

**LABORATORY CAPACITY OF BOTH HUMAN AND VETERINARY NATIONAL  
TUBERCULOSIS REFERENCE LABORATORIES IN EAST AFRICA  
TO DIAGNOSE *MYCOBACTERIUM BOVIS*.**

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## **BACKGROUND**

In many developed countries, tuberculosis (TB) is considered under control or eradicated. However, the impact of this disease can be overwhelming even today, especially in those resource-poor countries suffering from high burdens of both TB and human immunodeficiency virus (Parsons et al., 2011). For this and other reasons TB remains an important public health issue in the developing world. In Africa it is very important, it is e.g. being implicated as the biggest killer disease in Nigeria (Nwata et al., 2011) .

The main aetiology of human tuberculosis is *Mycobacterium tuberculosis* however; the disease can also be zoonotic where *Mycobacterium bovis* (bovine TB or bTB) is the most common cause of zoonotic TB in humans which manifests itself most commonly as extra pulmonary tuberculosis (Nwata et al., 2011). Areas characterized by high prevalence of bovine tuberculosis in animals are also likely to have a number of human tuberculosis cases caused by *Mycobacterium bovis* (Ates et al., 2015).

Available data suggest a gross underestimation of the burden of *Mycobacterium bovis* in causing human disease. Cosivi et al. (1998) suggest that a reason for this could be the lack of attention or resource allocations given to this disease by the research community. This misconception has resulted in the pathogen (*M. bovis*) being neglected in the human health sector and this is further exacerbated by the inability of most diagnostic tests in human laboratories to differentiate between *M. bovis* infections and *M. tuberculosis* infections (Müller et al., 2013). Therefore, in many instances, tuberculosis infections end up being assumed to be caused by *M. tuberculosis* (Cosivi et al., 1998 and Olea-Popelka et al., 2017).

The impact of this possible misconception is felt either in misdiagnosed and undiagnosed extra-pulmonary tuberculosis leading to delayed treatment (Ates et al., 2015) or in the wrong treatment of patients; indeed, *M. bovis* is naturally resistant to some of the first line drugs of choice for treatment of tuberculosis in humans such as pyrazinamide (Cosivi et al., 1998). In cases where treatment commences before bacterial species identification and antimicrobial susceptibility results, as is common practice, patients are frequently exposed to inadequate treatment (Olea-Popelka et al., 2017).

Reducing the number of undiagnosed and misdiagnosed cases should therefore be regarded as a priority. This can be achieved through the prevention of zoonotic tuberculosis and investing in new and more accurate diagnostic technologies (Olea-Popelka et al., 2017).

For this to be achieved both the veterinary and public health sectors have to be involved to understand the endemicity of bovine tuberculosis in animals as well as the prevalence among humans. In order to do this a country has to be equipped to diagnose the pathogen accurately so as to come up with a treatment, control or eradication strategy.

While some techniques are simple, others have complex requirements, and therefore, it is important to carefully determine how to link these new tests and incorporate them within a country's national diagnostic algorithm. Finally, the successful implementation of these methods is dependent on key partnerships in the international laboratory community and ensuring that adequate quality assurance programs are inherent in each country's laboratory network (Parsons et al., 2011).

This study aimed to investigate the state of the national laboratories that are mandated to carry out or manage tuberculosis diagnosis in East Africa (Kenya, Uganda and Tanzania). The study sought to understand the current functional diagnostic tests in the laboratories, whether or not zoonotic tuberculosis is diagnosed/ differentiated from *M. tuberculosis*, staffing and qualifications, coverage of each National Tuberculosis Diagnostic laboratory as well as any collaboration that exists between the laboratories in the veterinary sector and the human health sector.

## **METHODS**

### **Study area**

The study was conducted in both national central veterinary laboratories and human tuberculosis reference laboratories in East Africa (Kenya, Tanzania and Uganda).

