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African Journal of Microbiology Research

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African Journal of Microbiology Research

Full Length Research Paper

Use of thermal imaging for the early detection of signs of disease in pigs challenged orally with *Salmonella* typhimurium and *Escherichia coli*

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A total of 27 piglets were randomly assigned to three treatment groups (control, infection with *Salmonella enterica* serover Typhimurium KCTC 2515 (ST) and infection with *Escherichia coli* KCTC 2571 (EC)) in a completely randomized design to early detection of signs of disease based on body temperature. Totally three inoculations were done consisting of 14 days each and then thermal images were captured to calculate body temperature of pigs at 0, 2, 6, 12, and 24 h and then every 24 h up to 14 days post inoculation. A reduced average daily gain (ADG) was observed in the first week of all three post inoculation (P < 0.05), while the gain:feed ratio was decreased at first week during first inoculation in both ST and EC group compared to control (P < 0.05). Body temperature was elevated in ST infected piglets at 24 h, peaked at 72 h (P < 0.05) and remained elevated, however, the EC induced piglets showed a subnormal body temperature throughout the experimental period relative to the control (P < 0.05). Taken together, the results indicate that signs of disease following experimentally induced bacterial infection in pigs can be detected quickly and easily using thermal images.

Key words: Piglets, growth performance, thermal image, body temperature, disease diagonosis.

INTRODUCTION

Infections with enteric pathogens such as *Salmonella* or *Escherichia coli* are common during intensive pig production and are associated with poor performance and animal welfare (Pijpers et al., 1991; Greiner et al., 2000). Such infections may also pose a risk to human health and lead to expensive veterinarian intervention costs. Delays in detection may result in the outbreak of diseases and even more expenses. Detection of animal illness and providing individual and group-by-group mass therapy is not effective. Since infection is primarily spread via horizontal transmission from carrier animals to others in close contact (Schwartz, 1991), the best way to handle an illness is early detection of diseases followed by application of treatment before the disease can spread.

Healthy animals are important to profit and performance, as well as animal welfare. Timely interventions may interrupt the lifetime carriage of *Salmonella* and *E. coli* and improve the health and growth of weaned pigs.

Swine have an unusual means of regulating core temperature. Due to the low number of sweat glands (Moritz and Henriques, 1947; Montagna and Yun, 1964), pigs reduce their core temperature by increasing the rate of peripheral blood flow through the skin, panting, or moistening the skin with water. However, panting and evaporation are less efficient regulation systems under some environmental conditions and are not always possible in confined pig housing. Fever is the earliest and one of the main clinical signs of many diseases (Plonait, 2004). Previous investigations showed that *S. typhimurium* challenged piglets showed increased body temperature (Balaji et al., 2000), while *E. coli* endotoxin inoculation resulted in subnormal body temperature (Halloy et al., 2004) compared to controls. Based on the way heat is transmitted through the skin of swine, infrared images have the potential for use in the detection of body temperature (Ingram and Weaver, 1969; Godynicki et al., 1985).

Traulsen et al. (2010) concluded that infrared thermography allows routine measurements of body surface temperature that can be used for early disease detection. Röhlinger et al. (1979) reported the possibility of using infrared cameras (IRCs) to measure body surface temperature for early disease detection in various animals, including swine. Scolari et al. (2011) used an IRC to detect the rise and fall of vulvar skin temperature of sows during estrus. Therefore, the present study was conducted to measure the growth performance and potential for early detection of sign of disease based on body temperature patterns measured using a thermal camera as an early disease diagnostic tool in *Salmonella enterica* serover Typhimurium and *E. coli* infected piglets.

MATERIALS AND METHODS

All experimental procedures used in this study were approved by the Animal Care and Use Committee of Sunchon National University, South Korea.

Animals and experimental design

A total of 27 newly weaned piglets (crossbred ((Landrace x Yorkshire) x Duroc), and mean body weight 8 kg and 28 days of age) were used for three consecutive inoculations. The length of each post inoculation period was 14 days and the experiment was conducted for a total of 42 days. At the onset of the study, each animal was tested bacteriologically (fecal) before receiving the *Salmonella* and *E. coli* challenge and were found to be negative for the bacteria. The piglets were assigned to one of the three treatment groups (control, infection with *Salmonella enterica* serover Typhimurium (ST) and infection with *Escherichia coli* (EC)) in each post inoculation period in a completely randomized design based on their initial body weight. Each treatment had three replicate pens with three pigs per pen. The pigs in each group were

reared in three isolated pens (slatted floor and a space allowance of 0.75 m^2 per piglet) in an environmentally controlled room and allowed a 1-week adaptation period before commencing the study. Pigs in the ST and EC group were then orally infected with 10 mL mixed suspensions (1:1) of *S. enterica* serover Typhimurium KCTC 2515 (5.5 x 10^6 cfu/ml) and *E. coli* KCTC 2571 (3.7 x 10^6 cfu/ml). An ambient temperature of 24 ± 1.49°C was maintained in the pig houses via overhanging electric heaters. During the experimental period, pigs were provided with a commercial diet *ad libitum*. The pens were illuminated by artificial light, and ventilation was provided by ten air changes per hour. The body weight and feed intake were recorded on a weekly basis until the end of the experiment.

Infrared thermography of piglets

Thermal images of piglets were captured using an infrared camera (Thermo Tracer, Type- TH5104R CAT I, NEC San-ei Instruments, Ltd., Tokyo, Japan) at a fixed distance of approximately 1 m from the animal. The emissivity value was set to 0.985 and the thermograph resolution was calibrated to ambient temperature and humidity as per the manufacturerer's recommendations. The piglets were numbered temporarily and allowed to settle before infrared thermographic (IT) images were captured. Multiple images were captured and the most informative image for an individual piglet was used. Thermal images were captured at 0, 2, 6, 12, and 24 h and then every day for 14 days post inoculation (dpi) without handling the piglets. Images were stored in a memory card, then transferred to a computer for analysis using the InfRec Analyzer Lite software system. The temperatures acquired from the IT images were recorded from three locations in each piglet (head, body and tail region), and the average values were used to calculate the standard body temperature of piglets.

Statistical analysis

All recorded data were statistically analysed using the SAS system *v.* 9.1 (SAS Institute. Inc., Cary, NC, USA, 2003). The individual pen was considered as the experimental unit for growth performance and body temperature calculation. Treatment means were computed with the LSMEANs and significant treatment x time interactions were observed for body temperature using thermal camera. Longitudinal significant variations in body temperature due to *Salmonella* and *E. coli* infection in different time periods are denoted by abcde, while treatment effects on specific time period denoted by xyz. Duncan's multiple range tests were used to identify significant differences among treatment groups for growth performances and body temperature. A probability level of P < 0.05 was considered statistically significant.

