slaughtering sick animal at verge of death and consuming its meat. Most respondents (83.7%) claim not to consume meat from sick animals, mainly for fear of disease transmission (72.5%).

Nine persons reported death of family members associated with disease which they thought to be zoonotic. Upon further investigation, six persons mentioned anthrax, two rabies and one *maagaa*-

Table	3
-------	---

Awareness about zoonoses transmission among the respondents who know that diseases are transmissible from animals to humans (n= 80)

Variable	Source of information	n	%
Source of knowledge*	health professionals	12	12.2
	elders	34	34.7
	mass media (radio/	3	3.1
	television)		
	veterinarians	6	6.1
	formal education	15	15.3
	own personal observation	32	32.7
Received health education about zoonoses		10	11.1
Aware of transmission through meat consumption		77	96.3
Aware of transmission while slaughtering (contact)		57	71.3
Aware of transmission through milk consumption		41	51.3
Aware of transmission via inhalation from sick animals		12	15
Aware of transmission with direct contact with sick animals		5	6.3

\*The percentage does not sum to 100% because all responses are not shown.

Variable	Category	n	%
Meat consumption habit	cooked	41	41.8
	as available (raw or cooked)	57	58.2
Reason of cooking	fear of disease transmission	33	33.7
	custom (culture)	32	32.6
	to increase palatability	24	24.5
	dislike (psychological)	5	5.1
	no reason	4	4.1
Source of meat for home consumption*	butchery	15	15.3
	slaughtered at home	58	59.2
	backyard slaughtering (qircha)**	93 23	94.9
Slaughter of cattle for home consumption		23	23.5
Slaughter of sheep/goat for home consumption		77	78.6
Slaughtering and skinning of cattle by him/herself		21	21.4
Slaughtering and skinning of sheep by him/herself		55	56.1
Slaughtering and skinning of goats by him/herself		60	61.2
Milk consumption habit*	boiled	42	42.9
	fresh raw	56	57.1
	sour without heat treatment	91	92.9
Reason for boiling milk*	fear of disease transmission	27	64.3
	custom (culture)	15	35.7
	dislike (psychological)		16.7
	to increase palatability	2	4.8
	no reason	2	4.8

 Table 4

 Animal products handling and consumption habits (n= 98)

\*The percentage does not sum to 100% because of multiple response of the informants.

\*\*'Qircha' is a backyard slaughtering practiced by a group of people who share the meat.

fardaa (literally meaning 'ascaris of horse').

Specific factors associated with transmission of tuberculosis and brucellosis were assessed. Out of 98 respondents, 16 reported that they had observed a sick and emaciated animal for long time in their herds. Twelve of them had noticed an animal with chronic cough and only one identified long lasting cough as a cause of death. History of abortion during the last one year in cattle herd was reported by 18 persons. Seven of them leave the aborted materials where they find them and eleven of them give them to dogs.

## Discussion

Brucellosis serology in this study revealed low infection prevalence in livestock in Arsi-Negele, compared to some of the previous reports in extensive livestock husbandry systems in sub-Saharan Africa (7, 10). Given the low *Brucella* spp. seroprevalence in goats, it is unlikely that *Brucella melitensis* is present at all in the area: the positive result should be regarded as either a false positive or as a *Brucella abortus* infection. The prevalence of tuberculosis in cattle is also low compared to other areas in Ethiopia (1, 2). The tuberculosis prevalence is extremely low in small ruminants, which confirms that small ruminants are unlikely to play any role in the epidemiology of zoonotic tuberculosis.

In cattle, the SCIT test results indicate that bovine tuberculosis is present and well distributed in the area. However, an important proportion of the cattle seem to be free of Mycobacterium bovis, despite the absence of control measures. In addition, the infected animals do not seem to suffer much from the infection since their body condition is not affected. Therefore, infected animals are unlikely to excrete much pathogen, compared to clinical tuberculosis cases, bringing the transmission by the aerial, digestive and milk routes to a low level. The reasons why infections are low in cattle of the study area could be related to the breed (local and cross breeds are more resistant than performing breeds) (1) or to the obstacles of transmission among animals (9). In Arsi-Negele District, animals are usually kept in free range, in a mostly dry or warm environment, unfavourable to the survival of the mycobacteria (4). Close contact only occurs at night, when animals are brought together but, even then, animals stay outside, in well ventilated conditions. Finally, herds are small, which results in relatively little contacts between animals (4).

Despite the RBP test might lack sensitivity (6), the results should be indicative enough to evaluate the occurrence of *B. abortus* in cattle. Positive animals were not clustered, except for 3 animals which

belonged to the same herd. In the absence of vaccination, the epidemiological situation reflects a very low circulation of *Brucella* spp. in the study area. Lack of transmission could be explained again by the type of livestock and by the livestock husbandry that is practiced in the area. The fact that aborted material is usually left in the field is not associated to high brucellosis prevalence in cattle. A tentative explanation is that the environment is likely inappropriate for the survival of *Brucella* spp., given the hot and relatively arid conditions of the area.

The questionnaire survey has provided information regarding the knowledge and practices of livestock keepers about zoonotic diseases in Southern Ethiopia. Though the communities seemed to be aware of the risk of anthrax transmission to humans, a high number of respondents had no detailed and accurate knowledge about zoonotic tuberculosis and brucellosis. This low awareness is a limiting factor if control strategies are to be implemented. Experience is their main source of knowledge. This may indicate paucity in health education rendered to the community. The most important practices potentially supporting transmission of zoonotic diseases in the study area were backyard slaughtering of animals and consumption of raw animal products. Aborted foetuses, though rarely destroyed, are unlikely to play any role on human brucellosis since they are seldom handled by people.