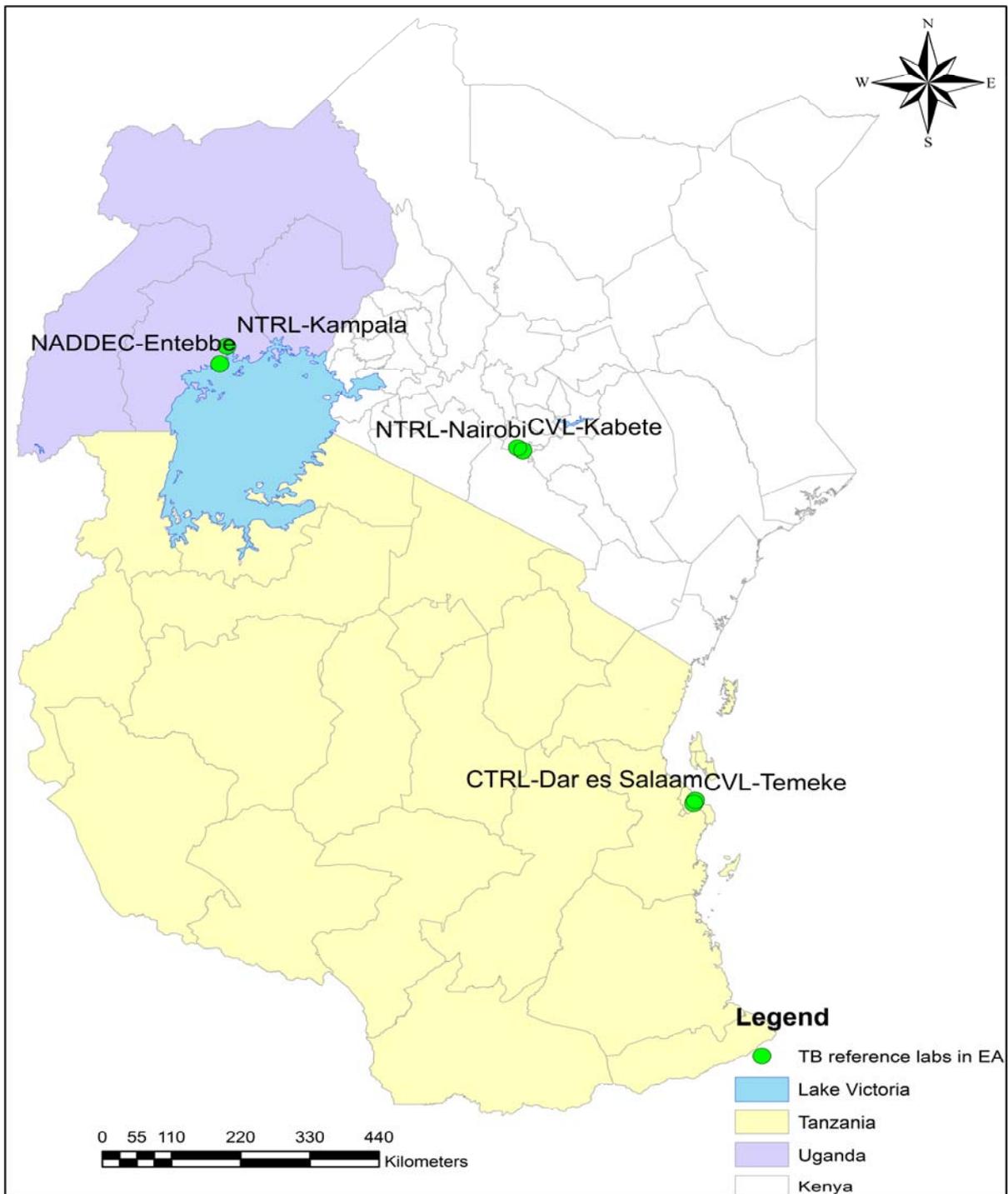
### **Study design and data collection**

A cross sectional survey was carried out to assess the laboratory capacity to diagnose *M. bovis* in veterinary and human tuberculosis reference laboratories. Questionnaires were administered to the *Central Veterinary Laboratories* (CVL) in Kabete, Kenya and the *National Tuberculosis Reference Laboratories* (NTRL) in Nairobi, Kenya, the Central Veterinary Laboratories (one of the *Tanzania Veterinary Laboratory Agency* - TVLA- sites) in Temeke, Dar es Salaam and the *Central Tuberculosis Reference Laboratory* (CTRL) located in Muhimbili National Hospital in Dar es Salaam, Tanzania, the *National Tuberculosis Reference Laboratory* (NTRL) - and Supranational Reference Laboratory - in Kampala, Uganda and the *National Animal Disease Diagnostic and Epidemiology Centre* (NADDEC) in Entebbe, Uganda.

The questionnaire was structured to collect information on the mandate of the institutions, the area covered by the laboratory i.e. national, regional, supra national etc, the biosafety levels of the institution, types and sources of samples, functional equipment present in the laboratory, diagnostic techniques used, laboratory prevalence of *M. bovis* and multi drug resistance cases, among other information.

A tour around the facilities was also given to the person administering the questionnaire providing an opportunity to observe the adherence to biosafety measures and the flow of work.

This was done for all laboratories except for the Supranational Reference Laboratory in Kampala which does not allow access of unauthorised personnel or any personnel without any affiliation to the institution.



