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Bovine Campylobacteriosis and Tuberculosis in Dairy Farming

Bovine campylobacteriosis (Cb) and *Bovine tuberculosis* (bTB) caused by bacterial pathogens have a large number of reservoir hosts, and environmental persistence with zoonotic importance. Despite the risk of huge economic penalties, these diseases are rather neglected in low and middle income countries including Bangladesh. Farmed cattle infected with *Campylobacter* spp. can infect humans. In Bangladesh, *Campylobacter* infections have been established to be a significant public health burden like diarrhea and the Guillain–Barré syndrome. On the other hand, bTB is a bacterial disease of cattle mainly caused by *Mycobacterium bovis*, a member of the *M. tuberculosis* complex. However, *M. orygis* has also been reported to be a major causative agent of bTB in Bangladesh in recent times. Cattle are the primary hosts for *M. bovis*, but other domesticated wild mammals as well as humans can also be infected. In under developed countries, bTB still prevails can cause severe economic loss arising from livestock deaths, chronic disease and reduced production. Bangladesh has a very high ruminant population density (145 large ruminants/ sq km). In the rural areas of the country people live in close contact with domesticated animals and birds sharing the same premises, and some people even consume raw milk (Islam *et al*., 2021). Lack of good farming practices (GFP) in livestock farming in combination with lack of food safety compliance and poor personal hygiene in slaughtering and meat processing activities aggravate transmission of zoonotic diseases like bTB and Cb. In this backdrop, a KGF sponsored project studied the prevalence of prevalence of Cb and bTB, identified potential risk factors for transmission of these zoonotic diseases estimated the economic impacts of the diseases associated dairy farming practices in Dhaka and Mymensingh districts of Bangladesh, and tried to develop a sanitation program complying with GFP.

Methodology

In all 1080 samples were collected from 90 dairy farms of two districts (Dhaka and Mymensingh) through a cross-sectional survey. The study included 4 breeding (crossbred-3 and indigenous-1) bull farms that comprised 410 bulls, of which 300 were sampled in this study. Three hundred and thirty stool specimens were collected randomly from patients suffering from gastroenteritis symptoms including diarrhea admitted to the Surya Kanta Hospital, Mymensingh Medical College. Samples collected from both animals and humans were evaluated by both basic (culture and biochemical tests) and advanced molecular based assays (PCR).

To estimate the prevalence of the two zoonotic diseases and to identify potential risk factors, 79 herds with 1865 animals from five districts (Dhaka, Mymensingh, Gazipur, Jamalpur and Munshiganj) were included for the tuberculin skin test (TST) in this study. The intradermal test (SICTT) was performed as per the standard procedure (Fig. 1).

Fig. 1. (a) Administration of tuberculin (both bovine and avian) in farmed cattle and subsequent data collection from animals in the process of SICCT for potential risk factors of bTB positive status, (b) animal-level sample collection for confirmation of *Campylobacter* positive status from breeding bulls

Along with the above, a cross-sectional survey was conducted to assess the knowledge and practices of cattle handlers on bTB. A list of dairy farms was obtained from the three relevant district livestock offices (Dhaka, Gazipur and Mymensingh) and 404 participants from the three districts were interviewed for the knowledge and practice assessment.

Samples (vital organs: lung, liver, kidney, etc. of cattle) from abattoirs/butchers' shops along with postmortem samples were collected from dairy farms and zoological gardens. The samples were processed as per standard protocol and grown in both solid (L-J slants) and liquid (MGIT 960) culture media. Staining of the samples were done though smear microscopy using the Ziehl Neelsen (Z-N) technique. The acid fast bacilli (AFB) stained as red and appeared slightly bended in positive cases. Positive mycobacterial cultures were identified on the basis of colony morphology and molecular methods. The line probe assay (LPA) was for genotyping of culture bacteria and later molecular characterization was done through whole genome sequencing (WGS) of culture mycobacteria.

Economic losses associated with bTB and Cb were assessed using a standard method. In this evaluation, data on production parameters were collected through face to face interviews of the relevant stakeholders. Different loss parameters were confirmed from secondary data.

