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1 Acknowledgments

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This work was conducted in partnership between the Burnet Institute, the Papua New Guinea Institute of Medical Research (PNG-IMR), PNG Provincial Health Authorities in Eastern Highlands (EH) province and East New Britain (ENB) province, the PNG National TB Program (NTP), PNG Central Public Health Laboratory (CPHL), Port Moresby General Hospital (PMGH), and the Victorian Infectious Diseases Reference Laboratory (VIDRL) in Australia.

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2 Executive summary

Papua New Guinea (PNG) is listed by the World Health Organisation (WHO) as one of 30 high TB burden countries with an estimated incidence rate in 2021 of 424 per 100,000 population, and an incidence of multi-drug resistant/rifampicin resistant (MDR/RR) TB of 24 per 100,000, which is high even amongst the high burden subset. TB case notification data from PNG (2008-16) show a higher-than-expected proportion of extra-pulmonary TB (EPTB) cases. TB lymphadenitis (TB-LN) is the commonest form of EPTB, but very few cases are bacteriologically confirmed. *Mycobacterium bovis* has been noted to be associated with higher proportions of TB-LN but is clinically indistinguishable from *M. tuberculosis* TB in humans. It is possible that *M. bovis* infection is occurring in humans and contributing to the high proportion of EPTB amongst TB notifications. Without bacteriological diagnosis, it is uncertain whether the true cause of illness is TB (due to *M. tuberculosis* or *M. bovis*), and if it is TB whether any of it is MDR/RR-TB. Without a confirmed diagnosis, antimicrobial treatment may be incorrect or insufficient, which could lead to additional drug resistance and poor treatment outcomes.

Therefore, we aimed to determine the proportion of cases of clinically presumptive TB-LN due to microbiologically confirmed *Mycobacterium tuberculosis* complex (MTBC), and the proportion of confirmed MTBC lymphadenitis due to *M. bovis* in two provinces (East New Britain and Eastern Highlands) and the National Capital District. We undertook a prospective cohort study of people presenting with clinically presumptive TB-LN, and when indicated took fine needle aspirates (FNA) from enlarged LN. These were assessed by GeneXpert for detection of MTBC and rifampicin resistance, and by mycobacterial culture with drug susceptibility testing for confirmation of MTBC species and drug resistance pattern. We assessed factors associated with confirmed TB-LN. We sought to understand how humans interact with animals and animal products including practices that may pose risks for zoonotic infections through community focus group discussions and in-depth interviews in the same provinces.

We found that 27% of 223 people presenting with clinically presumptive TB lymphadenitis in three provinces of PNG had bacteriologically confirmed TB due to *M. tuberculosis*. No people had TB due to *M. bovis*. Two people had specimens containing Mycobacterium Avium Complex (MAC). This indicates that *M. bovis* is not a common cause of enlarged LN in the facilities that participated in the study. We detected rifampicin resistance in 7 (12%) of 58 bacteriologically confirmed LN FNA. An additional 15 samples were reported as having cytology consistent with TB but were not confirmed as TB by bacteriological tests. Cytology assessments also resulted in 5 cancer diagnoses. Our findings indicated that clinical presentations with a higher likelihood of being bacteriologically confirmed as TB were visible LN on both sides of the neck with the largest being 5-10cm (diameter multiplied), and of a duration of at least 8 weeks. Bacteriological confirmation of TB was much more common in adults (15-49 years) compared to children.

Through the qualitative exploration of risks for zoonotic infections we found that interaction with both domestic and wild animals can be close, with animals used and highly valued in a variety of ways, including consumption of some. There was some awareness that animals may pose a risk of illness to humans, but also many interactions described where there was no perception of the possibility of harm and therefore no mitigation of risk. Animals were considered an essential part of participants everyday life. We conclude their value cannot be ignored when developing culturally responsive zoonotic risk mitigation strategies.

We recommend clinicians in PNG follow the National TB Guidelines and take LN FNA samples from people presenting with clinically presumptive TB-LN, prioritising Xpert MTB or Ultra testing. Passive surveillance for the presence of *M. bovis* can occur by monitoring species confirmation when any specimens for presumptive TB are cultured in a reference laboratory. Exploration of zoonotic illness risk should be carefully contextualised within the entangled living and high value placed on animals by members of the PNG community.

3 Background

TB is a leading cause of death amongst infectious diseases worldwide. In the Pacific region it is an important health issue, with Papua New Guinea (PNG) listed by the World Health Organisation (WHO) as one of 30 high TB burden countries with an estimated incidence rate of 424 per 100,000 population, and an incidence of multi-drug resistant/rifampicin resistant (MDR/RR) TB of 24 per 100,000 in 2021, which is high even amongst the high burden subset.[1,2] The high levels of MDR/RR-TB have prompted a national emergency response task-force.[3] Tuberculosis (TB) is one of the leading causes of mortality in PNG, with an estimated 51 deaths per 100,000 due to TB alone in 2021 (excluding HIV/TB co-infection).[1]

TB case notification data from PNG (2008-16) show a higher-than-expected proportion of extra-pulmonary TB (EPTB) cases.[2] In 2016, EPTB contributed to 42% of the total national TB caseload.[2] However, proportions of EPTB are heterogeneous with an extremely high proportion of 78.5% in Eastern Highlands Province, 43% in National Capital District, and 25% in East New Britain Province,[2] with similar findings more recently of 25-30% in East New Britain identified through operational research.[4] [1] Approximately two thirds of EPTB cases were in children under 15 years old. TB lymphadenitis (TB-LN) is the commonest form of EPTB,[2] but very few cases are bacteriologically confirmed. Further, drug resistant TB is now a major health security issue for PNG, with MDR/RR-TB identified in several hotspots in PNG including Port Moresby, National Capital District.[3] This has implications for the health security of both PNG and the region, including Australia, where cross-border transmission has occurred.[5] There is little information about rates of drug resistant TB amongst EPTB cases in PNG, partly because most notified cases are clinically diagnosed. If the proportion of MDR/RR-TB in EPTB is similar to that seen in pulmonary TB, there will be important implications for diagnostic and treatment approaches to EPTB in PNG.

The *Mycobacterium tuberculosis* complex (MTBC) is a group of closely related acid-fast bacilli including *M. tuberculosis* and *M. bovis*, with different lineages more adapted to causing TB in humans or a variety of both domestic and wild animal species. Both domestic and wild animal species may function as maintenance hosts, or spillover hosts for *M. bovis*.[6] While *M. tuberculosis* is avirulent in cattle and transmission back to humans is extremely rare, *M. bovis* can infect humans and humans can infect livestock, but transmission amongst immunocompetent individuals is uncommon. *M. bovis* can infect and maintain infectious cycles in a variety of species. It has been proposed that expansion of MTBC to new hosts has mostly been due to host geographic proximity and high host population densities.[6] In New Zealand, *M. bovis* transmission to cattle and potential risk to humans has been shown to be maintained through ongoing transmission among the introduced brushtail possums (*Trichosurus vulpecula*), with spillover infections amongst feral pigs and wild deer.[7] A range of wildlife reservoirs have been recognised worldwide (European badger in Great Britain, African buffalo in South Africa, wild boar in Spain, and white tailed deer in USA),[8] but it is unknown if there are reservoirs in PNG, and whether particular animal-human exposures in this context pose a risk for *M. bovis* spread to humans. Globally *M. bovis* prevalence, where accurate data exists, is extremely variable, with proportions as high as 26% of all TB cases in Mexico City to 0.4% in Ethiopia to zero out of 4000 cases in China.[9,10,11]

Mycobacterium bovis has been noted to be associated with higher proportions of EPTB lymphadenitis,[12] but is clinically indistinguishable from *M. tuberculosis* TB in humans. In PNG, the proportion of cases registered as EPTB is unusually high at 42% of

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[1] *This research was conducted as part of the first Operational Research Course for Tuberculosis in PNG, implemented by the Burnet Institute in collaboration with the PNG Institute of Medical Research (PNG-IMR), University of PNG (UPNG) and National Department of Health*

notifications,[2] compared to a global proportion of 16% in 2019.[13] Higher proportions of EPTB have been reported in TB-HIV co-infected compared to non-co-infected TB patients.[14,15] In the absence of a generalised HIV co-epidemic in PNG the reasons for the high proportion of EPTB are unknown, although it is noted that rates of EPTB and HIV infection are both higher in the highlands compared to elsewhere in PNG.[16] One possible cause is zoonotic TB (*M. bovis*), with other possibilities being non-tuberculous mycobacteria (NTM, a multi-species group of human pathogens including *M. avium* complex and *M. intracellulare*, that are ubiquitous in water and soil), other infectious causes (bacteria, fungi), or non-infectious causes of lymphadenitis.[17] These are not part of routine diagnostic workup for suspected EPTB in PNG, and almost all EPTB cases are diagnosed solely on clinical grounds,[18] which means not only is the true cause of illness uncertain, but also that rates of rifampicin resistant TB among EPTB cases are unknown, with potentially serious clinical consequences.