Map. Location of the 6 reference laboratories for tuberculosis diagnosis in the 3 countries visited.

## RESULTS

### Area of responsibility covered by the different laboratories

None of the three countries involved in this study have specific veterinary reference laboratories designated for assessing bovine tuberculosis or any livestock tuberculosis. This role is therefore performed by the existing national-level laboratories which are also responsible for diagnosis of other animal diseases. The two CVLs in Kenya and Tanzania, as well as the NADDEC in Uganda, all serve as National Veterinary Reference Laboratories and were therefore regarded as the reference tuberculosis diagnostic laboratories in the veterinary sector for the purposes of this study.

In contrast, all three countries have national reference laboratories specifically for diagnosis tuberculosis in the human health sector: the CTRL in Tanzania and the two NTRLs in Uganda and Kenya, as mentioned above. The NTRL in Uganda is primarily a national reference laboratory, but it also serves as a supra-national reference laboratory; meaning that tuberculosis cases are being referred for diagnosis from other countries as well, including Kenya, Tanzania, Rwanda, Burundi, Malawi, Zambia, Somalia and South Sudan. As part of their mandate, the reference laboratories in the human health sector all perform drug resistance testing on all samples processed. This situation is different from that of the veterinary laboratories where no drug resistance testing of tuberculosis is conducted.

### Sampling for tuberculosis in East Africa

Tuberculosis diagnosis in all six reference laboratories that were part of this study, is clearly separated by sector, with veterinary reference laboratories processing the samples from livestock and human tuberculosis reference laboratories processing human samples only. Given this clear distinction of species-handling the samples collected by the laboratories in the two sectors is slightly different.

The veterinary laboratories in all three countries largely use intradermal skin testing to detect tuberculosis in livestock. However, in cases of genuine laboratory-based diagnosis, diagnosis is based on suspect lung tissues collected at slaughter facilities or as part of post-mortems from different animal species. Transport requirements for the tuberculosis samples include triple packaging and transport in cold chain within 24 hours in Tanzania CVL and the Uganda's NADDEC. The CVL in Kenya does not receive samples from any of the satellite *Veterinary Investigation Laboratories* (VILs) but processes samples collected from its own post mortem room. The CVL in Tanzania also receives blood and plasma samples for the interleukin test and also reported to collaborate on several occasions with the Sokoine University of Agriculture (SUA) on bovine tuberculosis research.

Notwithstanding the above observations, laboratory diagnosis of tuberculosis in the veterinary sector in East Africa is rarely conducted and largely depends on ongoing personal projects of Masters or PhD students.

The national tuberculosis reference laboratories in the human health sectors of the three East African countries process a wider sample range, including sputum, the most common sample laboratories process in cases of pulmonary tuberculosis (in addition to bronchial lavages). Other samples received and processed in these public health reference laboratories are

*cerebral spinal fluid* (CSF), aspirates from different organs (mostly lymph node aspirates), gastric lavages and tissue biopsies; these are mostly collected in suspect cases of extra-pulmonary tuberculosis in human patients. All three human tuberculosis reference laboratories receive samples from their inland satellite laboratories and in addition the NTRL in Uganda receives samples from the two other reference laboratories in Kenya and Tanzania. The requirements for sample transport to these laboratories are triple packaging and transport in a cool box within a maximum of 72 hours (to cater for weekends during which the laboratories do not receive samples).

Upon reception, the samples are inspected and those with breaks in the triple packaging are rejected and a request for a resampling is issued.

The volume of samples processed varies greatly in the East African reference laboratories. In the veterinary sector reference laboratories, very few samples are processed. The Kenyan CVL did not provide a frequency. The NADDEC in Uganda provided a frequency of approximately 1 (one) sample per year, while the CVL (TVLA) in Tanzania processes about 3 samples per month. This makes the TVLA the most active in tuberculosis sample processing and diagnosis in East Africa, though even this level of activity is to be regarded as very low.

In the public health sector, both NTRLs and the CTRL however, have 100% of their activities focused on diagnosis of tuberculosis thus process a much larger sample number ranging from 25 samples per day to 60 samples per day in the supranational reference laboratory (Kampala). These numbers correspond to the normal daily routine throughput. However, whilst this study was conducted, the NTRL in Nairobi was undertaking a national tuberculosis surveillance activity where the daily volume of samples increased to about 150 samples.