RESULTS

The average daily gain (ADG), average daily feed intake (ADFI) and gain:feed ratio of piglets are shown in Figures 1, 2 and 3. In the present study, we observed a decreased ADG at the first week of each post inoculation period in the ST and EC groups (P < 0.05) (Figure 1). A reduced ADFI was found in the second week of first inoculation and in the first week of second inoculation in the ST and EC infection groups relative to the control (P < 0.05) (Figure 2). A reduced gain:feed ratio was observed in the first week of first inoculation (P < 0.05) (Figure 3). Although the gain:feed ratio was decreased in

Control ⬚   *E. coli* ▨   *S. typhimurium* ▨
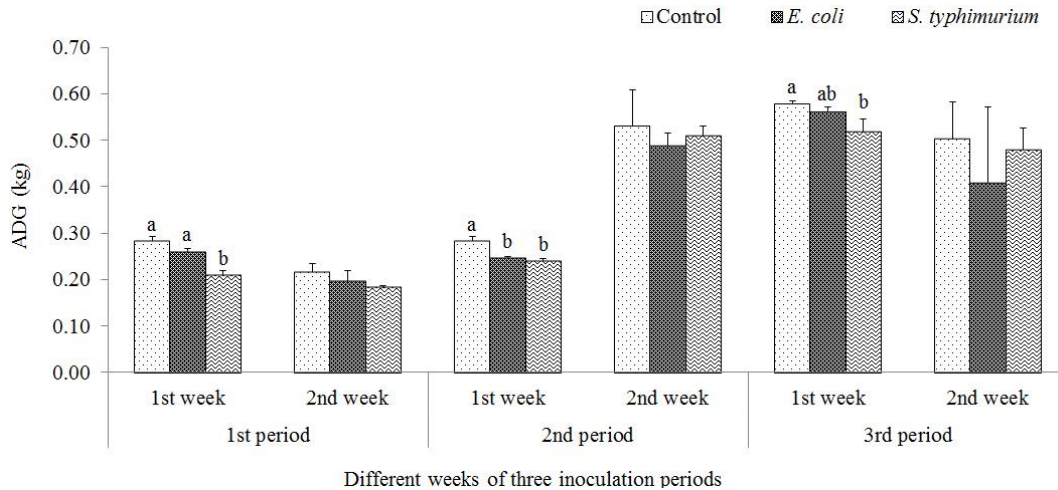
Figure 1. Mean average daily gain (ADG) (kg) of piglets in different weeks during the three inoculation periods**.** Data are presented as the mean ± SE. Bars at a week without a common letter differed significantly (P < 0.05).
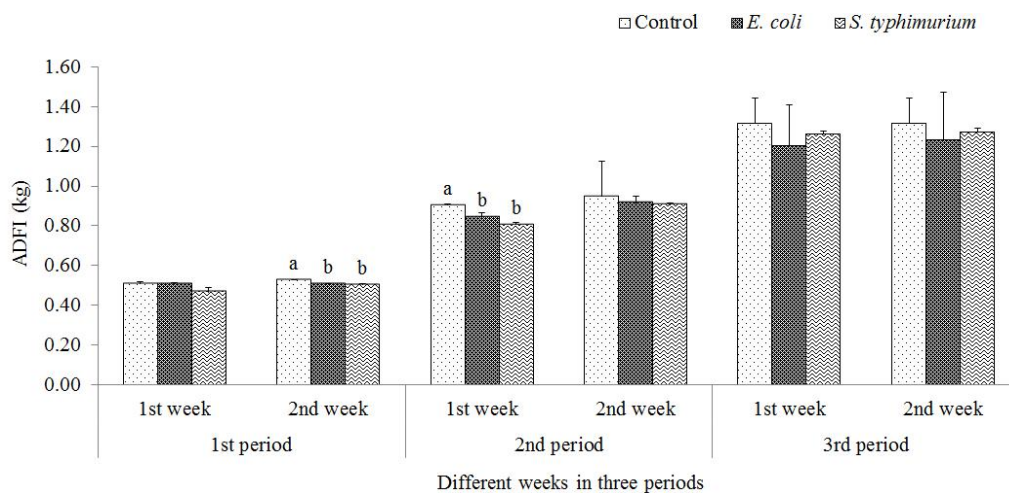
Figure 2. Mean average daily feed intake (ADFI) (kg) in different weeks during the three inoculation periods**.** Data are presented as the mean ± SE. Bars at a week without a common letter differed significantly (P < 0.05).

EC group, it was increased in ST group during second week of first inoculation and second inoculation period compared to control (P < 0.05) (Figure 3). Thermal images of piglets for control, infected with *Salmonella enterica* serover Typhimurium KCTC 2515 and *Escherichia coli* KCTC 257 are shown in Figure 4.

The body temperature patterns of piglets with bacterial infections are shown in Figure 5, 6 and 7. During the first inoculation, no significant variations were observed until 24 h post inoculation, while piglets challenged with ST showed a gradual increase in temperature by 24 h. The maximum temperature increase relative to the control was observed at 72 h (P < 0.05), and this value remained

elevated until the end of the study period (Figure 5). However, the EC induced piglets showed a subnormal body temperature pattern throughout the experimental period during all three inoculation periods relative to the control (P < 0.05) (Figures 5, 6 and 7). Average daily body temperature was significantly lower in EC group while it was alleviated in ST group compared to control (P < 0.05) (Figure 8).

DISCUSSION

In most animals, illness is commonly expressed by their
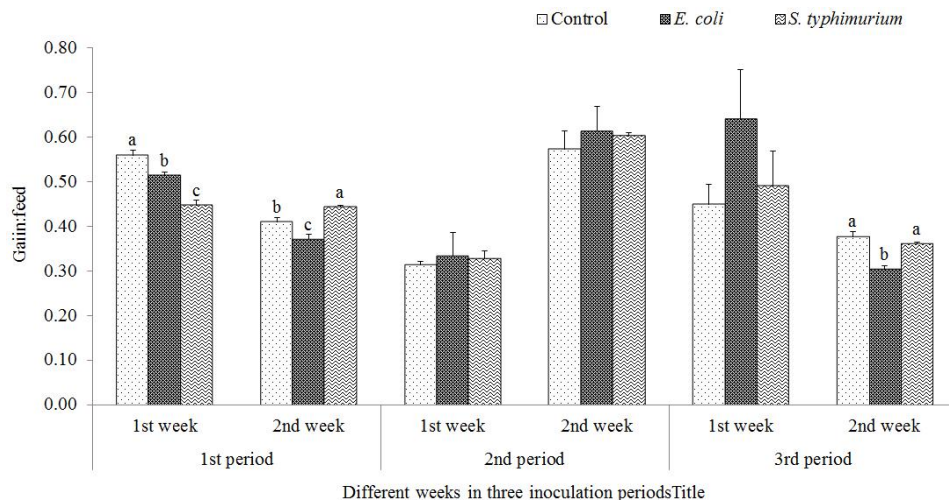
Figure 3. Mean average gain:feed ratio in different weeks during the three inoculation periods. Data are presented as the mean ± SE. Bars at a week without a common letter differed significantly (P < 0.05).