Xpert® MTB/RIF (Xpert) is a WHO recommended, rapid, automated, nucleic acid amplification assay that is used widely for simultaneous detection of MTBC and rifampicin resistance in sputum specimens.[19] Rifampicin resistance is a primary marker of multi-drug resistance used to inform clinicians whether a longer and different course of TB treatment is required than for fully drug-susceptible strains. A recent Cochrane review on the use of Xpert for EPTB concluded with high-certainty that Xpert is accurate for detection of rifampicin resistance, while detection of MTBC had high specificity for most extrapulmonary specimens but variable sensitivity.[19] The pooled sensitivity of Xpert MTB/RIF for lymph node aspirates was 87.6% (95% credible interval 81.7 – 92.0%), and specificity 86.0% (95% credible interval 78.4 – 91.5%).[19] A similar sensitivity (87.0%) but slightly higher specificity (92.0%) was reported in an earlier meta-analysis.[20] Fine-needle aspiration (FNA) of lymph nodes is well suited for use in resource-limited settings because the procedure is simple, easy to learn, minimally invasive, and inexpensive.[21] Xpert MTB/RIF Ultra (Xpert Ultra) has an improved sensitivity for MTB diagnosis compared with Xpert MTB/RIF,[22] and the WHO recommended Xpert Ultra as an alternative to Xpert, stating that all recommendations concerning use of Xpert with selected extrapulmonary specimens (cerebrospinal fluid (CSF), lymph nodes, and tissue specimens) also apply to Xpert Ultra.[23] Fine needle aspirate biopsies, histology and culture for lymph nodes from the head and neck region have been reported to have high sensitivity and specificity for TB.[24]

Standard laboratory methods including culture and Xpert do not distinguish *M. bovis* from *M. tuberculosis*, with Xpert detecting the MTBC that contains both organisms. *M. bovis* is intrinsically resistant to pyrazinamide, one of the key drugs in first-line TB treatment. While identification of pyrazinamide monoresistance in an MTBC indicates potential *M. bovis*, it cannot solely be relied on as confirmation. In a surveillance study in the United States, pyrazinamide susceptibility was found to be only 80% sensitive for identification of *M. bovis*.[25] More recently, whole genome sequencing and phylogenetic analysis has been used for classification of *M. bovis.* In addition, treatment of *M. bovis* with standard first-line TB treatment has been associated with a higher mortality rate than for *M. tuberculosis*.[26]

It is estimated that 142,000 cases of zoonotic TB occur globally each year, with nearly 5000 cases in the Western Pacific region.[13] There has been a strong call to address zoonotic TB, with a clear need to improve the scientific evidence base.[27] However, the global estimates are based on limited data and there have not been recent studies in PNG. In Ethiopia, where proportions of reported EPTB and lymphadenitis are similar to PNG, recent studies have highlighted an increased risk of TB-LN associated with regular and direct animal contact, despite very low proportions of *M. bovis*.[28,10] It is unclear whether different exposures to specific animals or animal products are a significant factor in the high proportions of clinically diagnosed TB-LN in PNG. Other zoonoses may also have a role in lymphadenitis clinically diagnosed as EPTB. While the TB incidence in Fiji is not extremely high (66 per 100,000 in 2021 [29]), a Brucellosis outbreak in cattle has recently been reported in Fiji and human cases in Futuna and Wallis,[30,31] raising the

question of whether other island nations such as PNG could be at similar risk for outbreaks of Brucella, or bovine TB. Improved understanding of the role of zoonotic pathogens could guide animal health, human education and public health interventions and improved regional estimates.

The reasons for the high proportion of EPTB in PNG are not known, nor do we understand factors associated with suspected TB-LN. PNG is a location with pockets of high population density in poor living conditions and frequent contact with a range of animal species. Given the potential confluence of human and ecological risk factors in settings such as PNG that could drive disease spill-over from animals to humans, there could be both immediate clinical benefits and broader benefits from a One Health perspective from determining the proportion of suspected TB-LN that is due to MTBC and assessing potential local risk factors, such as animal exposure, that are associated with presentations of TB-LN. There could be implications for programmatic management and patient care by improving capacity in routine clinical practice for taking lymph node aspirates for Xpert testing, and determining the proportion of bacteriologically confirmed TB-LN that is resistant to rifampicin, i.e., requires treatment for MDR/RR-TB. If a large proportion of clinically diagnosed cases are negative for TB by molecular and culture methods, suggesting that other pathogens may be responsible, a review of clinical diagnostic algorithms may be necessary.

Therefore, we aimed to determine the proportion of cases of clinically presumptive TB-LN due to microbiologically confirmed MTBC (both drug susceptible and rifampicin resistant), and the proportion of confirmed MTBC lymphadenitis due to *Mycobacterium bovis* in two provinces (East New Britain and Eastern Highlands) and the National Capital District. We undertook a prospective cohort study of people presenting with clinically presumptive TB-LN, and when indicated took fine needle aspirates from enlarged LN. These were assessed by Xpert MTB/RIF or Ultra for detection of MTBC and rifampicin resistance, and by mycobacterial culture with drug susceptibility testing for confirmation of MTBC species and drug resistance pattern. Through clinical documentation we assessed factors associated with confirmed TB-LN. We also sought to understand how humans interact with animals and animal products including practices that may pose risks for zoonotic infections, explored through ethnographic research in the form of community focus group discussions and patient in-depth interviews in the same provinces. This study was conducted in partnership by the Burnet institute, the PNG Institute for Medical Research, and the PNG Central Public Health Laboratory, and the Victorian Infectious Diseases Reference Laboratory.

4 Objectives

Aim:

To evaluate whether presumptive TB lymphadenitis (TB of lymph node, TB-LN) in three provinces of Papua New Guinea (PNG) is caused by *Mycobacterium tuberculosis* complex (MTBC) and assess whether there are important zoonotic infection risks.

Objectives:

1. Determine the proportion of presumptive TB lymphadenitis attributable to *Mycobacterium tuberculosis* complex in East New Britain and Eastern Highlands provinces, and National Capital District, Papua New Guinea.
2. Determine the proportion of confirmed TB lymphadenitis attributable to *Mycobacterium bovis* in East New Britain and Eastern Highlands provinces, and National Capital District, Papua New Guinea.
3. Explore animal-human interactions that may pose a risk for zoonotic infections.

5 Methodology

The study was conducted in two parts: 1) evaluation of consecutive patients diagnosed with clinically presumptive TB-LN, and 2) qualitative exploration of animal-human interaction risks through in-depth interviews (IDI) and focus group discussions (FGD).

5.1 Study setting

The study was conducted in three provinces in PNG – East New Britain (ENB) Province, Eastern Highlands (EH) Province, National Capital District (NCD).

Figure 1: Map of PNG, showing the capitals of the provinces selected (Rabaul in ENB, Goroka in EH, Port Moresby in NCD)

In East New Britain Province, health facilities which are TB Basic Management Units (BMUs) enrolled patients. Five sites were initially selected, which in 2018 reported 60% of 785 TB cases notified in ENB Province and were within a sufficiently limited geographic area to make sample transport and supervision feasible. After interruptions due to the COVID-19 pandemic, patients were recruited from three sites (Nonga General Hospital, Rabaul Clinic, Butuwin Clinic) which were still managing relatively high case numbers. The Nonga General Hospital is the provincial hospital and has microscopy, Xpert MTB/RIF, chest radiography and provides treatment for drug-susceptible and drug-resistant TB. Care is provided by the internal medicine unit. Prior to the study, FNA and Xpert was not being utilised for TB-LN diagnosis.

In Eastern Highlands Province patients were enrolled through Goroka Base Provincial Hospital inpatient and outpatient services. In 2018, 47% of 1812 TB cases notified in the province were in Goroka district. FNA of LN had previously been in use at Goroka Base Provincial Hospital, however had not been utilised for several years prior to this study being implemented.

In National Capital District patients were enrolled through Port Moresby General Hospital inpatient or outpatient services. These services manage a high caseload, including patients referred in from Central Province. FNA are regularly performed by the PMGH Pathologists for presumptive TB-LN following a study demonstrating the benefits of testing FNA with Xpert.[32]

These settings were selected because they were expected to include a high proportion of cases in each province, and it was feasible to acquire test and transport samples. It was pragmatically accepted that they may not be representative of the entirety of each province due to potential variation in patient presentation at each treatment site.

5.2 Participants, sample size and duration

5.2.1 Patients with presumptive TB lymphadenitis

We aimed to recruit all consecutive patients presenting with clinically presumptive TB-LN to participating BMUs for the duration of the study in that location. People were included if clinical staff confirmed they had signs of TB-LN including enlarged lymph nodes appropriate for an FNA sample, and the person provided informed consent for FNA samples and tests to be conducted and for inclusion in the analysis of their medical and sociodemographic data (re-identifiable: study ID, excluding name and address details) recorded on a case investigation form (CIF). Children aged less than 18 years were included if informed consent was provided by the child's guardian, together with verbal assent from older children (13-17 years). Patients who did not want to have the recommended FNA sample taken were asked permission to include a copy of the CIF data in the study. Any patient who did not want to participate was told they would still receive standard of care including clinical assessment and routine diagnostic evaluation for TB as per PNG guidelines. An anonymous record of refusals was kept (date, age, sex, BMU and refusal reason if given) to allow an assessment of representativeness of the final participants compared to all TB-LN patients during the study period.