**Table 1: Coverage, species from which samples are processed and the tuberculosis diagnostic throughput of each reference laboratory**

Country	Name of laboratory	Coverage	Species sampled	Volume per year (estimates)
Uganda	NADDEC	National	Animals	1
Uganda	NTRL	Supra-national	Humans only	6,000 - 12,000
Tanzania	CVL	National	Animals	36
Tanzania	CTRL	National	Humans	5,000 – 6,000
Kenya	CVL	National	Animals	-
Kenya	NTRL	National	Humans	7,000

## Diagnostic procedures and confirmation of tuberculosis in the laboratories

The standard test used for confirmation of tuberculosis in the East African reference laboratories are:

- in the three human health reference laboratories : culture and isolation on Lowenstein Jensen media ,
- in the two CVLs : comparative intradermal tuberculin skin test
- in the NADDEC : Ziehl-Neelsen staining

The CVLs and NADDEC only perform the above-mentioned tests for *M. bovis* identification, for the purposes of livestock trade or for research purposes which explains the level of diagnostic activity in these national veterinary reference laboratories. None of the laboratories performs *M. bovis* identification as part of their routine laboratory diagnostic processes.

None of the reference laboratories for the human health sector in the three East African countries perform any diagnostic test for the specific identification of *M. bovis* in (human) patients.

Other tests used in the reference laboratories for tuberculosis diagnosis include the commercial GeneXpert, the *Line Probe Assay* (LPA), the BACTEC MGIT liquid culture, light microscopy, LED fluorescent microscopy and the BOVIGAM ELISA test (in CVL Tanzania). For the NTRLs and the CTRL, the nucleic acid based tests i.e. the GeneXpert and the LPA are able to identify members of the *Mycobacterium tuberculosis complex* (MTBC) other than *Mycobacterium tuberculosis*. It is this group that *M. bovis* falls under. However, no further identification is done to species level for the MTB complex members and neither is the prevalence of MTBC cases other than *M. tuberculosis* calculated in any of the laboratories.

## Quality assurance and standardisation

All veterinary national laboratories are regarded as Biosafety level 2 (BSL2) facilities. The supranational NTRL in Uganda and the CTRL in Tanzania are both Biosafety level 3 facilities (BSL3), while NTRL in Kenya is a BSL2 facility with a new upcoming BSL3 facility about to be completed for tuberculosis diagnosis. Only one of the reference laboratories benefits from international accreditation i.e. the NTRL supranational laboratory (Kampala) which is ISO:15189 accredited.

All laboratories except NADDEC (Entebbe) and CVL (Kabete) have quality management systems in place for tuberculosis diagnostics and all the human health sector reference laboratories carry out inter-laboratory comparisons (proficiency testing) as part of their quality assurance.

## Equipment servicing and maintenance

Monitoring of the functionality of all operational equipment is done in all the reference laboratories however; maintenance is quoted as being a challenge in the CVL (Kabete, Kenya) and NADDEC (Entebbe, Uganda) laboratories.

Table 2: Standard confirmatory diagnostic tests used in the laboratories, diagnosis of bovine tuberculosis (bTB), and the prevalence of bovine tuberculosis and antimicrobial resistant cases in the laboratory.

Country	Name	Percentage dedicated to TB diagnosis	Diagnosis of BTB	Standard confirmatory diagnostic test used	Instances for bTB screening	Prevalence of bTB (lab diagnosis)	Prevalence of MDR-TB	Prevalence of drug resistant bTB
Uganda	NADDEC	5%	Not currently	Ziehl-Neelsen staining	Livestock trade and movement of livestock	Not known	Not known	Not done
Uganda	NTRL	100%	Identify complex	Culture on Lowenstein Jensen	N/A	Not known		Not done
Tanzania	CVL	10%	Yes	Comparative intradermal tuberculin skin test.  Occasionally <i>polymerase chain reaction</i> (PCR)	Livestock trade and research	3-4%	Not known	Not done
Tanzania	CTRL	100%	Identify complex	Culture on Lowenstein Jensen	N/A	Not known	1.1%	All members of the MTB complex other than <i>M. tuberculosis</i> are resistant to first line treatment (rifampicin and isoniazid)
Kenya	CVL	<5%	Not currently	Comparative intradermal tuberculin skin test	Livestock trade	Not known	Not known	Not done
Kenya	NTRL	100%	Identify complex	Culture on Lowenstein Jensen	N/A	Not known		Not done

**Table 3: Accreditation for each laboratory and presence of quality assurance systems.**

Country	Laboratory	Biosafety level	Accreditation for TB diagnosis	Quality management system for TB diagnosis	Inter laboratory testing
Uganda	NADDEC	BSL2	None	-	-
Uganda	NTRL	BSL3	ISO:15189	+	+
Tanzania	CVL	BSL2	None	+	-
Tanzania	CTRL	BSL3	None	+	+
Kenya	CVL	BSL2	None	-	-
Kenya	NTRL	BSL2 *	None	+	+

(\*) BSL3 underway

### Staffing and staff development

All new staff in all the six laboratories underwent a mandatory biosafety and biosecurity training as well as an introduction to the *standard operating procedures* (SOPs) for each laboratory section they work in. These trainings were given by the safety officers, section heads or more experienced staff in the laboratories.