Results and Outputs

Study of the Pathogens

Serotyping using Penner's scheme was sufficient for identification of about 57% of the dairy *C. jejuni* isolates, and about 61% C. jejuni isolates of bull origin. However, serotyping using Penner's scheme was sufficient for identification of about 68% *C. jejuni* isolates of human origin. Different virulence and toxin genes were identified in *C. jejuni*, *C. coli* and *C. fetus* isolates. The 16S rRNA gene sequences of *C. jejuni* (n=17), *C. coli* (n=1) and *C. fetus* (n=3) analyzed in this study were registered with the Gene Bank. Many *C. jejuni, C. coli* and *C. fetus* isolates were found to be multidrug resistant.

The project successfully applied the Gamma interferon assay (GIA) for the first time to detect bTB in Bangladesh as a parallel testing protocol along with TST. Among animals tested (n=148), the ratings with TST for bTB were: positive (n=125), inconclusive (n=17) and negative (n=6); the same groups tested positive for bTB with GIA in the order 83.2% (n=104), 64.7% (n=11), and 16.67% (n=1), respectively. The agreement obtained between the two tests was substantial (Kappa agreement: 77.7%, 95% CI=70.9-84.5%, P=0.004). Since a considerable proportion of the TST-inconclusive animals (64.70%, n=11) presented as positive with the GIA test, it is recommend that parallel tests with GIA be done along with TST to increase the sensitivity of the bTB test protocol.

Of 1310 samples, 209 were human sputum samples, 942 cattle samples (vital organs: lung, liver, spleen, lymphnode, milk, feces and pharyngeal swab) from slaughtered, TST positive and post mortem cattle and 159 wild species samples (deer, n=1; wildebeest, n=8; lion=8; goyal, n=5; common eland, n=1; impala, n=7; and monkey, n=128 and girrafe, n=1) that included vital organs collected during post mortem examination and feces samples of wild species in zoological gardens. Fifteen animals (cattle: 6 and wild species: 9) were found provisionally positive as MTBC through acid fast technique.

Of 15 animal samples, 11 (5 cattle and 6 wild species) showed bacterial growth in culture media, both in solid (LJ Slant) and liquid (MGIT). The culture organisms were genotyped via the Line Probe Assay (LPA) and confirmed as *Mycobacterium africanum (M. orygis)*. Of 209 sputum samples (cattle handers and butchers), 2 were confirmed as *Mycobacterium tuberculosis* through genotypying.

Disease prevalence

Bovine campylobacteriosis

Overall, farm-level prevalence of bovine *Campylobacter* was found to be 53.3% (95% CI: 42.5-63.9). The feces samples had a high level (30.9%) of contamination ((95% CI: 27-35%) followed by the manure swab (pooled) at 15.6% (95% CI: 8.8-24.7%). *Campylobacter* jejuni was documented as an abundant species (12.6%), followed by *Campylobacter coli* (5.1%), and *Campylobacter fetus* (0.3%). Farm age >5 years with zero or minimum cleaning and disinfection practices, and animals roaming outside the farm were documented as significant risk factors for farm-level Campylobacter occurrence.

Nearly one in three persons hospitalized with diarrhea was found to be infected with *Campylobacter* spp. The overall prevalence of *Campylobacter* spp. was estimated to be 31.5% (104/330) among which the prevalence of C. jejuni was 21.8% (n = 72), and that of C. coli was 9.6% (n = 32). Among the positive cases, the prevalence of *Campylobacter* was higher in the age group 0-5 years (52%) followed by 6-18 years (42.7%), 19-40 years (34.0%), 41-60 years (25.4%), and >60 years (10.5%). Age, family level personal hygiene, and involvement with animal husbandry came out as potential determinants of a Campylobacter positive status.