Target sample size was based on the number of presumptive TB-LN patients who have samples sent for laboratory confirmation and the anticipated study duration of 12 months. Study duration was extended and repeatedly interrupted by responses to the COVID-19 pandemic. We aimed to include 200 presumptive TB-LN patients, with at least 50 from ENB province given the higher number of TB cases and higher proportion of EPTB in EH province and NCD. We estimated that if 50% of 200 presumptive TB-LN was MTBC positive, the 95% confidence intervals for this point estimate would be 43 - 57%. If 1% of confirmed MTBC lymphadenitis was *M. bovis* the confidence intervals would be 0 - 4%.[2]

5.2.2 Qualitative exploration of risks for zoonotic infections

Focus group discussions (FGD) were conducted in Eastern Highlands province and East New Britain province with members of the community residing near BMUs in these two provinces. Participants were recruited through purposive sampling, which allowed for the exploration of different practices amongst different subgroups within the communities. The composition of the FGDs was based on the characteristics urban/rural, male/female, and age 16-24 years/40+ years, with 14 FGD conducted in the two provinces. The interviewer was the same gender as the group. FGDs examined urban/rural, gendered, age or temporal differences in animal and human interactions, general health issues in the community, disease transmission risk, and the cultural value of animals.

Participants in in-depth interviews (IDI) to explore potential risks for zoonotic infections were selected from amongst patients who had FNA taken for testing and consented to further contact. Purposive sampling was done from amongst those consenting, aiming for representativeness by age, sex and rural/urban residence. Selected patients were provided an explanation of the interview purpose and requirements, before being asked if they consented to participate in an IDI. If the selected patient refused, another patient from the cohort who had FNA sampled and similar characteristics was approached instead. IDI were conducted in Eastern Highlands province.

[2] A higher or lower proportion of confirmed MTBC would lead to lower confidence intervals for estimate of confirmed MTBC proportion. With this sample size zero cases of *M. bovis* provides a 95% confidence interval of 0-5% and does not exclude low proportions of *M. bovis*.

5.3 Study procedures

5.3.1 Clinical documentation, samples and tests

Patients with presumptive TB-LN who consented to participate after initial screening and consent were assessed by clinical study staff and medical and sociodemographic data recorded on a standardised CIF. A lymph node FNA was taken by trained medical staff (pathology registrars or health extension officers trained by pathologists). Sampling was done with a 23 or 25G needle after first cleaning the palpable LN site with an alcohol swab, with two separate samples usually taken. Drops of FNA fluid were put on slides directly from the needle and immediately smeared, then stored before sending for pathology staining. A second sample was expelled using a syringe into sterile saline, then sent to the laboratory for testing for TB.

Pathology staining and reporting was performed by pathologists at PMGH and Goroka hospital using routine methods. Assessments included macroscopic appearance, Ziehl–Neelsen (ZN) stain for mycobacteria and May Grunwald-Giemsa (MGG) stain for microscopic cytological features. Local laboratory (on-site for PMGH and Goroka hospital, transported within PNG for clinics in ENB) testing for MTBC was done according to manufacturer instructions using Xpert MTB/RIF or Xpert Ultra for detection of MTBC and rifampicin resistance. The remaining sample in sterile saline was stored and transported at 2-8 degrees Celsius to an Australian Reference Laboratory (VIDRL) for culture and further identification.

At VIDRL, samples were cultured using both solid (Löwenstein-Jensen agar) and liquid media (Mycobacterial Growth Indicator Tubes (MGIT)) in accordance with standard protocols for mycobacterial growth. ZN stain and TB MPT64 antigen tests were performed on positive cultures. Drug susceptibility testing (DST) was performed using the MGIT system. After review of four aspects of routine tests, any isolates suspected to be *M. bovis* would be further investigated via MIRU-VNTR to confirm identification.

Clinical management was through routine care by clinicians at participating BMUs. Xpert laboratory test results were provided to the diagnosing clinician to inform clinical management of the individual patient according to existing PNG guidelines. If the DST results provided information that would influence treatment choices beyond the results of the Xpert test, the diagnosing physician was advised. If any sample was confirmed as *M. bovis* infection then the result would be notified to the treating physician and a modified regimen suggested on best evidence[33], which extends duration from 6 to 9 months with use of either isoniazid-rifampicin or isoniazid-rifampicin-ethambutol in the continuation phase to take into account the intrinsic pyrazinamide resistance of *M. bovis*.

5.3.2 Qualitative exploration of risks for zoonotic infections

IDIs and FGDs aimed to develop an understanding of cultural perceptions and practices regarding varying interactions with and exposures to different animal species and animal products. Broad topic guides were used to guide the interviews and facilitate discussions. IDIs and FGDs were audio-recorded with consent. Pseudonyms were used to protect participants' identities. Aspects explored included health issues in the community and knowledge of TB symptoms, and interactions with animals and beliefs around animal to human disease transmission. Topics related to interactions with animals included domestic, incidental and occupational exposure to domestic and wild animals, targeting some animal species (cattle, deer, marsupials and pigs) and cultural food practices (e.g., handling and consumption of raw animal parts or products) where there may be intensive and/or potentially high-risk exposures for zoonotic infection. IDIs and FGDs were conducted in Tok Pisin or Tok Ples as necessary, by staff trained in qualitative research

techniques including research ethics and managing confidential and sensitive data. Participants were not paid but were provided with refreshments and a small location-dependent amount to cover bus/transport fare.

5.4 Data collection, management and analysis

5.4.1 Data collection and sources

A *Case Investigation Form (CIF)* was developed to collect clinical signs and possible exposure risk history for people with presumptive TB-LN as there was no standard clinical forms for assessment of possible EPTB. Forms were completed by a clinician during clinical assessment, and a copy of the data excluding the patient's name and address details stored for use in the study if the patient gave permission. Study clinicians completed an anonymous log of refusals for participation.

Specimen collection details were recorded on a *Specimen Collection and Processing Form* developed for the study, which included a study ID code for each participant but no identifying information such as patient name and address. It also included a *TB suspect register* number to allow trace back of results if required to provide additional clinically relevant results from the testing performed at VIDRL. The study form included space to record laboratory test results extracted from routine laboratory reports. The standard PNG NTP *Laboratory Request Form – AFB Microscopy and Xpert MTB/RIF* for diagnosis or monitoring of DS-TB treatment was used for routine details to be provided on patients with presumptive TB infection, including the smear and Xpert results recorded by the laboratory. The form is focused on sputum samples but can be used for other specimens such as LN-FNA. A log was kept of FNA samples stored and transported to the reference laboratory for culture and additional testing.

5.4.2 Data management and confidentiality

All clinical and test result data for the study were stored on paper-based forms and entered in a password protected electronic database by study investigators or staff. Completed standard NTP *Laboratory Request Form – AFB Microscopy and Xpert MTB/RIF* were stored as per standard practice. Paper study-specific forms were securely stored in locked cabinets in each provincial central site (PNG-IMR in EH, Burnet office in ENB, IMR/Burnet laboratory at PMGH in NCD).

Personal information was collected to the extent required for routine clinical care. The paper and electronic data exclusive to the study did not retain patient names or addresses beyond village/area of residence. Patient consent forms include contact details for patients who consent to follow-up contact and were kept securely only in paper format at each study site in PNG. A unique study identification (ID) code was assigned to each patient and used as the identifier on all other study data. A separate key of unique study IDs to lab/NTP IDs was kept in an encrypted electronic format and used only if validation of data or case trace-back was needed to provide additional laboratory results to inform patient care. Thus, data is classed as re-identifiable. Data was managed by the investigator team and study staff for the duration of the study. The electronic study database is stored securely on a password-protected computer. Data including only study ID codes entered in a computer for final analysis is backed up and stored on external password-protected hard drive or secure server location to protect against loss of data. Only members of the research team have access to the data. Data will not be used for purposes other than this research project.

Qualitative interviews were audio-recorded with permission of participants, then transcribed and translated verbatim into English by experienced qualitative researchers

from PNG-IMR. Transcripts were stored on password protected computers at PNG-IMR and UNSW Sydney and only available to the study team. Audio recordings were deleted after quality assurance of the transcriptions and translations was completed. All participants were provided with a pseudonym which is the only name retained in the stored transcripts, and no identifiable information shared in reports or publications.

5.4.3 Data analysis plan

Analysis was undertaken using statistical software (STATA version 17 or R). Categorical variables were described by frequency and proportion. Numerical variables were described by mean and standard deviation or median and interquartile range depending on normality assumptions. Differences in variables were assessed by province and bacteriological confirmation status, with categorical variables assessed by chi-square test and numeric variables by unpaired t test or Wilcoxon rank sum test depending on normality assumptions. Level of statistical significance will be set at 5%.

Analysis of qualitative ID and FGD transcripts was undertaken using a thematic analysis approach, where all data transcripts were coded in NVIVO 12 in a process of analytical induction, focusing on the identification of recurrent patterns[34]. Coding of transcripts were cross-checked for consistency by research staff at PNG-IMR and UNSW (under the guidance of co-investigator Dr Angela Kelly) and revised considering emergent themes. Initial codes were analysed in detail to identify sub-themes as well as differences and similarities within and across the transcripts. This is an iterative and reflexive approach with each piece of data building on the next, to develop overall thematic concepts.

5.5 Ethics, community engagement, partnerships

Ethical approval for the study was obtained from the relevant Human Research Ethics Committees of the investigators in PNG and Australia: the PNG IMR's Institutional Review Board, the Medical Research Advisory Committee (MRAC) of the PNG National Department of Health, and Alfred Hospital Medical Research Committee in Melbourne.