Control piglets   *E. coli* infected piglets   *Salmonella* infected piglets
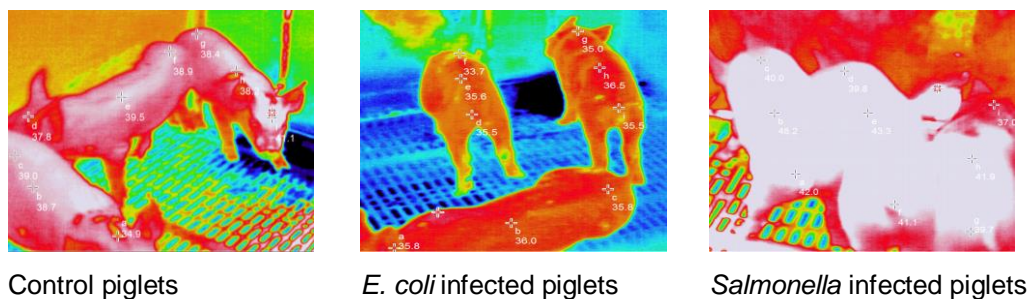
Figure 4. Thermal images of piglets for control, infected with *Salmonella enterica* serover Typhimurium KCTC 2515 and *Escherichia coli* KCTC 2571.
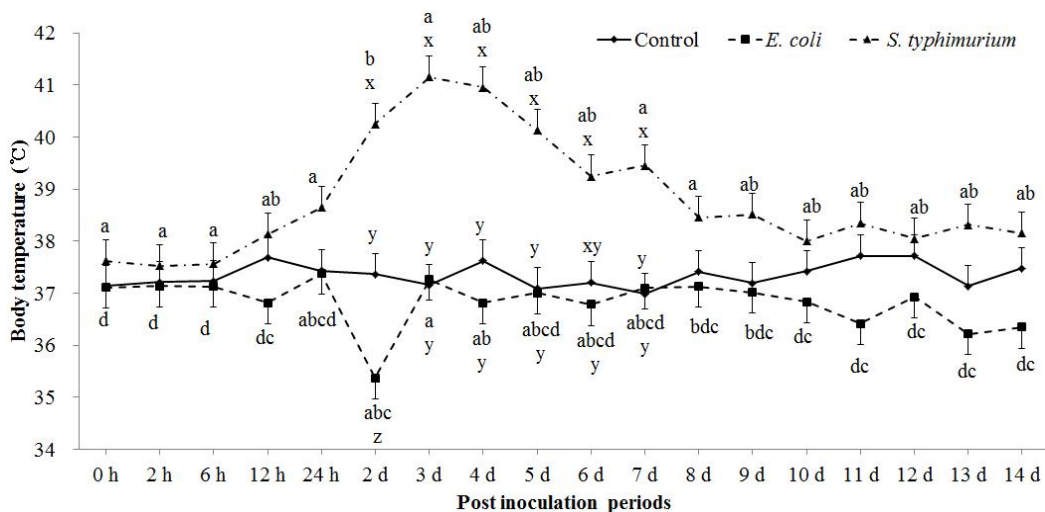
Figure 5. Body temperature pattern of piglets during the first inoculation period (14 days). Data are presented as the mean ± SE. Lines at a particular time period without a common letter differed significantly (P < 0.05).
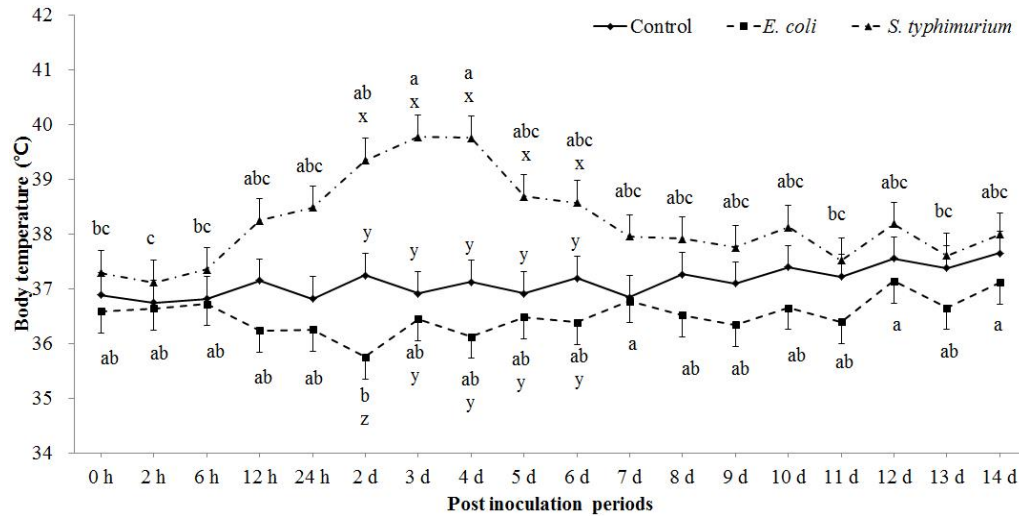
Figure 6. Body temperature pattern of piglets during the second inoculation period (14 days). Data are presented as the mean ± SE. Lines at a particular time period without a common letter differed significantly (P < 0.05).
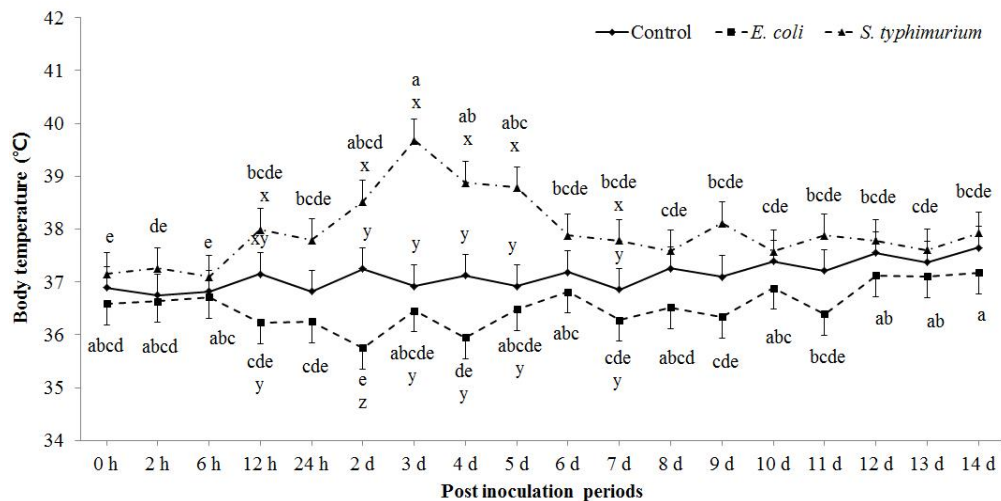
Figure 7. Body temperature pattern of piglets during the third inoculation period (14 days). Data are presented as the mean ± SE. Lines at a particular time period without a common letter differed significantly (P < 0.05).

behaviour (Weary et al., 2009). Farm animals express a wide range of behaviours such as loss of appetite, drinking and social behaviours, many of which may impact their health and welfare directly or indirectly. In the present study, we observed a decreased ADG at the first week of each post inoculation period in the ST and EC groups (Figure 1), which is consistent with the results reported by Van Heugten et al. (1994). Additionally, reduced body weight gain of pigs was observed over 14 days in response to bacterial infection (Balaji et al., 2000), and decreased weight gain, feed intake and efficiency of feed utilization were observed after repeated

challenge with non-infectious agents in broilers (Klasing et al., 1987). The reduced ADG of piglets during post inoculation period of piglets might be due to effect of *Salmonella* and *E. coli* infection (Van Heugten et al., 1994; Balaji et al., 2000). ADFI did not affect in the first week, it was decreased in the second week during first inoculation which also extended in the first week of second inoculation (Figure 2). Feed intake was depressed in ST induced pigs in the 120 h period following the challenge, with the maximum decrease being observed 48 h after challenge. In a previous study, intake was returned to control levels at between 120 and
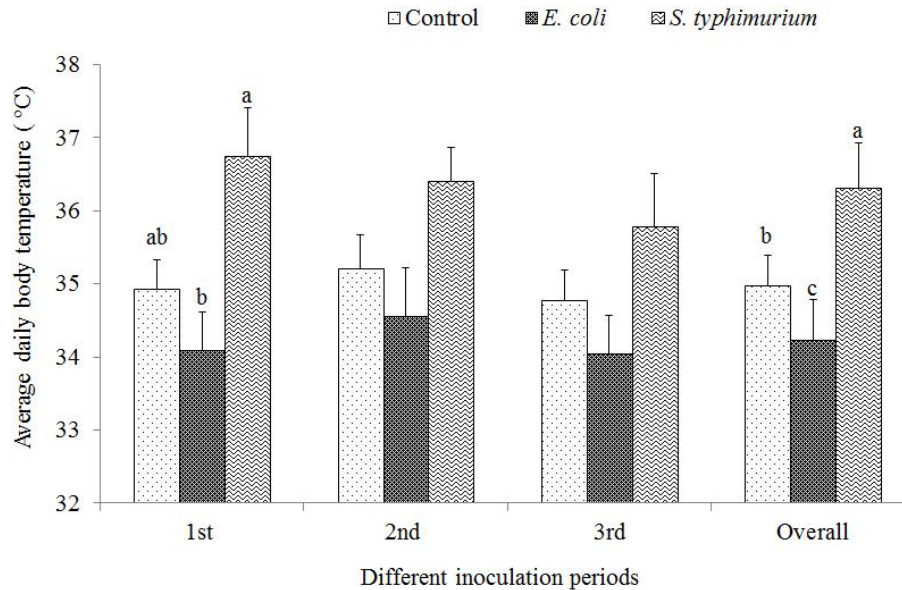
Figure 8. Average daily body temperature pattern of piglets during the three inoculation periods (42 days). Data are presented as the mean ± SE. Bars at a particular inoculation period without a common letter differed significantly (P < 0.05).