Among the surveyed farms, 75% (95% CI: 19.4-99.4%) were confirmed to have bulls infected with *Campylobacter fetus* at herd level. However, animal level occurrence of *C. fetus* was estimated to be 8.7% (26/300) (95% CI: 5.7-12.4%). Natural service increased the odds of *C. fetus* infection and Cb in bulls by 38.18 times (95% CI: 13.89-104.94) in comparison with artificial insemination.

The study confirmed an annual estimated economic loss due to Cb in farmed cattle to the tune of BDT 1,282.26 million (95% CI: 1,120.41-1,448.00), equivalent to USD 15.09 million (95% CI: 13.18-17.04). The study showed that the cost restocking of heifer and cows (BDT 703.89 million, 95% CI: 615.12-794.98) contributed most to the overall loss.

Bovine tuberculosis

The herd level prevalence of bTB was estimated to be 45.6% (95% CI: 34.3-57.2%) and animal level prevalence was found to be 11.3 (95% CI: 9.9-12.8%) using the OIE recommended >4 mm cut-off. The greatest risk of bTB was found to be in older and pregnant cattle within large herds (>20) which highlighted an urgent need for continued surveillance and implementation of bTB control programs in Bangladesh.

Inappropriate practices such as; not using protective equipment (98%); smoking, drinking or eating food whilst working with cattle (69%); and sharing the same premises with animals (83%) were identified as high bTB risk factors for humans. Those rearing animals for 1-5 years were 2.67 times (95% CI: 1.44-4.91) more likely to have adequate knowledge of bTB compared with those who had reared animals for >15 years. Cattle handlers with a monthly income of 10,000-20,000 taka were significantly (odds ratio = 0.36, 95% CI: 0.14-0.92) less likely to have adequate knowledge compared with those with monthly incomes <10,000 taka. Cattle handlers with high school or higher education were 6.98 times (95% CI: 2.47-19.71)

more likely to apply appropriate bTB control and prevention practices compared with those without formal education. Overall, education, duration of cattle rearing and monthly income predicted bTB knowledge and practices well.

The total economic caused by bTB was estimated to be BDT 107.33 million (95% CI= 76.17-149.05 million, equiv. US$ 12.78 million, 95% CI= 9.07 -17.74 million) in the two districts. Decreased milk production accounted for the greatest loss (BDT 55.74 million, 95% CI= 39.61-77.55 million).

Expected Impact

Results of this study will help reduce the risk of introduction of Campylobacter pathogens into animals, farms and the environment. The findings will be helpful in handling the public health burden (diarrhea and AMR status) in people at high risk through framing control measures in livestock rearing and dairy farming, and will support livelihood and food security among marginal communities in rural Bangladesh.

This study confirmed the presence of circulating mycobacteria causing bTB through SICTT and Gamma interferon assays and identified the risk factors. The information will help frame policies to mitigate the health and economic burdens by curbing human exposures and controlling bTB in animal-human interface.

Recommendations

- Stringent biosecurity and hygienic measures need to be enforced to lessen the load of the *Campylobacter* pathogen in the farm environment and prevent transmission to other animals and humans
- Campylobacter spp. was found to be the major causative agent of diarrhea among hospitalized patients, and proper measures need to be taken to reduce the burden
- Official guidelines for *C. fetus* control and prevention in Bangladesh including mandatory artificial insemination in reproductive cows and heifers, routine screening of breeding bulls for C. fetus free status need to be developed
- High herd and animal level prevalence of bTB found in the five districts highlights an urgent need for continued surveillance and implementation of bTB control programs in the country
- In view of the fact that *M. orygis* is circulating in diverse animal species like farmed cattle, captive wild species the Government should consider providing compensations for the culled animals if tested positive and ensure routine screening
- There is an urgent need to educate those at high-risk of bTB transmission on issues including the handling of infected animals, and general hygiene in a One Health approach.

This Technical Bulletin has been prepared on the basis of technical information available from a completed BKGET-KGF Funded CGP Project, the details of which are given below:

Project Code and Title: TF-45-L/17. Epidemiological Investigation on Tuberculosis and Campylobacteriosis Associated with Dairy Farming Practices in Selected Districts of Bangladesh

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