5.5.1 Informed consent

Written informed consent was sought from all patients diagnosed with presumptive TB-LN and recommended to have a FNA taken for testing. An explanation of the study was first provided, and any questions answered. In the case of children, consent was sought from their guardian. Trained study staff were responsible for obtaining informed consent for study specific requirements from participants/guardians as appropriate. Study and clinic staff ensured the participant/guardian was aware of study procedures and risk and provided time to ensure participant's/guardian's questions were answered. Participants aged <18 years required an adult guardian to provide consent and were asked for their assent if aged over 12 years. Illiterate participants (or those unable to sign) had the consent form read aloud to them by study staff. If they then consented, the staff member signed the consent form and noted that verbal consent was given, and a witness or guardian would be asked to countersign. All study material was available in English and Tok Pisin and copies available to participants/guardians. Consent could be withdrawn by any participant or guardian at any time, and participants would be asked but not required to provide a reason for withdrawing. Any participants withdrawing consent would be told they were able to continue accessing health services outside of the study and should receive standard care.

Participants in IDIs and FGDs were asked to provide written informed consent after an explanation of what participation involved and having any questions answered.

5.5.2 Research Governance and partnerships

This work was conducted in partnership between the Burnet Institute, the PNG IMR, PNG provincial health authorities, the PNG NTP, PNG CPHL, PMGH, and the VIDRL in Australia.

5.5.3 Community engagement & knowledge dissemination

The qualitative component of the study included the community through FGD in EH province. In ENB, updates have been provided to the Provincial Research Advisory Ethics Committee. Interim findings were presented at the 2022 PNG Medical Symposium. A plain language summary of study results will be developed for dissemination at various levels: interested participants and local community, healthcare staff at the participating sites, national level stakeholders.

6 Achievements against activities and outputs/milestones

Objective 1: Determine the proportion of presumptive TB lymphadenitis attributable to Mycobacterium tuberculosis complex in East New Britain and Eastern Highlands provinces, and National Capital District, Papua New Guinea.

Objective 2: Determine the proportion of confirmed TB lymphadenitis attributable to Mycobacterium bovis in East New Britain and Eastern Highlands provinces, and National Capital District, Papua New Guinea.

No.	Activity	Outputs/ milestones	Completion date	Comments
1.1	Protocol Development	Protocol finalised	30/10/2019	
1.2	Ethics Approval	Approved by MRAC, IMR IRB, Alfred HREC	22/05/2020	
1.3	Stakeholder engagement	Stakeholders in all 3 sites consulted.	Ongoing	Final feedback on outcomes to be provided
1.4	Site Assessment and training	3 sites assessed	10/09/2021	Multiple staff trained on FNA to facilitate recruitment in ENB
1.5	Participant Enrolment	3 sites completed	23/09/2022	Multiple interruptions related to COVID-19 (surges, restrictions, staffing)
1.6	Sample Testing	3 sites completed	Final cytology results 14/07/2023	Culture completed Dec 2022
1.7	Analysis and write up	Core analysis completed	Ongoing	Interim results presented at 2022 PNG Medical Symposium; Abstract submitted to 2023 The Union World Conference on Lung Health (not accepted).

Objective 3: Explore animal-human interactions that may pose a risk for zoonotic infections.

No.	Activity	Outputs/ milestones	Completion date	Comments
3.1	Protocol Development	Qualitative protocol and interview guides	30/10/2019	
3.2	Ethics Approval	3 ERB approvals	22/05/2020	
3.3	Data collection through Focus group discussions (FGD)	FGD conducted in EH (6 total) and ENB (8 total).	Dec 2021	FGDs conducted. Recordings transcribed, translated & quality checked
3.4	Data collection through In-Depth Interviews (IDI)	IDI conducted in EH (20).	Feb 2023	IDIs conducted. Recordings transcribed, translated & quality checked
3.5	Analysis and write up		Ongoing	All FGD data has been analysed. IDI analysis not yet complete. Abstract submitted to 2022 World One Health Congress (accepted in-person poster but lead author unable to attend). Research article being prepared to submit to a peer-reviewed journal.

All aspects of the study were challenged by repeated delays mostly resulting directly or indirectly from the COVID-19 pandemic, and some reduction in scope was necessary. Nonetheless, key activities were completed, with more than the target of 200 people with presumptive TB-LN recruited and FNA samples taken for laboratory testing. The project facilitated training in taking LN FNA by PMGH chief pathologist Dr Joseph, one of the senior investigators, to ten healthcare staff in ENB, and training in qualitative research methods by specialists from IMR to staff in ENB. FGD were completed in both ENB and EH provinces, and IDI were conducted with 20 people who had previously been assessed for presumptive TB-LN and had an FNA taken as part of the clinical component of the study.

7 Key results and discussion

Results will be presented in two parts: 1) evaluation of consecutive patients diagnosed with clinically presumptive TB-LN, and 2) qualitative exploration of animal-human interaction risks through in-depth interviews (IDI) and focus group discussions (FGD).

7.1 Patients with presumptive TB lymphadenitis

7.1.1 Cohort description

On the outpatient clinic days when study staff were in attendance and enrolling eligible consenting patients, they kept a screening log of people presenting with presumptive EPTB. Among 247 people with presumptive TB-LN who may have been eligible to participate, 17 were not screened for the study for various reasons including being unwilling to engage with the information process, and only two were screened but declined to participate, such that the proportion of people who participated amongst those potentially eligible was more than 91% in all three provinces. Amongst the 228 people who were enrolled, three agreed to participate but an FNA sample was unable to be obtained, and two had a sample taken but no results provided by the local laboratory. All subsequent results are for the 223 samples that were tested using Xpert MTB/RIF or Xpert Ultra. The proportion of female participants was 60% and not significantly different across the 3 provinces. The median age was 20 years, and was significantly different between sites, ranging from 25 in NCD to 11 in ENB (Table 1).

Table 1: Cohort demographics of patients with presumptive TB lymphadenitis enrolled in the study, by province.

	Total	NCD	EH	ENB
Participants enrolled	228	85	86	57
Participants with LN Xpert-MTB result *	**223**	**83**	**84**	**56**
Female (%)	134 (60%)	47 (57%)	56 (67%)	31 (55%)
Age [median; IQR] **	20 (10-32)	25 (16-36)	22.5 (11-35)	11 (8.5-14)
Age <15 years**	93 (42%)	18 (22%)	29 (34%)	46 (82%)

* Subsequent data includes only those that had a LN sample taken and an Xpert-MTB result recorded (n=223); ** Difference between sites ($p<0.05$)

Most participants (91%) had previously received a BCG vaccine (based on scar, vaccination card or verbal report). More than one fifth (21%) had previously been diagnosed with TB, which varied significantly by site with a much lower proportion (9%) in ENB. The type of TB was EPTB in almost two thirds (61%) of those with a previous diagnosis, and only one was reported to have been DR-TB, which was EPTB. Most (19%) of those who had been diagnosed also started treatment for that diagnosis. but the proportion was significantly lower in ENB (60% amongst 5 diagnoses). For those previous treatments the proportion completing treatment was only 65% across all sites and lower in ENB than the other sites. Nearly half (45%) of the participants had close contact with someone who had been diagnosed with TB in the last two years (Table 2). Only 18 people

had both previously been diagnosed with TB and had close contact with someone recently diagnosed with TB.

Table 2: TB history of patients with presumptive TB lymphadenitis enrolled in the study*, by province.

	Total (n=223)	NCD (n=83)	EH (n=84)	ENB (n=56)
Received BCG **	204 (91%)	79 (95%)	69 (82%)	56 (100%)
Previous diagnosis TB** ‡	45 (21%)	22 (27%)	19 (23%)	5 (9%)
Previous EPTB	28 (61%)	12 (55%)	14 (74%)	2 (40%)
Previous DR-TB**	1 (2%)	1 (5%)	0	0
Started treatment during previous episode of TB**	42 (91%)	22 (100%)	17 (89%)	3 (60%)
Completed treatment during previous episode of TB	26 (65%)	18 (82%)	7 (47%)	1 (33%)
Close contact of someone with TB (in last 2y)	101 (45%)	46 (55%)	30 (36%)	25 (45%)

* and who had a LN sample taken and an Xpert-MTB result recorded; ** Difference between sites ($p<0.05$); ‡ Proportions are of those with a previous diagnosis, except completion which is of those who started treatment.

The most common (100%) symptom recorded amongst participants was persistent enlarged LN, as expected for presumptive TB-LN. Other commonly recorded symptoms were fever or night sweats (48%), weight loss or infant failure to thrive (46%) and malaise (37%), all of which were significantly different across the three provinces. The median duration of enlarged lymph nodes was 12 weeks, which varied significantly across the study sites at 20 weeks in EH compared to 4 weeks in ENB. Duration of fever, weight loss

and malaise were also longer in EH and shorter in ENB compared to the median across all sites (Figure 2).