144 h of the challenge and was supported by Balaji et al. (2000). A number of infectious diseases of swine are characterized by fever, cachexia, inactivity and anorexia (Hart, 1988). The reduced gain:feed ratio might have been due to the effect of reduced weight gain in the respective periods in each post inoculation period. The overall decreased piglets growth performance of this study can be explained as the effect of bacterial infection which might be the indication of illness in pig (Klasing et al., 1987; Balaji et al., 2000).

Physiological responses of infection and inflammation in animals are the result of neuroendocrine and immune systems interactions. Adaptive responses include induction of proinflammatory mediators and fever, reduced feed intake and diminished growth performance, as well as development of a regulated, specific, immune response to ward off pathogens. *Salmonella* and *E. coli* are the most successful at colonizing the gastrointestinal tract under stress conditions, when nutrition and immunity are suboptimum, as is the case in newly weaned pigs (Aumaitre et al., 1995). A recent review described changes in peripheral blood flow resulting in alterations of skin temperature of livestock that could be detected by infrared thermography (Stewart et al., 2005). In the present study, piglets challenged with ST and EC showed remarkable temperature variations at different hours and days in all three post inoculation periods. The body temperature patterns of piglets with bacterial infections are shown in Figures 5, 6 and 7. During the first inoculation, no significant variations were observed until 24 h post inoculation, while piglets challenged with ST showed a gradual increase in temperature by 24 h. The

maximum temperature increase relative to the control was observed at 72 h, and this value remained elevated until the end of the study period (Figure 5). However, the EC induced piglets showed a subnormal body temperature pattern throughout the experimental period relative to the control (Figures 5, 6 and 7). The increased body temperature of ST challenged piglets observed in the present study is consistent with the results reported by Walsh et al. (2012), who found a tendency for increasing body temperature with increasing time of bacterial inoculation. The febrile conditions in our present study started at 12 h post inoculation, then gradually increased until becoming significantly higher on day 8. These increased levels then continued throughout the experimental period. Balaji et al. (2000) reported that the fever associated with ST was gradual in onset, and was sustained for 5 days following challenge. The infrared thermographies used to detect bacterial infection in pigs in the present study have also been applied in many other studies (Loughmiller et al., 2001; Schaefer et al., 2004; Johnson et al., 2011). Infrared techniques were used by Loughmiller et al. (2001) to measure the body temperature of pigs, and the results showed that it is possible to detect a febrile response using body-surface temperature. Pigs have few sweat glands and are therefore forced to cool down by increasing the rate of blood flow through their skin (Moritz and Henriques, 1947; Montagna and Yun, 1964); accordingly, infrared technology may be a good method for detection of increases in body temperature. Manno et al. (2006) reported that surface temperature increases with increasing ambient temperature. *E. coli* are commonly

found in human and animal intestinal tracts and can survive outside the gastrointestinal tract for considerable lengths of time in several environments (Ashbolt, 2004). In the present study, EC induced piglets showed a subnormal body temperature throughout the experimental period in all three post inoculation periods, which is normal and occurs due to starvation and dehydration due to diarrhoea. Feed intake of the EC infected group of this experiment was lower during most of the week in each inoculation period. The body temperature was also lower during the first week, indicating that starvation or lower feed intake can be a cause of subnormal body temperature. Additionally, some pigs infected with EC showed diarrheal symptoms, although we did not record the diarrheal score, which may also have led to subnormal body temperature. The detection of subnormal body temperature in piglets of this study is in agreement with the results of studies conducted by Vianna and Carrive (2005), who investigated changes in temperature at the rat tail in connection with a fear reaction and concluded that it was possible to detect decreased temperature via infrared images. The increased body temperature due to *salmonella* and subnormal body temperature due to *E. coli* infection of this present study could be helpful for the pig farmers to early detection of bacterial infection which is important to take measures for further severe damage. Average daily body temperature was significantly lower in EC group while it was alleviated in ST group compared to control (Figure 8). The overall body temperature in ST group was also alleviated while EC group was decreased compared to control which might be due to the effect of weekly body temperature in pigs. Overall, growth performances and body tem-perature pattern of challenged piglets could be more beneficial for the farmers in pig monitoring system.

Conclusions

Reduced ADG, ADFI and gain:feed ratio, as well as variations in body temperature are general indicators of bacterial infections in pigs. In the present study, thermal image analysis of heat emissions from pig skin made early detection of ST and EC infection in pigs possible. This method provides the opportunity to measure body temperature continuously without contracting or stressing the animals and with a minimal risk of injuries. Thus, it can be an effective tool to minimize costs and save time while ensuring animal welfare during pig farming.

Conflict of interests

The authors did not declare any conflict of interest.

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Full Length Research Paper

Prevalence of triple viral infections of human immunodeficiency virus (HIV), hepatitis B and C among tuberculosis patients and associated risk factors: The case of West Arsi Zone, Ethiopia

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Tuberculosis, human immunodeficiency virus and hepatitis B and C infections are serious public health problems in Ethiopia. This study was conducted to assess the prevalence of triple viral infections and associated factors among tuberculosis patients who were on anti-tuberculosis treatment in the year 2011/12 in West Arsi Zone, Ethiopia. A cross sectional study design was conducted among 374 TB patients who were on first line anti-TB treatment. Simple random sampling technique was employed to draw the study subjects until the calculated sample size was achieved. Blood samples were collected to determine the HIV serostatus by rapid HIV testing kits; whereas the hepatitis B and C sero-status was determined by instant one step HBsAg and Flavicheck-HCV$_{WB}$ kits, respectively. Socio-demographic and risk factors information was collected and analysed using univariate and multivariate logistic regression. Injection drug users were excluded from this study. From a total of 374 tuberculosis patients enrolled, TB-HIV co-infection was found to be 56 (14.97%) among which 5 (8.92%) hepatitis B and 4 (7.14%) were hepatitis C triple infected. The overall TB-HIV-Hepatitis triple infection prevalence was 2.4% (9/374). Though the current level of triple viral infections among TB patients seems to be low, an integrated program for treatment, prevention and control strategy is crucial to be considered in TB patient management.

Key words: Co-infection, HBV, HCV, HIV, TB, triple infection, West Arsi Zone, Ethiopia.