Even though the prevalence of zoonotic tuberculosis and brucellosis was relatively low, surveillance and prevention may be warranted taking into account possible animal genetic improvement programs, unrestricted animal movement in the area and low awareness of the community about zoonoses, which might result in an increased transmission to humans.

## **Acknowledgments**

The Belgian Directorate-General for Development Cooperation (DGCD) is acknowledged for funding this study in the framework of the project: Development of a framework for the improved control of zoonoses in developing countries – 97402 (Institute of Tropical Medicine, Antwerp). The Office of Associate Vice President for Research, Extension and Publication (AVP-REP) of Hawassa University is thanked for facilitating the implementation of the research project and providing vehicle. The Aklilu Lemma Institute of Pathobiology, Addis Ababa University, is acknowledged for technical support.

## Literature

- Ameni G., Aseffa A., Engers H., Young D., Gordon S., Hewinson G. & Vordermeier, M., 2007, High prevalence and increased severity of pathology of bovine tuberculosis in Holsteins compared to zebu breeds under field cattle husbandry in central Ethiopia. Clin. Vaccine Immunol. 14, 1356-1361.
- Ameni G. & Erkihun A., 2007, Bovine tuberculosis on small-scale dairy farms in Adama Town, central Ethiopia, and farmer awareness of the disease. Rev. Sci. Tech. 26, 711-719.
- Asfaw Y., Molla B., Zessin K.H. & Tegegne A., 1998, A cross-sectional study on bovine brucellosis and test performance in intra and peri-urban dairy production system in and around Addis Ababa. Bull. Anim. Hlth. Prod. Afr. 46, 217-224.
- Cleaveland S., Shaw D.J., Mfinanga S.G., Shirima G., Kazwala R.R., Eblate E. & Sharp M. 2007, *Mycobacterium bovis* in rural Tanzania: risk factors for infection in human and cattle populations. Tuberculosis, 87, 30-43.
- Etter E., Donado P., Jori F., Caron A., Goutard F. & Roger F., 2006, Risk analysis and bovine tuberculosis, a re-emerging zoonosis. Ann NY Acad Sci. 1081, 61-73.
- Gall D. & Nielsen K., 2004, Serological diagnosis of bovine brucellosis: A review of test performance and cost comparison. Rev. Sci. Tech. 23, 989-1002.
- Kubuafor D.K., Awumbila B. & Akanmori B.D., 2000, Seroprevalence of brucellosis in cattle and humans in the Akwapim-South district of Ghana: public health implications. Acta Trop. 76, 45-48.
- 8. Marcotty T., Matthys F., Godfroid J. et al., 2009, Zoonotic tuberculosis and brucellosis in Africa: neglected zoonoses or minor public-health

issues? The outcomes of a multi-disciplinary workshop. Ann. Trop. Med. Parasit. 103, 401-411.

- 9. Morris R.S., Pfeiffer D.U. & Jackson R., 1994, The epidemiology of *Mycobacterium bovis* infections. Vet. Microbiol. 40, 153-177.
- Muma J.B., Samui K.L., Siamudaala V.M., Oloya J., Matope G., Omer M.K., Munyeme M., Mubita C. & Skjerve E., 2006, Prevalence of antibodies to *Brucella* spp. and individual risk factors of infection in traditional cattle, goats and sheep reared in livestock-wildlife interface areas of Zambia. Trop. Anim. Health Prod. 38, 195-206.
- Nicholson M.J & Butterworth MH., 1986, A guide to body condition scoring of zebu cattle. International Livestock Center for Africa (ILCA), Addis Ababa, Ethiopia.
- 12. OIE, 2004, Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees). Office International des Epizooties (OIE).
- O'Reilly L.M. & Daborn C.J., 1995, The epidemiology of *Mycobacterium* bovis infections in animals and man: a review. Tubercle Lung Dis. 76, 1-46.
- Radostits O.M., Gay C.C., Blood D.C. & Hinchclif K.W., 2000, Veterinary medicine: a text book of the disease of cattle, sheep, pigs, goats and horses. W.B Saunders, London.
- 15. Seifert H.S.H., 1996, Tropical Animal Health. Kluwer Academic Publishers.
- 16. StataCorp., 2008, Stata Statistical Software: Release 10. Texas: Stata Corporation.
- 17. Thrusfield M., 1995. Veterinary Epidemiology, 2<sup>nd</sup> ed. Black Well Science Ltd, London.

K.K. Amenu, Ethiopan, DVM, MSc, Assistant Professor, Department of Veterinary Medicine, Hawassa University, Hawassa, Ethiopia.

E. Thys, Belgian, DVM, PhD, Senior researcher, MSc coordinator, Department of Animal Health, Institute of Tropical Medicine, Antwerp, Belgium.

- A. Regassa, Ethiopan, DVM, MSc, Associate Professor, Department of Veterinary Medicine, Hawassa University, Hawassa, Ethiopia.
- T. Marcotty, Belgian, DVM, PhD, Senior researcher, Department of Animal Health, Institute of Tropical Medicine, Antwerp, Belgium.