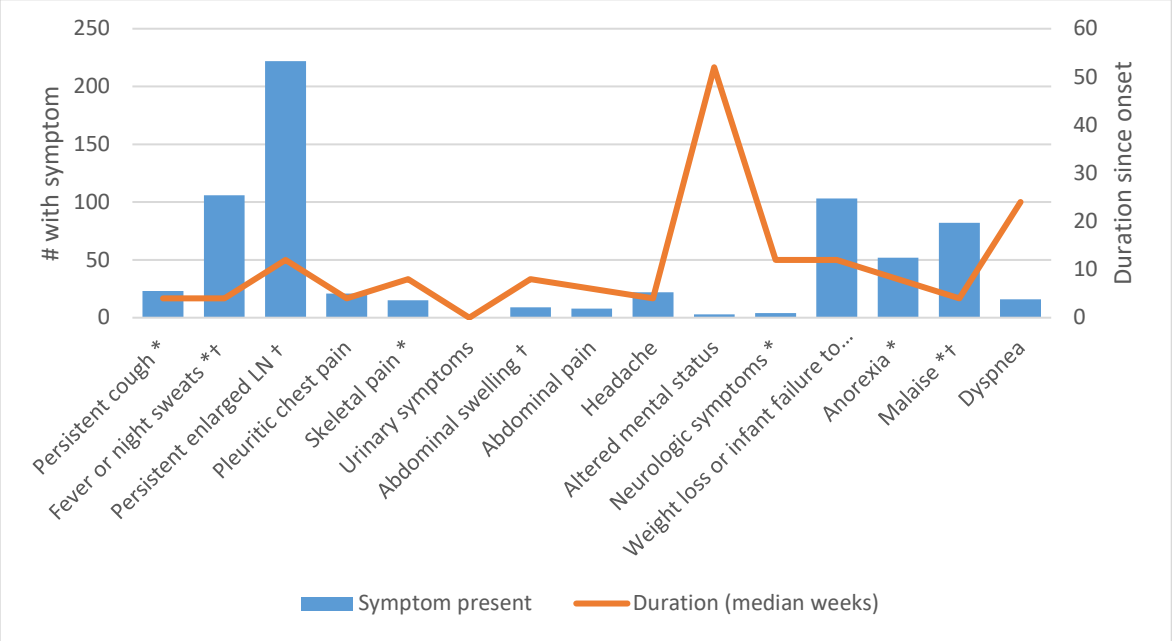

Figure 2: Symptom history amongst patients with presumptive TB lymphadenitis enrolled in the study.

* Difference in proportion between study sites (p<0.05), † Difference in duration between study sites (p<0.05)

Most participants (91%) presented with enlarged LN in only one site, which was mostly the neck (92%). The LN were usually palpable and visible, with similar proportions of people having the nodes on both vs. one side of the neck. The median size of nodes (measured as longest diameter and perpendicular diameter multiplied) was 4cm (Table 3).

Table 3: Lymph node presentation in patients with presumptive TB lymphadenitis enrolled in the study*, by LN site.

	Neck n (% of 223)	**Axilla** n (% of 223)	**Groin** n (% of 223)
Enlarged	205 (92%)	23 (10%)	11 (5%)
Visible	156 (70%)	15 (7%)	5 (2%)
Palpable	203 (91%)	23 (10%)	11 (5%)
Bilateral	104 (47%)	5 (2%)	5 (2%)
Unilateral	95 (43%)	17 (8%)	5 (2%)
Size (diameter multiplied, cm) [median; IQR]	4 (2-9)	4 (1-15)	6.5 (2-15)

* and who had a LN sample taken and an Xpert-MTB result recorded; 91% of participants had only 1 physical site type with enlarged LN; Maximum size at any site [median; IQR] = 4 [2-10]; 3 samples taken from other sites – 2x breast, 1x abdomen.

Relatively few other clinical findings were reported, except that 30% of participants had other indications of EPTB, ranging from 10% in NCD to 55% in ENB. The main other form of EPTB that was a presumptive co-diagnosis with TB-LN was disseminated TB in 30 (13%) of participants. Nutritional measures indicated healthy body mass index (BMI) (median 21.9, IQR 19.1 - 24.8) in participants aged 15 years and older. Only 3 children were recorded as having a middle upper arm circumference (MUAC) less than 125cm, as indicator of malnutrition. All participants had a presumptive diagnosis of EPTB, with 6% also having presumptive pulmonary TB, and 14% having presumptive disseminated TB recorded in additional to LN TB.

7.1.2 Laboratory test results

Approximately one quarter (26%) of LN FNA samples tested positive on Xpert MTB/RIF or Xpert Ultra indicating that MTBC was detected. This varied significantly by site, from 43% in NCD to only 7% in ENB (Table 4). The proportion of specimens that were positive was not significantly associated with the type of Xpert cartridge used (36% positive with Xpert MTB/RIF, 24% positive with Xpert Ultra, p = 0.15). Amongst the 58 samples positive on Xpert, 7 (12%) had rifampicin resistance detected. Other laboratory test result types were received on a smaller number of the total specimens due to tests not routinely being done (AFB smear) or remaining sample not being sent for additional tests at higher level facilities (PMGH for pathology when relevant and VIDRL for culture) or lost before processing (pathology tests). The number of samples with test results for each type are shown as denominators in Table 4. Nearly one quarter (22%) of AFB smear results were reported as positive for the presence of Mycobacteria, more than one third (36%) of cytology results were reported as being consistent with TB, but only 17% of culture results were reported as positive isolation of *M. tuberculosis*. No culture results were reported as isolating *M. bovis*. Two samples were reported to contain *Mycobacterium avium* complex (MAC), both from people recruited in ENB, and both Xpert MTB tests were negative. All *M. tuberculosis* isolates underwent drug sensitivity testing and four were resistant to one or more antimicrobial drugs, with all four resistant to rifampicin, including one also resistant to isoniazid, one also resistant to ethionamide, and one resistant to all three drugs (Table 4).

Table 4: Laboratory test results on LN FNA samples from patients with presumptive TB lymphadenitis enrolled in the study*, by province.

	Total (n=223)	NCD (n=83)	EH (n=84)	ENB (n=56)
LN Xpert MTB positive ** ‡	**58 (26%)**	**36 (43%)**	**18 (21%)**	**4 (7%)**
LN Xpert RIF positive	7 (12%)	5 (14%)	1 (6%)	1 (25%)
LN AFB smear **	27/124 (22%)	25/66 (38%)	2/33 (6%)	0/25
LN cytology consistent with TB **	**63/175 (36%)**	**35/72 (49%)**	**25/76 (33%)**	**3/27 (11%)**
LN cytology other findings reactive lymphadenitis non-specific inflammation cancer	 39 (22%) 16 (9%) 5 (3%)	 9 (13%) 7 (10%) 0	 13 (17%) 6 (8%) 5 (7%)	 17 (63%) 3 (11%) 0
Culture positive *M. tuberculosis* **	**34/205 (17%)**	**26/83 (31%)**	**7/66 (11%)**	**1/56 (2%)**

	Total (n=223)	NCD (n=83)	EH (n=84)	ENB (n=56)
Culture positive *M. bovis*	0			
Culture positive other (MAC)	2 (1%)			2 (4%)
DST of *M. tuberculosis* †	(n=34)	(n=26)	(n=7)	(n=1)
Rifampicin resistant	4 (12%)	4 (15%)	0	0
Isoniazid resistant	2 (6%)	2 (8%)	0	0
Ethambutol resistant	0	0	0	0
Pyrazinamide resistant	0	0	0	0
Ethionamide resistant	2/5	2	0	0
Bacteriologically positive by Xpert MTB or culture	61 (27%)	37 (45%)	20 (24%)	4 (7%)

* and who had a LN sample taken and an Xpert-MTB result recorded; ** Difference between sites ($p<0.05$); ‡ Quantitative results not provided. 2 specimens have comment noting 'trace' result: 1 where trace repeated and report positive; 1 where trace repeated and report 'not detected'. † resistance in 4 samples in total with resistance patterns of: (1) R-H, (2) mono R, (3) R-Eto, (4) R-H-Eto; MAC = *Mycobacterium avium* complex; DST = drug susceptibility testing

There were 25 samples that tested positive for MTBC on Xpert MTB but were negative when cultured, and three where MTB was not detected on Xpert MTB but was isolated from culture. Delays in handling of specimens were reviewed as days between the steps of 1) sending from the clinic to the laboratory, 2) storage at the laboratory (of remainder sample), 3) shipping to the reference laboratory (VIDRL), and 4) receipt by the reference laboratory. There was a significant difference between samples that were culture negative vs *M. tuberculosis* positive in the days from specimen collection until storage (median 1, IQR (0-4) vs. med 0 IQR (0-0) respectively; $p<0.05$) and for the days from shipping until received by VIDRL (med 7 (IQR 5-8) vs. med 8 (IQR 7-13) respectively; $p<0.05$).

Using the results from Xpert MTB testing and culture to identify all specimens where *M. tuberculosis* was detected, a total of 61 (27%) of participants had confirmed TB (Table 5). Most of these (81%) were also reported to have cytology consistent with TB. An additional 15 samples were reported as having cytology consistent with TB but were not confirmed as TB by bacteriological tests (Table 5), of which three had a past diagnosis of TB. With combined bacteriological and cytological results, a total of 76 (34%) participants had laboratory test evidence consistent with a TB infection. Amongst the 61 people from whom their LN FNA tested positive for *M. tuberculosis* on bacteriological tests, 10 reported they had previously been diagnosed with TB and 51 did not. All 10 who were previously diagnosed had started treatment. Amongst those with bacteriologically confirmed TB, the proportion with RR amongst those previously treated was 4/10 (40%), and amongst those not previously treated was 3/51 (5.9%).

Table 5: Combined key laboratory test results on LN FNA samples from patients with presumptive TB lymphadenitis enrolled in the study*

	Bacteriologically positive**	Bacteriologically negative or no result**	Total
Cytology consistent with TB	**48 (22%)**	**15 (7%)**	**63 (28%)**
Cytology not consistent with TB	**4 (2%)**	108 (48%)	112 (50%)
No finding or no result	**9 (4%)**	39 (17%)	48 (22%)
Total	**61 (27%)**	162 (73%)	223

* and who had a LN sample taken and an Xpert-MTB result recorded; ** Bacteriological result is combined data from Xpert MTB and culture of LN FNA, with positive being *M. tuberculosis* detected on one or both tests, and negative being a negative or no result on each test.