INTRODUCTION

Human immunodeficiency virus (HIV) continues to be a major global public health problem, having claimed more than 39 million lives so far. In 2013, there were 1.5 [1.4–1.7] million people died from HIV-related causes globally. In the same year there were approximately 35.0 [33.2–37.2] million people living with HIV with 2.1 [1.9–2.4]

million people becoming newly infected globally. Sub-Saharan Africa is the most affected region, with 24.7 [23.5-26.1] million people living with HIV that accounts for almost 70% of the global total of new HIV infections (WHO, 2014).

Ethiopia is one of the Sub-Saharan African countries affected by high burden of HIV/AIDS pandemic. Since the first report of HIV case in 1984 in the country, the disease remained as a major public health concern of the country (FMoH, 2010). There is an estimated prevalence of 2.4% with 1.9% and 2.9% in male and female respectively. There is also difference in prevalence among urban (7.7%) and rural (0.9%) communities (Ministry of Health of Ethiopia, 2010).

According to the World Health Organization, Ethiopia is one among 22 TB high burden countries   with an estimated 220,000 (258 per 100,000) incident and 200,000 (237 per 100,000) prevalence cases. There were an estimated 15,000 deaths (18 per 100,000) due to TB, excluding HIV related deaths in 2011 (World Health Organization, 2012).

HIV/AIDS pandemic has caused a resurgence of TB, resulting in increased morbidity and mortality worldwide. HIV and TB have a synergistic interaction; each accentuates progression of the other. Unfortunately, Sub-Saharan Africa region is part of the world where TB has been flourishing unhindered reaching the proportion of 82% TB cases co-infected with HIV in 2010. In the same year an estimated 1.1 million deaths occurred among HIV positive cases of TB including 0.32 million deaths among women. In addition, there were an estimated 0.35 million deaths among incident TB cases that were HIV positive (World Health Organization, 2011).

Though the prevalence of hepatitis co-infection with HIV varies widely across different studies mainly due to the variation in the distribution of risk factors, geographic location and other factors specific to the study population; it is one of the leading causes of morbidity and mortality in individuals with HIV (Iser and Sasadeusz, 2008). The co-infection of HIV with hepatitis (B and C) is attributable due to similar routes of transmission with evidence of significant clinical implications of complicated clinical course, case management and may also adversely affect therapy for HIV infection and vice versa (Padmapriyadarsini et al., 2006; D'Souza and Foster, 2004; Hennessey et al., 2009).

The coexistence of TB, HIV and viral hepatitis infections in the same patient poses a unique challenge to the patient and clinicians. Here, the major concern is most commonly available anti-TB and antiretroviral agents have associated risk of liver damage. In situation of concurrent treatment of these infections is inevitable, carefully chosen and tailored therapy is needed to minimize drug-drug interactions and related toxicities. When this condition is superimposed with HBV and/or HCV infection, the treatment and patient management challenge will be further complicated (Marzuki et al., 2008;

Yee et al., 2003).

Studies revealed that TB/HIV co-infected patients demonstrated a doubling level of liver enzymes among 14% of HBV and 12 % HCV infected patients. Moreover, 45% of patients with both HCV and HIV infection were developed drug-induced hepatitis during TB treatment (Wajiso, 2003; Byrd et al., 1979).

HIV-infected TB patients demonstrated a doubling in liver enzyme levels in 14% of patients with HBV and 12% of patients with HCV infection, although few had symptomatic hepatitis. About 5 of 11 (45%) patients with both HCV and HIV infection developed drug-induced hepatitis during TB treatment; the risk of hepatitis was over 3 times greater than in those with HIV infection alone and over 14 times greater than in those with neither HIV nor HCV infection (Wajiso, 2003; Byrd et al., 1979).

There is very limited or no information on the prevalence of HIV and Hepatitis (B and C) among TB patients thereby very little is known about the clinical burden of triple viral infection among TB patients and subsequent impact on TB treatment outcome in the country.

Shashemene, the capital of West Arsi Zone, is a crossroad town with a high prevalence of TB and HIV infection. According to the 2011(2004 E.C) Ethiopian Ministry of Health report the TB-HIV co-infection rate in the age group of 15-49 years was estimated to be 47% in the area. Therefore, this study was conducted to address the prevalence of triple viral infections and associated risk factors among TB patients in Shashemene, West Arsi zone, Ethiopia.

MATERIALS AND METHODS

The study area

West Arsi zone is one of the 14 zones of Oromia Regional State (the largest of 9 regional states of Ethiopia). The estimated population of the zone is 2,286,987. Shashemene is the capital of the zone with a population of 127, 798. Shashemene Referral Hospital is the only referral hospital in the zone. It is situated at 240 km away from capital of Ethiopia, Addis Ababa to the southern part of the country on the way to Moyale-Kenya. The hospital was established by missionaries as TB and Leprosy clinic in 1950. In 1976 it was upgraded to general public hospital and then in 2008, to referral hospital. The Health Centers used in this study were located within 12 to 35 km radius from the hospital with estimated catchment population of 750,000 (Figure 1).

Study design

A health facility based cross-sectional study was conducted to assess the prevalence of triple viral infection of HIV, HBV and HCV among TB patents at one referral hospital (Shashemene Referral Hospital) and three health centers (Shashemene, Aje and Arsi Negele) from September 2011 to June 2012.

Ethical considerations

The protocol was approved by Hawassa University Department of
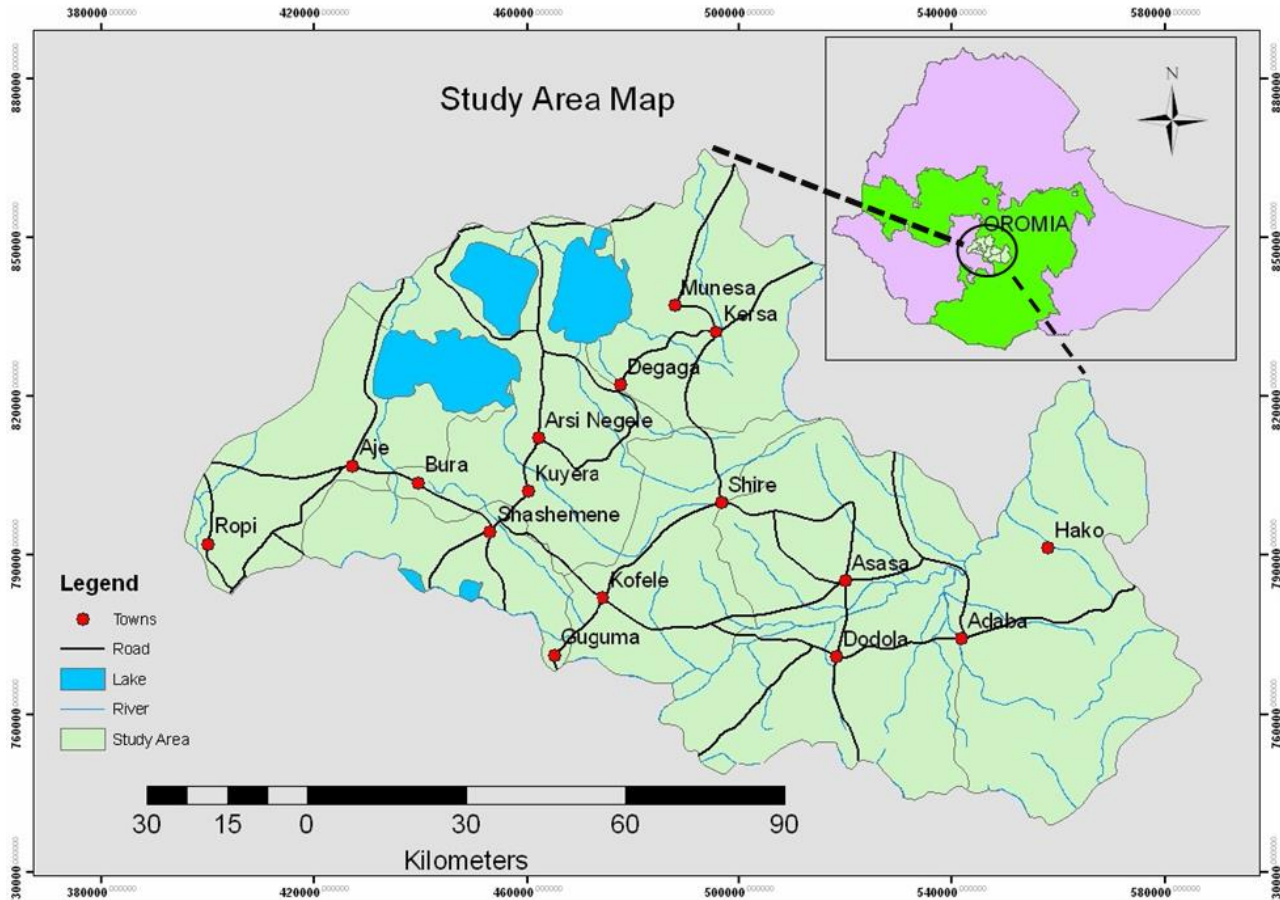
Figure 1. Map of the study area.