There is also a provision for continuous development of skills for the employees that range from in-house workshops, ministry-coordinated trainings to opportunities for attending different professional training courses / workshops or conferences of interest. Such avenues are encouraged in all the laboratories involved in tuberculosis diagnosis that were interviewed in the framework of this study.

Only the NTRL / supranational laboratory in Kampala, Uganda reported having experienced a single case of laboratory-acquired tuberculosis in the last 7 years.

### DISCUSSION AND CONCLUSION.

All laboratories in the three East African countries are involved or have been involved in to some degree in tuberculosis testing. However, laboratory diagnosis of bovine tuberculosis (*M. bovis*) is not done in the reference laboratories except for the Ziehl - Neelsen acid fast staining, which is done in all the laboratories, and also the Tanzania CVL which occasionally performs the blood-based BOVIGAM test (gamma interferon test) and PCR testing. This demonstrates that the capacity for laboratory diagnosis of bovine tuberculosis in East Africa is almost non-existent. The reason for this is probably because the veterinary sector in East Africa largely depends on the comparative tuberculin skin test for detection of *M. bovis* positive animals. This test is easy to perform and the main cost is labour as it requires the repeat restraint of animals for the measuring of the skin area, as well as experience in interpreting the skin swellings. The intradermal tuberculin skin test is one of the recommended tests *World Organisation for Animal Health* (OIE) for testing of bovine tuberculosis. Another contributing factor may be that bovine tuberculosis is not of great importance to animal health as it rarely shows any symptoms and is usually only diagnosed at slaughter. Hence, laboratory diagnosis in the veterinary national laboratories may not have been given much priority in the past.

The main importance of the disease remains its public health impact.

There are other tests, which are recommended by the OIE for diagnosis of this condition that are less labour intensive and are more confirmatory. Nucleic acid based tests exist and are allegedly used very occasionally in the two central veterinary laboratories in this study (Tanzania and Kenya).

Novel technologies such as the use of isothermal amplification is sometimes being employed in veterinary reference laboratories for the diagnosis of bovine tuberculosis, e.g. in Tanzania. The CVL Tanzania has equipped its laboratory with an isothermal amplification machine that is used for tuberculosis diagnosis in the field. However, this technology is only employed during externally funded research projects.

Improving the capacity of tuberculosis laboratory diagnosis in the veterinary laboratories is of importance since these facilities should be the source of information on disease prevalence / endemicity for the countries. Currently there are no records of prevalence of bovine tuberculosis in any of these countries. Equipping these laboratories should be prioritized and the diagnosis of certain “neglected” diseases, amongst which tuberculosis in veterinary laboratories should also be given more importance to enhance collaborative efforts (One Health) in tackling the disease at the human – livestock interface.

As seen in the results of this study, the human diagnostic sector is undoubtedly more advanced in tuberculosis diagnosis. However, the challenge in differentiating *M. bovis* from *M. tuberculosis* is quite pronounced. Only the Tanzania CTRL has information pointing to the fact that almost all infections caused by members of the *Mycobacterium tuberculosis complex* (MTBC), other than *M. tuberculosis*, are resistant to the first line of treatment for human tuberculosis. This only stresses the importance of zoonotic tuberculosis in human health and brings out the importance in differentiating *M. bovis* from *M. tuberculosis* infections. When this is implemented, then a more accurate estimation of the number of *M. bovis* infections will be obtained from the diagnostic data and better tailored treatments of zoonotic cases will be achieved, thus reducing morbidities and mortalities.

This assessment concludes that the diagnosis of bovine tuberculosis in the three East African countries is neglected. The human health sector definitely has more developed capacity for tuberculosis diagnosis compared to the veterinary sector in all three countries but even so, *M. bovis* is not diagnosed at all in clinical cases. The only diagnosed cases so far have been identified by individual, focused research projects. This report thus calls especially for efforts to stress the importance of this pathogen to both animal health and human health. Secondly, this paper calls for improvement of the diagnostic capacity (i.e. efficient and affordable techniques and equipment) to differentiate the pathogen from other members of the MTBC in both pulmonary tuberculosis and extra-pulmonary tuberculosis cases.

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