7.1.3 Bacteriologically confirmed TB

There was no association found between a result of bacteriologically confirmed TB and the sex of people with a presumptive TB-LN diagnosis. A larger proportion of bacteriologically confirmed TB occurred in people aged 15-49 compared to other age groups (p<0.001), in those living in urban rather than rural settings (p<0.001), and in those presenting in NCD compared to the other provinces (p<0.001) with a very low proportion positive in ENB (Table 6).

Table 6: Key characteristics of patients with presumptive TB lymphadenitis enrolled in the study, by bacteriological confirmation of their LN FNA

	Total	Bacteriologically confirmed TB	Not bacteriologically confirmed TB	p-value*
Sex				0.10
Female	134 (60.1%)	42 (68.9%)	92 (56.8%)	
Male	89 (39.9%)	19 (31.1%)	70 (43.2%)	
Age group				<0.001
<5 years	10 (4.5%)	1 (1.6%)	9 (5.6%)	
5-14 years	83 (37.2%)	6 (9.8%)	77 (47.5%)	
15-49 years	114 (51.1%)	50 (82.0%)	64 (39.5%)	
>=50 years	16 (7.2%)	4 (6.6%)	12 (7.4%)	
Home setting				<0.001
Urban	122 (54.7%)	46 (75.4%)	76 (46.9%)	
Rural	101 (45.3%)	15 (24.6%)	86 (53.1%)	

Study province				<0.001
NCD	83 (37.2%)	37 (60.7%)	46 (28.4%)	
EH	84 (37.7%)	20 (32.8%)	64 (39.5%)	
ENB	56 (25.1%)	4 (6.6%)	52 (32.1%)	

* chi-square test of proportions

A finding of bacteriologically confirmed TB was not significantly associated with self-report of a history of a TB diagnosis or close contact with a person diagnosed with TB in the previous 2 years. Most symptoms were not associated with the LN sample being bacteriologically confirmed as TB. A higher proportion of positive samples were observed with relatively uncommon symptoms of skeletal pain (p=0.003) and headache (p=0.045), and the more common symptom of weight loss (p=0.003) (Table 7). The self-reported duration of each symptom was recorded and was only significantly associated with a bacteriologically confirmed diagnosis of the duration of enlarged LN (median 16 weeks vs 8 weeks; p=0.029) (Table 7).

Table 7: Key clinical characteristics of patients with presumptive TB lymphadenitis enrolled in the study, by bacteriological confirmation of their LN FNA.

	Total	Bacteriologically confirmed TB	Not bact. confirmed TB	p-value*
Symptoms †				
Skeletal pain	15 (6.7%)	9 (14.8%)	6 (3.7%)	0.003
Headache	22 (9.9%)	10 (16.4%)	12 (7.4%)	0.045
Weight loss	103 (46.2%)	38 (62.3%)	65 (40.1%)	0.003
Duration of enlarged LN	12 (4-28)	16 (8-24)	8 (4-48)	0.029
Neck LN characteristics †				
Visible	156 (70.0%)	53 (86.9%)	103 (63.6%)	<0.001
Palpable	203 (91.0%)	58 (95.1%)	145 (89.5%)	0.19
Bilateral	104 (46.6%)	35 (57.4%)	69 (42.6%)	0.049
Unilateral	95 (42.6%)	22 (36.1%)	73 (45.1%)	0.23
Maximum LN size **				<0.001
<2 cm	19 (8.5%)	1 (1.6%)	18 (11.1%)	
2-<5 cm	96 (43.0%)	19 (31.1%)	77 (47.5%)	
5-<10 cm	47 (21.1%)	24 (39.3%)	23 (14.2%)	
>=10 cm	61 (27.4%)	17 (27.9%)	44 (27.2%)	

* chi-square test of proportions or Wilcoxon rank-sum non-parametric test of continuous data; † numbers show those with this presentation noted as a proportion of all presentations bacteriologically confirmed or not confirmed; ** largest size of LN in each person, in diameter multiplied (cm)

There was no significant difference in the proportion of confirmed diagnoses based on the body location of the enlarged LN, with most people presenting with enlarged LN in the neck. A high proportion (87%) of visible neck LN were bacteriologically confirmed

(p<0.001), and a higher proportion of samples were bacteriologically confirmed when enlarged neck LN were bilateral (p=0.049) rather than unilateral. FNA specimens from LN of larger size were more likely to be bacteriologically confirmed as TB, and this association was significant for neck LN in participants aged 5-14 years (p=0.015) but there were few confirmed diagnoses in that age group (Figure 3). The maximum diameter of LN at all body sites was associated with bacteriologically confirmed TB in a non-linear fashion, so sizes were categorised pragmatically as <2cm, 2-<5cm, 5-<10cm and ≥10cm. A larger proportion of bacteriologically confirmed TB occurred in LN of size 5-<10cm compared to LN that were smaller or larger (p<0.001) (Table 7).

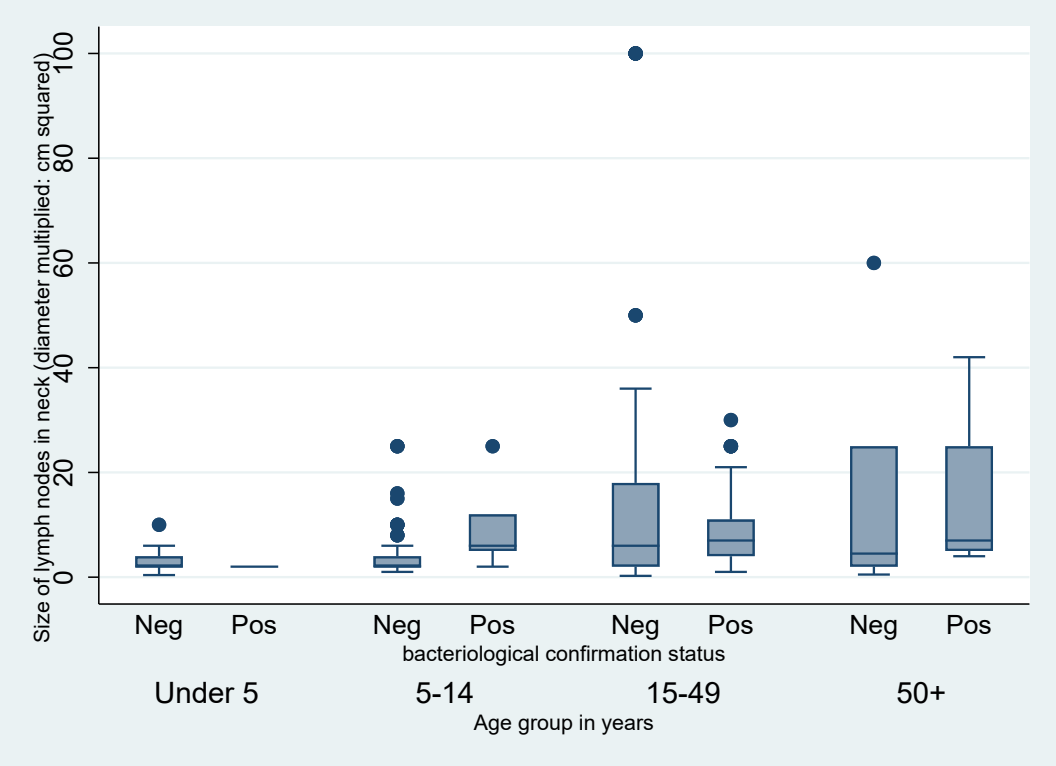

Figure 3: Size of neck lymph nodes by age group and bacteriological confirmation status amongst patients with presumptive TB lymphadenitis enrolled in the study.

7.1.4 Discussion of LN FNA results

We confirmed TB through bacteriological tests (Xpert MTB or Ultra and culture) in 61 (27%) of 223 LN FNA acquired from people presenting with clinically presumptive TB-LN in three provinces of PNG. A further 15 samples were reported as having cytology consistent with TB but were not confirmed as TB by bacteriological tests, resulting in 76 (34%) of participants having laboratory test evidence consistent with a TB infection. None of the LN specimens that were successfully cultured were found to contain *M. bovis*, but two specimens contained *Mycobacterium avium* complex (MAC).

Amongst the 34 specimens that had *M. tuberculosis* isolated through culture, all underwent DST and 4 (12%) were resistant to at least rifampicin. Xpert MTB also detected 12% of samples with rifampicin resistance (RR), however this was 7 of 58 samples. Xpert MTB detected the 4 RR isolated through culture, all of which were from NCD, and Xpert MTB detected one additional resistant sample in each of the study sites. Of these three additional RR samples, two did not have *M. tuberculosis* isolated through culture, and one had *M. tuberculosis* isolated through culture but no resistance detected on DST; that specimen was detected as 'low' on Xpert quantification. RR of 12% was a relatively high

yield, and while the estimates of 5.9% RR amongst new (not previously treated) cases and 40% in previously treated cases are also relatively higher, they are within the uncertainty intervals of the estimates for 2021 provided by WHO, of 4% (1.6-8.4) in new cases and 23% (6.2-53) in previously treated cases.[1] TB-LN in much of PNG has usually been diagnosed clinically, which would mean RR would not be detected and correctly treated. LN FNA is done by pathologists at PMGH (the location of the NCD study site), and if Xpert MTB/RIF is always successfully done then our results (5 of 7 RR were in NCD) indicate that a high proportion of the RR TB-LN in PNG would be detected, however there was RR detected in both other study sites and there are many other provinces and facilities in PNG. Isoniazid and ethionamide resistance were also detected in 2 samples each, and co-incident with RR. The number of these resistance profiles detected was small, but it is reassuring that there was no isoniazid mono-resistance and all cases of resistance would have been detected by use of only Xpert MTB/RIF or Ultra (without subsequent culture) because all included RR.