Biology and College of Health Sciences institutional Ethical Review Committee. Support letters were obtained from the Zonal and district health offices. Informed consent was obtained from all adults (> 18 years) and for children (<18 years) informed consent was obtained from their parents or guardians. A verbal assent was obtained from children of 12-17 years. For all the study subjects, laboratory diagnosis and treatment (if needed) were given free of charge and all results were kept confidentially.

Sample size determination

The sample size was determined using a single population formula with P=37.2% from previous study in the area:

$$N=\frac{(Z1_{-\alpha/2})^2 *P\,(1-P)}{d^2}$$

$$N=\frac{(1.96)^2*(0.372)*(0.628)}{(0.05)^2}$$

N = 359 with 5% non-response rate; N = 359+18=377.

We considered the confidence limits = 95% (α= 0.05), Power =80%, P= 37.2 % (Wajiso, 2003).

Source population and study population

The source population was all types of TB cases following anti -TB treatment in TB clinics of the study area. The study subjects were recruited by randomly selecting TB cases from Shashemene Referral Hospital and Shashemene, Arsi Negele and Aje health centers.

Sampling procedure

A simple random sampling technique was used to select the study subjects. All TB patients those put on first line anti-TB treatment in three health centres and Shashemene Referral Hospital were taken as sampling frame. An identification numbers were given for all registered patients and then the study subjects were selected randomly until the calculated sample size is achieved.

Inclusion and exclusion criteria

All types of TB patients who visited the health facilities and registered for anti-TB treatment were included in the study. However, pediatrics and referred out cases were not included in the study. Individuals who were not willing to give their consent for participation and injection drug users were also excluded.

Socio-demographic and patient data collection

Data on socio-demographic and risk factor were collected from each

consented participants in a well-designed and pre-tested questionnaires.

Nurses working at the respective health facilities were assigned to counsel the patients before blood sample collection for HIV testing through PIHCT (Provider Initiated HIV Counselling and Testing) and hepatitis testing.

Clinical sample collection and transportation

Vein blood samples of 2-5 ml was collected aseptically by the assigned laboratory technologist or technician. The collected blood specimens were transported appropriately to Shashemene Referral Hospital Laboratory for HIV and hepatitis testing within 2 hof collection.

HIV testing

HIV testing was done on whole blood sample by rapid testing kits KHB (Shanghai Kehua Bio-Engineering Co., China, 100% sensitivity and 98% specificity), Stat-pack (Chembio Diagnostics Systems, USA, 99.7% sensitivity and 99.9% specificity) and Uni-Gold (Trinity Biotech Plc, Ireland, 100% sensitivity and 100% specificity) following the national HIV testing algorithm.

Hepatitis testing

HBV was tested for HBsAg using Instant One step test kit (SD BIOLINE, Korea, >99% specificity and >99% sensitivity) and HCV was tested by using Flavicheck®-HCV WB (Qualpro diagnostics, India, 100% Sensitivity and 99.6% specificity) test kits using the manufacturer's guideline at Shashemene Referral Hospital Laboratory.

Quality assurance of data

To assure the quality of data to the level maximum, the questionnaires were validated by pre-testing on about 10% of sample size on some randomly selected patients who were not included in the study. Beside manufactures' instruction, there was strict adherence to good laboratory practice principles and standard operational procedures during laboratory analysis as.

Study variables

Independent

These included clinical signs and symptoms, risky behaviours for HIV and Hepatitis infection, Socio-economic and socio-demographic variables.

Dependent

HIV, HBV and HCV serostatus, TB-HIV co-infection and TB-HIV-Hepatitis triple infection were considered as outcome variables.

Statistical analysis

All the collected laboratory and socio-demographic data were properly coded and labelled for easy of entry. All data were double entered into an Excel spread sheet, cleaned, verified using STATA version 9.0 and made ready for analysis.

Descriptive analysis

Means, standard deviations, frequencies, standard error of the

means, proportions, percentages as well as descriptive graphs and tables were used with respect to the given variables.

Univariate analysis

Explanatory variables were individually cross tabulated with the outcome variable and statistical significance was assessed using chi-square, Odd ratio (OR) and 95% confidences interval (CI) were calculated to determine the strength of the association.

Multivariate analysis

Explanatory variables significantly associated ($p \leq 0.05$) with the outcome variable in Univariate analysis were included in a multivariate logistic regressions analysis using the program packages SPSS (SPSS Inc., Chicago, Illinois, USA, version 15.0 and STATA version 9.0) to detect confounding effects and to evaluate the relative influence of the different co-variates outcome variables.

RESULTS AND DISCUSSION

Socio-demographic characteristics of study subjects

A total of 374 TB patients were included in the study. Of the total TB patients, 224 (59.9%) were male while 150 (40.1%) of them were female with male to female ratio of 1.5:1. Among the total study participants, 203 (54.3%) of them were urban residents while 171(45.7%) were rural residents. The mean (±SE) age of the respondents was 30.12 ±0.645 years with a median age of 27 years. The average family size of the study subjects was 5.29+0.157SE with median of 5 (range 1-19) and the mean number of housewife per husband was 1.69+0.037SE with median of 2 and range of 1-5.

Concerning the educational status of the study subjects, 143 (38.2%) were illiterate (unable to write and read) and 231 (61.2%) were literate. About 192 (51.3%) of study subjects were married, 148 (39.6%) were single, 12 (3.2%) were divorced, 15 (4.0%) were widowed and 7 (1.9%) were separated from their partner (due to work, education etc.). Occupationally majority of study subjects; 163 (43.6%) were farmers followed by students, 79 (21.1%) and only 3(0.8%) were commercial sex workers (Table 1).

Socio-demographic variables versus seropositivity of study participants

The overall prevalence of TB-HIV co-infection was 56 (15%). Among 224 male and 150 female TB patients tested for HIV, 32 (14.3%) male and 24 (16.0%) female were seropositive respectively.

The prevalence of Hepatitis (HBV and HCV) in TB-HIV co-infected patients was found to be 9 (16.07%). Of the 9 hepatitis positive TB-HIV co-infected patients 5(8.9%) were with HBV and 4 (7.1%) with HCV co-infection. The

Table 1. Socio-demographic characteristics of the study subjects for the study of triple viral infections of human immunodeficiency virus (HIV), Hepatitis B and C (HBV and HCV) among tuberculosis patients and associated risk factors in West Arsi zone, Ethiopia in 2011/12.

Variable	Category	N=374	%
Sex	Female	150	40.1
	Male	224	59.9
	Total	374	100.0
Age	15-24	147	39.3
	25-34	117	31.3
	35-44	69	18.4
	45-54	15	4.0
	55-64	16	4.3
	>64	10	2.7
	Total	374	100.0
Residence	Urban	203	54.3
	Rural	171	45.7
	Total	374	100.0
Education	Illiterate	143	38.2
	1st cycle	120	32.1
	2nd cycle	94	25.1
	12 grade complete	12	3.2
	College and above	5	1.3
	Total	374	100.0
Occupation	Farmer	164	43.9
	Student	79	21.1
	Daily laborers	28	7.5
	Merchants	40	10.7
	Commercial sex workers	3	0.8
	Government employee	13	3.5
	House wife	26	7.0
	Others	21	5.6
	Total	374	100.0
Marital status	Single	148	39.6
	Married	192	51.3
	Divorced	12	3.2
	Partner died	15	4.0
	Partner separated	7	1.9
	Total	374	100.0

mean (±SE) age of HIV positive TB patients was 30.41(±1.14) and the mean age of Hepatitis positive TB/HIV co-infected patients was 30.56(±0.86SE). There is no statistically significant difference between the age groups of study participants (P>0.05) in HIV seropositivity.

Among the total 56 HIV positive TB patients, 32(57.1%) were male while 24 (42.9%) were female and from 9 hepatitis positive TB-HIV co-infected patients, 4 (44.44%) of them were male while 5 (55.56%) were female with the overall TB-HIV-Hepatitis triple infection prevalence of 9(2.4%) observed in the current study area. About 45 (80.4%) of HIV positive TB patients were urban residents while 11 (19.6%) of them were rural residents. The statistical analysis showed that statistical significant difference between the urban and rural residents (P<0.05) with regard to HIV seropositivity among TB patients. Similarly, majority 6(66.7%) of triple infected patients were reside in urban areas while 3 (33.3%) of them were reside in rural area. The HIV seropositivity of respondents were statistically significantly different (P<0.05) with respect to their educational status, occupation and marital status (Table 2).

Characteristics and clinical features of study subjects

TB patients were categorized as new, relapse, and defaulter cases. Of the total 374 TB patients enrolled in the study 347 (92.8%) of them were new cases, 17 (4.5%) were relapse and 10 (2.7%) were defaulters. The prevalence of HIV seropositivity in relapse TB cases was 5 (29.4%) and 49 (14.5%) in new TB cases (Table 3). The prevalence of triple infection (TB-HIV and Hepatitis) was found to be 8 (16.3%) in new TB cases and 1 (5.9%) in relapse TB cases. No triple infection was detected in defaulters. From the total of 300 TB patients tested for AFB microscopy, 138 (46.0%) of them were positive for the presence of AFB in the sputum samples. Among the 200 (53.5%) TB patients tested for X-ray, lung infiltration reported in 102 (51.1%), whereas, cavities were reported in 51(25.5%) and 49 (24.5%) were with no findings or not reported. The prevalence of HIV seropositivity was 23 (16.7%) and 25 (15.4%) for AFB positive and negative study participants respectively (Table 3). Among the AFB positive and AFB negative TB-HIV patients, 3 (13.04%) and 4 (16.0%) of them were positive for hepatitis test respectively.

Seropositivity versus sampling area

The TB-HIV co-infection prevalence for study subjects from Shashemene Referral Hospital, Shashemene Health Center, Arsi Negele Health Center and Aje Health Center was 26 (13.9%), 14 (21.9%), 11 (8.92%) and 5 (8.9%) respectively. The HIV seropositivity was slightly higher in Shashemene Health center 14 (21.9%) compared to the other centers. The prevalence of triple infection were 4 (15.4%), 3 (21.4%) and 2 (18.2%) Shashemene Referral Hospital, Shashemene Health Center, Arsi Negele Health Center and Aje Health Center respectively. Triple

Table 2. Socio-demographic characteristics of study subjects versus HIV and Hepatitis (HBV and HCV) Serostatus among tuberculosis patients and associated risk factors in West Arsi Zone, Ethiopia in 2011/12.

Socio-demographic characteristics of TB patients of study area		HIV Positive, n= 56				HIV Negative n=318	P-value
		Hepatitis					
		Positive		Negative			
		HBV	HCV	HBV	HCV		
Sex	Female	2	3	22	21	126	0.649
	Male	3	1	29	31	192	
	Total	5	4	51	52	318	
Age	15-24	0	0	11	11	135	
	25-34	5	2	22	25	91	
	35-44	0	2	17	15	62	0.709
	45-54	0	0	0	0	17	
	55-64	0	0	1	1	8	
	>64	0	0	0	0	5	
	Total	5	4	51	52		
Residence	Urban	3	3	42	42	158	
	Rural	2	1	9	10	160	0.000
Education	Illiterate	1	0	10	11	132	
	1st cycle	2	2	21	21	98	
	2nd cycle	3	2	18	18	73	0.026
	Grade 12 completed	0	0	1	1	11	
	College and above	0	0	1	1	4	
Occupation	Farmer	2	2	11	11	150	
	Student	0	0	2	2	77	
	Daily laborers	0	0	12	12	16	
	Merchants	2	2	14	14	24	
	Commercial sex workers	0	0	1	1	2	0.000
	Government employee	0	0	4	4	9	
	House wife	0	0	3	3	24	
	Other	1	0	4	5	16	
Marital status	Single	1	1	14	15	133	
	Married	1	2	19	20	172	
	Divorced	2	0	7	9	3	0.000
	Partner died	0	0	5	5	10	
	Partner separated	1	1	6	7	0	

infection was not reported from Aje Health center (Table 4).

Risk factor analysis for HIV and hepatitis seropositivity

On Univariate analysis, behavioural factors such as having multiple partner and having unprotected sex with commercial and non-commercial sex workers were found to be risk for HIV seropositivity. From host factors tested in the analysis, marital status (partners separated, divorced and widowed), being female, residing in urban, having poor HIV knowledge and being illiterate associated with high risk of acquiring HIV. On multivariate analysis, being urban dweller, marital status (partners separated), and being illiterate were associated with increased seropositivity (Table 5).

DISCUSSION

The observed prevalence of TB-HIV co-infection (15%)

Table 3. HIV and Hepatitis Serostatus, Tuberculosis cases category, form of tuberculosis and TB laboratory diagnosis status among tuberculosis patients and associated risk factors in West Arsi Zone, Ethiopia in 2011/12.

Category	Diagnosis	HIV sero-status		
		Positive n (%)	Negative n (%)	Total
HIV serostatus	HIV rapid test	56 (15%)	318 (85%)	374
Hepatitis	HBsAg	5 (8.9%)	51 (91.1%)	56
	HCV antibody	4 (7.1%)	52 (92.9%)	56
TB category	New	49 (14.1)	298 (85.9)	347
	Relapse	5 (29.4)	12 (69.6)	17
	Defaulter	2 (20)	8 (80)	10
Form of TB	PTB	48 (16.2)	248 (83.8)	296
	EPTB	8 (10.5)	68 (89.5)	76
	Disseminated TB	0	2 (100)	2
AFB Result	Positive	23 (16.7)	115 (83.3)	138
	Negative	25 (15.4)	136 (83.9)	162
	Not performed	8 (10.96)	65 (89)	73
X-ray Result	Infiltrations	15 (14.7)	87 (85.3)	102
	Cavities	9 (17.7)	42 (82.3)	51
	No finding	7 (14.3)	42 (85.7)	49
	Not performed	25 (14.5)	147 (85.5)	172

Table 4. Distribution of Hepatitis B, C (HBV and HCV) and HIV seropositivity among tuberculosis patients and associated risk factors in West Arsi Zone, Ethiopia in 2011/12.