No LN specimens that were successfully cultured were found to contain *M. bovis*. It is possible that organisms were present but not detected amongst the 27 FNA samples that were positive on Xpert but on culture were negative (n=25), contaminated (n=1) or not tested (n=1). *Absence of evidence* is not the same as *evidence of absence*, however our findings do not provide any evidence that zoonotic TB (*M. bovis*) is contributing to the high proportion of EPTB in PNG. In a published summary of PNG 2016 TB notifications[2], the highest proportion of EPTB in the 3 provinces that participated in this study was 78.5% in EH, followed by 42.8% in NCD and 25% in ENB. We did not attempt to sample proportionately by province, but the relatively low proportion of bacteriologically confirmed clinically presumptive TB-LN in EH province (24%) might indicate that the historically high proportion of notified TB that was EPTB may include a notable proportion of overdiagnosis of TB on clinical grounds, with other causes therefore being missed. In ENB province a very low proportion (7%) of the clinically presumptive TB-LN was bacteriologically confirmed, and where cytology was completed a high proportion (63%) was reported as reactive lymphadenitis, again an indication that other causes of illness are being missed, a high proportion of which were in children.

In ENB all participating sites were BMUs where a broad range of people present for healthcare, and which notify TB diagnoses to the National TB Program. The cohort of participants from ENB had a large proportion of children and thus was much younger compared to EH, which included patients from the provincial hospital TB outpatient (predominantly adult) and inpatient facility. NCD had a very low proportion of children compared to both other provinces, because participants were primarily recruited from the PMGH TB outpatient clinic (predominantly adult) which also received referrals from elsewhere in NCD and Central province for further assessment of possible TB, and thus may have been a somewhat pre-selected population with a greater likelihood of a positive TB test. The people recruited to participate in our study were a reasonable reflection of the people presenting to the facilities that contributed to the study. The relative proportion of bacteriologically confirmed TB by province was also within what might be expected based on the presenting patient populations from which they were recruited.

Obtaining a LN FNA specimen was very important for bacteriological confirmation of TB infection, and there was additional benefit from making slide smears for cytological examination by pathologists. Cytology identified an additional 15 specimens where the examination findings were consistent with a diagnosis of TB, 39 with a finding of reactive lymphadenitis which indicates further follow up and/ or investigation is warranted, and 5 cancer diagnoses were made. This will have facilitated better decisions on further tests and treatment. Reactive lymphadenitis can be due to a range of different causes. For example, cervical reactive lymphadenitis may be due to infective causes including bacteria, viruses, dental infections as well as non-infective causes such as allergic, chemical, pollutants amongst other causes.

The age group where the greatest proportion of patients were found to have bacteriologically confirmed TB were those aged 15-49 years, aligning with the high proportion of adults in NCD where the highest proportion of specimens were bacteriologically confirmed as TB. A higher proportion of those living in an urban setting had bacteriologically confirmed TB, which may reflect denser living conditions and more exposure to TB than in rural settings but may also be influenced by differences in access to healthcare. Symptoms that were more common in those with bacteriologically confirmed TB, were skeletal pain, headache and weight loss, and a longer duration of enlarged LN. Our findings indicate that clinical presentations with a higher likelihood of a bacteriologically positive result would be visible LN on both sides of the neck with the largest of 5-10cm (diameter multiplied), and of a duration of at least 8 weeks. This may aid with prioritisation if required, noting that other presentations may also return positive results. This study has confirmed TB in a relatively low proportion of children presenting with enlarged LN, the cytology results where available were mostly non-specific inflammation or reactive lymphadenitis. We are therefore unable to diagnose the cause of enlarged LN in these children.

7.2 Qualitative exploration of risks for zoonotic infections

7.2.1 Community Focus Group Discussions

A total of 14 FGD were held with 141 community members from ENB and EH, with groups formed based on gender (men or women) and community setting (urban or rural) and a range of 5 to 13 people per group. Data analysis showed that common health issues in the community included typhoid, respiratory illness, lack of clean water supply, malaria, diabetes, and skin itching, with the latter an issue raised in most groups as a problem.

Participants were asked about swelling in the body, and swollen lymph nodes in the neck. For many, it was the first time they had heard it could be a sign of TB. Most attributed swelling in the body to other health issues or caused by custom (e.g., sorcery if the swelling increases).

> *“What you are now saying, we normally see a lot of [swelling] but we don’t usually think that they have this kind of sickness (TB).” Woman, Aiyura, EHP*

> *“But when this thing gets worse [swelling is large, increases], the mindset of the whole community goes straight to (sorcery).” Man, Aiyura, EHP*

When asked about their knowledge of TB, some participants related TB transmission to spitting, sharing food/plates, being passed down the family line, airborne, coughing, and custom.

Regarding perceptions about animal to human disease transmission, it was shared that specific animals may transmit specific illnesses and diseases.

> *“I think that the pigs are transmitting this swine flu. When they cough or when they have runny nose, and if we are staying close to them, I think that they are spreading the swine flu through it.” Woman, Emagave, EHP*

> *“I usually think that perhaps the cat might pass asthma sickness to me. I often think like that.” Woman, Aiyura, EHP*

Some people felt that consuming animals that died of illness was the cause of some illness in people:

> *“I don’t allow the children eating the dead animals [who were ill] why, because we might be getting the sick which the animal died of” Woman, Vunapalading, ENB*

Others thought that animals could be killed and eaten without considering if they are infected with anything. A range of animals are consumed, with various ways described for preparation, cooking and consumption. Interaction with wild animals usually included consumption as well as other uses (Table 8).

Table 8: Community interaction with and purposes of animals (wild)

Animal	Purpose
Cuscus	• consumption • furs are twisted into bilums, grass skirts for singsing, bones for traditional attire
Wild cats	• consumption
Birds	• consumption • feathers for traditional dress
Wallabies	• consumption • bones for sharp needles
Green snake	• consumption • boiled with greens and eaten • snake oil for cooking • remedy for women to ease body aches and pains
Bandicoot	• consumption • roasted over open fire and eaten
Fish	• consumption • common food protein that is sold for cash
Green frog	• consumption
Echidna	• consumption
Fly beetle	• consumption

In relation to community interaction with and what they viewed the purpose of domesticated animals, they were seen to serve multiple purposes and have high value. The FGDs revealed that the spaces animals, particularly pigs and dogs, and humans occupy were fluid and entangled, resulting in relationships that are highly valued. Animals served multiple purposes including for protection, problem-solving, consumption, income, and companionship.

> *"Regarding all those animals that we are looking after, we think that we look after them so that they can help us…But we don't know whether there's going to be any sickness or not because we think that it's only helping us…We only think about how it's going to help us with the school fees or the problems and things like that and we don't think about the sickness." Man, Aiyura, EHP*

Often, the animals identified were not viewed as vectors of disease to be avoided, but instead were given a great deal of care, affection, and attention.

> *"The dog that we look after, we do not let them be on their own to grow, but we normally hug them, kiss them, and talk to them. For pig as well, we do hold them and scratch their skin. The pig is my money so I must hold them, like them, kiss them, talk to them, and scratch their skin." Woman, Aiyura, EHP*

Within the context of our study, animals and humans could not be viewed as occupying distinct conceptual spaces with strict boundaries. Animals were considered an essential part of participants everyday life.

7.2.2 In-depth interviews with people who had presumptive TB-LN

Amongst the 28 participants in EH province who consented to follow-up contact, 25 were successfully contacted, all agreed to participate in an IDI and 20 IDI were successfully conducted. This included at least 4 people in each of the 4 subcategories encompassing male/female and urban/rural living. Thematic analysis has not been completed at the time of finalising this report; the findings will be shared with stakeholders when available.

7.3 Discussion

Our investigation of people presenting to healthcare facilities with enlarged LN and diagnosed with clinically presumptive TB LN found that 27% had bacteriologically confirmed TB, with another 7% having cytology consistent with TB but not bacteriologically confirmed. All samples that had MTBC successfully isolated by culture in the reference laboratory were *M. tuberculosis*, with no evidence of *M. bovis* causing presumptive TB-LN. That does not mean *M. bovis* might not be a cause of illness in other parts of PNG, or in other people in these provinces, but it would not appear to be common in these locations.

The findings from our study indicate the benefit of FNA being routinely utilised with Xpert testing for TB-LN diagnosis. This is currently recommended in PNG guidelines but is often not done except for in major facilities. The additional benefit of confirmed diagnoses of cancer highlights the importance of FNA with cytology for potentially synergistically improving care for both TB and for cancer. Strengthening the diagnostic capacity and network through the leading role of PMGH Pathology department and CPHL is shown by this study to be feasible. This study is a reminder that in settings lacking adequate diagnostic facilities, empiric treatment for TB may incorrectly diagnose someone with cancer as having TB and not only fail to treat the cancer, but also provide drugs for TB with unnecessary toxicity risk.