Parameter	HIV serostatus		Hepatitis sero-status			
	Positive N (%)	Negative N (%)	Positive N (%)		Negative N (%)	
			HBV	HCV	HBV	HCV
Shashemene Referral Hospital	26 (13.9)	161 (86.1)	2 (7.7)	2 (7.7)	24 (92.3)	24 (92.3)
Shashemene Health center	14 (21.9)	50 (78.1)	2 (14.3)	1 (7.1)	12 (85.7)	13 (92.9)
Arsi Negele Health Center	11 (18.9)	56 (81.1)	1 (9.1)	1 (9.1)	10 (89.9)	10 (89.9)
Aje Health center	5 (8.9)	51 (91.1)	0 (0)	0 (0)	5 (100)	5 (100)
Total	56 (15.0)	318 (85.0)	5 (8.9)	4 (7.1)	51 (91.1)	52 (92.9)

Table 5. Univariate and multivariate logistic regression analyses of risk factors for HIV infections among tuberculosis patients in West Arsi Zone, Ethiopia in 2011/12.

Factor	Univariate analysis			Multivariate analysis		
	Crude OR	CI	P-value	Adjusted OR/ AOR	CI	P-value
Sex						
Male	1			1		
Female	1.14	0.643-2.031	0.065	1.054	0.371-3.003	0.085
Residence						
Rural	1			1		
Urban	4.143	2.068-8.30	0.000*	2.439	1.282-7.246	0.049*
Educational status						
Illiterate	1			1		
Literate	0.344	0.272-0.691	0.000*	0.141	0.096-0.682	0.000*

Table 5. Contd.

Factor	Univariate analysis			Multivariate analysis		
	Crude OR	CI	P-value	Adjusted OR/ AOR	CI	P-value
Marital status						
Married	1			1		
Single	1.631	0.549-4.811	0.06	1.003	0.537-3.897	0.889
Partners separated	10.466	3.568-31.312	0.001*	2.638	1.077-6.466	0.034*
Divorced	4.121	1.532-9.847	0.005*	1.021	0.899-4.0123	0.654
Widowed	2.193	1.685-11.349	0.044*	1.001	0.0123-2.014	0.998
HIV knowledge						
Yes	1			1		
No	2.54	1.581-10.883	0.042*	3.288	0.637-16.982	0.155
Unprotected sex with commercial sex worker (high risky groups)						
No	1			1		
Yes	1.268	0.673-2.392	0. 562	2.410	0.657-8.843	0.185
Unprotected sex with noncommercial partner						
No	1			1		
Yes	2.255	1.779-6.528	0.035*	1.217	0.331-1.999	0.653

reported in this study is lower than those studies conducted previously in the study area (37.2%; 25.7%; 20%). This might be attributed to behavioral changes brought by health education on HIV/AIDS prevention and control program. The program has been implemented by the Federal Ministry of Health at national level (Sharma et al., 2005; Wajiso, 2003; Arega, 2007).

The triple infection of TB-HIV-Hepatitis prevalence had little attention or was no longer been addressed in Ethiopia. The TB-HIV-Hepatitis triple infection, HBV (8.9%) and HCV (7.1%), in the present study pinpoints the need to consider triple infection for future prevention and control strategies in the study area in particular. The prevalence of HBV among TB-HIV co-infected patients obtained in this study is comparable with other findings in other countries that reported the prevalence of HBV among TB-HIV co-infected patients: in Thailand was 9% while the prevalence of HCV was 31%. According to these authors the main route of HCV transmission is injection and drug use while sexual intercourse is the main route for HBV transmission. The low HCV prevalence in this study may be accounted for the low prevalence of the main route of transmission. However this needs further investigation. The prevalence of triple infection of TB-HIV-Hepatitis was higher in this study (TB patients) compared to the studies conducted in other populations (blood donors, heath care providers etc., data not indicated) in the same area. This higher prevalence may be due to the fact that HIV and Hepatitis shares common routes of transmissions (Yee et al., 2003).

In this study, there was a significant difference in HIV seropositivity between urban and rural residents (p=0.000; adjusted OR=2.439). There was also a difference in triple infected between urban (10.7%) and rural (5.4%) dwelling study participants. This might be due to the differences of the available risk factors in both areas. In addition, the current study shows that, a statistically significant difference in HIV seropositivity between different educational levels (p=0.000). HIV seropositivity was lower among literate than illiterates (adjusted OR=0.141) and the same for triple infection. This is probably literates can easily understood the ways of transmission and prevention measures from different sources such as newspaper and periodicals. However, the presence of pre-disposing risk factors especially in urban settings where more literate people are found should not be underestimated. In such cases, target oriented education which induces behavioral changes should be tailored to minimize the high prevalence of HIV sero-prevalence in urban settings.

The result of this study showed that a significant difference of HIV seropositivity among different occupations and the same for triple infection. It was indicated that the leading seropositivity was seen in daily laborers, merchants, commercial sex workers and governmental employee with descending order. Similarly, a study conducted in India indicated that unemployed and business professionals took the larger percentage HIV/TB co-infection (Kumar et al., 2002), another study suggested that higher seropositivity in manual laborers and some

studies conducted in Ethiopia indicated that, these groups of patients were seropositive to HIV (Wajiso, 2003; Devi et al., 2005; UNAIDS, 2009; Kumar et al., 2002).

HIV seropositivity was highest among those partners who live separately for various reasons. The high HIV seropositivity in this group is probably due to the fact that partner separation eventually leads to risky behaviors. The other reason might be when partners know their HIV status, they prefer to live separate in order to make ART treatment more confidential.

The major risk factors for triple infection of HIV-TB-Hepatitis were found to be having multiple sexual partners, followed by unsafe sexual intercourse (data not presented). This is probably due to shared routes of transmission of HIV and hepatitis (B&C), and TB is the opportunistic disease which comes after immune suppression like in HIV patients (Sterling and Sulkowski, 2004; Sy and Jamal, 2006; Solomon et al., 2008; Sirinak et al., 2008).

Conclusions

This study reveal relatively high prevalence rate of triple infection even if TB-HIV co-infection is getting declined than previous reports. The HBV-HCV co-infection was not found. Some behavioral and socio-demographic risk factors such as partners being separated, having multiple sexual partners, doweling area (being urban) and occupation (unemployed) were found to be strongly associated with triple infection. In general the current study showed the need for more information on the triple infection in order to base therapeutic decisions and to set integrated prevention and control program in the country. This underline the need for further epidemiologic and clinical studies to optimize the management of this complicated medical conditions.

Authors' contributions

EM, EN, TA and MK formulated the study questions and designed the study protocol; EM conducted the field and all laboratory activities. EN, TA and MK supervised the field and laboratory works; EN and TA drafted the manuscript; EM, EN, TA and MK contributed to critical review of the manuscript. All authors equally contributed to the interpretation of the data and writing of the manuscript and read and approved the final version.

Conflict of interests

The authors did not declare any conflict of interest.

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