The study also highlights that overdiagnosis is probably occurring. It is important to note that EPTB including LN-TB is not always possible to confirm bacteriologically and may still require empiric treatment. For example, a review in Australia showed only 58% of EPTB was bacteriologically confirmed, albeit with greater investigational capacity than available in the present study context.[35] Nevertheless, the majority of LN sampled in this study were not confirmed as TB and may suggest that further training about appropriate diagnosis and selection of patients for further investigation, especially for children, is needed. Those with small lymph nodes could potentially be observed over time before having FNA if there are no significant concerning symptoms or examination findings. In addition, those with a finding of reactive lymphadenopathy may not warrant empiric TB treatment if Xpert results are negative and may instead be able to be observed.

Our study enrolled people from facilities selected due to the proportion of TB cases historically notified from those facilities and that they were logistically feasible to include. We had aimed for enrolment of consecutive patients presenting with clinically presumptive TB-LN, but repeated COVID-19 related interruptions and some staff availability challenges meant that this was not possible, but on individual clinic days we assessed most people meeting the inclusion criteria. We had aimed for presumptive diagnoses to be based on clinical assessment by clinic staff, however due to workloads these assessments were performed by qualified health extension officers (HEO) employed for the study. Therefore, we cannot be certain that those recruited to the study accurately represent the routinely detected presumptive TB diagnoses from these clinics, nor that the people presenting to these clinics are representative of the wider provinces. Furthermore, the study did not record whether participants were started on TB treatment following presumptive or

confirmed diagnosis of TB, and therefore cannot draw conclusions as to the impact of an unconfirmed presumptive TB diagnosis on actual treatment practice. These were limitations of the study due largely to the impact of the COVID-19 pandemic on the clinical staff available at participating study sites and the time limitations on the study.

The type of interaction that community members have with animals, both domestic and wild, indicates there is the potential for zoonotic illness – bovine TB or other illnesses such as swine flu – to occur and be unrecognised if people are not considering that there may be risk associated with their contact with animals and their body parts. Any attempt to raise awareness about potential risks must be contextualised within the entangled living and high value placed on animals. Strategies designed to reduce zoonotic disease risk should include an exploration into the cultural value of animals, along with the relationships developed at the human-animal interface in an effort to explore the sociocultural value animals hold to humans, so that we develop effective culturally appropriate strategies that consider these relationships.

8 Impacts

8.1 Scientific impacts – now and in 5 years

Preliminary findings of the clinical sampling of LN of people with presumptive TB LN were presented at the PNG Medical Symposium in 2022 and associated meetings of laboratory scientists. This generated interest in the methods and findings and we understand that FNA samples from ENB from regular clinic activities have been independently sent to PMGH for testing after the study concluded, suggesting that this activity is recognised as clinically useful and is likely to be sustainable.

8.2 Capacity impacts – now and in 5 years

A total of 11 people were trained in taking FNAs during the study. In September 2021, EZARET supported PMGH chief pathologist Dr Joseph to facilitate training with 10 doctors, HEOs and laboratory staff from 5 facilities in ENB. The one-day workshop covered theory and supervised practice in the outpatient clinic at Nonga Hospital. In May 2022, Burnet HEO Dorish Walsh was trained in FNA by pathologist Dr Chanoan over three weeks, with weekly supervised practice at the TB clinic at Goroka Base Hospital. Most FNA for participants from ENB were then performed by Dorish Walsh, however after skills training and equipment provision (FNA 'guns') in ENB, after the study concluded FNA have been sent from clinics in ENB to PMGH for laboratory testing.

In June 2021, PNG IMR staff trained Burnet staff from ENB in qualitative research methods, including FGD facilitation. Burnet staff supported PNG IMR in organising and facilitating FGDs and provided local language translation, providing valuable research experience for these staff. With strengthened skills from the training, the staff have continued undertaking IDIs and FGDs with other projects in ENB.

Findings from this study will be shared with the PNG National Agriculture and Quarantine Inspection Authority (NAQIA) to contribute to the knowledge-base on detection of zoonotic pathogens in PNG. Findings will also be provided to the Fleming Fund team who are implementing a laboratory information management system (LIMS) at 6 sites in PNG. While *M. bovis* was not found in the study, the possibility of it being present warrants discussion of what actions should be taken if it is detected and any implications for ongoing surveillance and how human laboratory information can be linked and provided to animal health specialists in PNG to guide targeted response.

Three key staff members in PNG who worked on this study and were given opportunity for greater leadership in the project have also worked jointly on the Indo-Pacific Centre for Health Security-funded grant for capacity development: Papua New Guinea and Republic of Indonesia Micro-Elimination (PRIME-TB) project. While aimed specifically at human TB disease, strengthening capacity of staff who have worked on both projects has facilitated broader understanding of One Health and communication of these issues to colleagues, patients and community members.

8.3 Community impacts – now and in 5 years

The project has engaged regularly with the East New Britain Local Implementers Group (LIG), consisting of PHA directors, District Health Coordinators, and OICs and representatives from ENB health facilities. These meetings promote local engagement and ownership of the study and ensure that findings can be discussed with key stakeholders who can influence future steps.

One unanticipated community impact has been an improvement in cancer diagnosis in EH province. The FNA sampling in Goroka has improved links between the TB service and

the pathology service. The introduction of FNA for possible TB lymphadenitis has resulted in cytological diagnosis of cancer which was not being offered as a service prior to this project commencing. The strong links developed in this project between TB service, laboratory and pathology services is a useful example for other provinces for improvements in provision of diagnostic services to the community.

8.3.1 Economic impacts

There are no direct economic impacts from this study.

8.3.2 Social impacts

There are no direct social impacts from this study.

8.3.3 Environmental impacts

There are no direct environmental impacts from this study. Participants in FGDs and IDIs may have reflected on their interactions with wild animals as a result of the discussions, but these activities were not attempting to stimulate behaviour change.

8.4 Communication and dissemination activities

A newsletter and list of key stakeholders was developed to provide regular updates. Regular LIG meetings have facilitated communication with local stakeholders in ENB.

Preliminary findings of the clinical sampling of LN of people with presumptive TB LN were presented at the PNG Medical Symposium in 2022. An abstract was submitted to the 2023 International TB Union conference. Full findings are being summarised to share with stakeholders in PNG and prepared to submit for publication in a peer-reviewed journal.

Findings from FGDs were submitted to the 2022 World One Health Congress, and while accepted it was not possible for the lead author to attend and present. An article based on the FGD findings is also being prepared to submit for publication in a peer-reviewed journal. The article will examine how certain animals are treated and prioritised in contemporary PNG, and what the implications are for developing zoonotic risk mitigation strategies within this context.

9 Conclusions and recommendations

9.1 Conclusions

In this study we aimed to evaluate whether presumptive TB lymphadenitis (TB-LN) in three provinces of PNG is caused by Mycobacterium tuberculosis complex (MTBC), specifically *M. tuberculosis* or *M. bovis*, and assess whether there are important zoonotic infection risks. We found that 27% of 223 people presenting with presumptive TB lymphadenitis had bacteriologically confirmed TB due to *M. tuberculosis*. No people had TB due to *M. bovis*. This indicates that *M. bovis* is not a common cause of enlarged LN in the facilities that participated in the study. These facilities have historically seen a relatively high proportion of TB cases in each province but may not represent all people with presumptive TB-LN in these provinces, or in the remainder of PNG. Therefore, we cannot conclude that *M. bovis* is not present and causing illness in PNG, but we found no evidence that it is. Through the qualitative exploration of risks for zoonotic infections we found that interaction with both domestic and wild animals can be close, with animals used and valued in a variety of ways. Animals were valued for protection, problem-solving, consumption, income, and/or companionship. There was some awareness that animals may pose a risk of illness to humans, but also many interactions described where there was no perception of the possibility of harm and therefore no mitigation of risk. Many people interviewed did not actively consider risk because animals served such an essential sociocultural role, whether for consumption, livelihoods, or other cultural reasons. These discussions on the value and purpose of animals are important as it demonstrates the role animals play in the lives of people within these contexts, which is crucial to developing (effective) culturally appropriate risk mitigation strategies.

9.2 Recommendations

Clinicians in PNG should try to follow the National Guidelines and take LN FNA samples from people presenting with clinically presumptive TB-LN. This will require training and support. The priority for testing should be to use Xpert MTB/RIF or Ultra because of the possibility of TB that is drug resistant. In addition, GeneXpert machines are available in every province and use requires less expertise than cytology. However, where cytology can be performed there may be added benefit in providing an alternative diagnosis when Xpert MTB is not positive. The finding of similar proportions of drug resistant TB amongst TB-LN as amongst Pulmonary TB is an important finding for PNG and highlights that a significant proportion of TB-LN, especially in those with a previous history of TB, will be incorrectly treated with an inadequate regimen that may amplify resistance.

M. bovis has not been found, however whenever samples (LN FNA or sputum) are sent to a reference laboratory for culture then the tests will confirm Mycobacterium species. The possibility of *M. bovis* as a cause of illness can be monitored passively through this diagnostic pathway.

There are a range of potential zoonotic illness that may be a threat to humans in PNG. Any attempt to raise awareness about potential risks of interactions with animals and animal products should be carefully contextualised within the entangled living and high value placed on animals.

10 References

10.1 List of publications produced by project

None to date.

10.2 References cited in report

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