# Isolation of Bacterial causes of Respiratory Infections in Calves in Smallholder farms in and around Gonder Town, Ethiopia

**Tefera Manaye**
Sede Woreda Veterinary University College of Fishery Science
**Pawlos Wasihun Asnake** ( pwasihun@gmail.com )
Haramaya University https://orcid.org/0000-0002-3124-9082
**Ashebr Abraha**
Haramaya University
**Tsegaw Fentie**
university of Gondar

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# Abstract

**Background:** Bovine respiratory disease (BRD) is considered as the major cause of severe respiratory tract infections in calves. Pasteurellosis is a multifactorial respiratory disease, which mainly affect calves within four weeks of weaning. A cross-sectional study was conducted from October 2017 to April 2018 in and around Gondar town, Amhara Regional State, North West of Ethiopia. The aim of the study was to isolate *Mannheimia* and *Pasteurella* species from calves up to six months old, and to assess the associated risk factors with the occurrence of respiratory disease. Sex, age (< 16 weeks and > 16 weeks), body condition status (poor, medium, good), breed (local and cross breed), livelihood (mixed crop and urban), farming systems (semi intensive and intensive), herd size (small medium, and large), maternity pens (present or absent), and method of colostrum feedings (hand bucket and suckling) were the examined risk factors.

**Results:** A total of 84 nasopharyngeal swab samples were collected from calves with any signs of illness related to pasteurellosis. The overall isolation rate of the respiratory pathogens was 64/84 (76.2%) (95% CI=65.7-84.8), with 46.4% of *Mannheimia haemolytica* and 28.8% *Pasteurella multocida* isolates. The distribution of pathogens was statistically higher (P< 0.001) in calves with respiratory problems (93.6%; 95% CI= 82.5-98.7) compared to those with no symptoms of respiratory illness (54.1%; 95% CI= 36.9-70.5). Among the examined risk factors age, sex, breed, farming system were found to be potential risk factors and significantly associated with *Pasteurella* infection of calves (p<0.05). The higher isolation rate of *Mannheimia haemolytica* indicated that it is the major cause of respiratory disease in the study area.

**Conclusion:** The present finding revealed that pasteurellosis is one of the major diseases of calves in the study area in which *M. haemolytica* and *P. multocida* were found to be commonly involved in respiratory infections. Improved farm management including timely feeding of colostrum, appropriate hygiene of the calf house and training of farmers is recommended to prevent and control of respiratory diseases in the study area.


# Background

Bovine respiratory disease (BRD) is considered as the major cause of severe respiratory tract infections in calves [1]. It causes great economic losses such as reduced average daily gain, feed efficiency, and overall performance of beef calves and finally calves morbidity and mortality [2, 3]. Several infectious agents are commonly isolated from the respiratory tracts of clinically sick and healthy animals [4]. Many factors can weaken the host's immune system and/or damage the lining of the respiratory tract to such an extent that these pathogens are able to progress deeper into the respiratory tract and cause disease [5–6].

Calf pneumonia refers to infectious respiratory disease in calves. It is primarily a problem in calves less than 6 months old with peak occurrence from 2–10 weeks, but it may be seen in calves up to 1 year of

age [7]. Pasteurellosis is a multifactorial respiratory disease [6, 8], which mainly affect calves within four weeks of weaning. Infected calves manifest the clinical signs when they are sorted and sold to different farms. The bacteria that causes pasteurellosis are part of the normal microbiota in the upper respiratory tract [9], making the disease difficult to prevent. *mannheimia haemolytica* and *pasteurella multocida* are most commonly associated with pneumonia in cattle calves [10, 11]. These pathogens can easily spread between animals, especially when calves are crowded (as in shipment) or closely confined (as in a dairy calf nursery) [12].

In practice, deep nasopharyngeal swabs (DNS) [13], transtracheal aspiration (TTA) [14], and broncho alveolar lavage (BAL) [15] have been used for sampling the respiratory tract. Deep nasopharyngeal swab is the easiest, fastest, and cheapest technique and therefore most suitable for sampling large numbers of animals [16].

In Ethiopia, calf pneumonia has been reported as the second most disease syndrome associated with calf morbidity and mortality next to calf diarrhea [17, 18]. Even though bovine pneumonic pasteurellosis is one of the most economically important infectious diseases in Ethiopia [19], there is limited data on the status of the disease and potential risk factors. This is particularly important in the study area, because dairy farms are an integral part of the economy. Hence, the study was conducted to identify the main bacterial respiratory pathogens and to assess major risk factors in bovine calves managed under dairy farms of Gondar town.

## Methods

# Study area

Sample collection was held in and around Gondar town, which is the capital of central Gondar administration zone, Amhara regional state. The area is located in the north west of Ethiopia at 740 km from Addis Ababa, capital city of the country. It is situated between $12^{o}36'$N and $33^{o}$ 28'E at an altitude of about 2300 m above mean sea level with an average temperature of $20^{o}$c and an average annual rain fall of 1800 mm.

# Study Population

The study population constitutes bovine calves up to 6 months of age that shows any respiratory sign or having general illness (suspected to have respiratory pathogens) found in dairy farms located at urban as well as peri-urban, which is characterized by mixed crop livestock production system. Study animals are selected regardless of breed, sex, body condition, herd size, and weaning status.

# Study Design and Animal Selection

A cross-sectional study was employed to isolate bacterial pathogens causing bovine respiratory disease of calves and assess its major risk factors from November 2017 to April 2018. Purposive type of

sampling was applied based on the availability of calves and willingness of farmers to take the nasopharyngeal swabs for bacteriological culture of *Pasteurella* species. Thus, a total of 84 calves were included in the study.

# Sampling Procedures

Animals were restrained by an assistant and then the external part of the nose was disinfected with 70% alcohol. After the alcohol has evaporated, a sterile cotton-tipped swab was inserted in to the nostril and rotated against the wall of the nasal cavity as described by [20]. The swab was then placed in a labeled sterile test tube containing 3 ml of tryptose soya broth. Samples were then kept in a box containing ice for transport to University of Gondar veterinary microbiology laboratory.

# Isolation and identification of Pasteurella species

The isolation and identification was performed as described by [21]. Briefly, the specimen in tryptose soya broth was incubated for 24 hours at 37°c and a loop full of the broth culture was streak on petri-dish of blood agar base supplemented with 5% sheep blood then incubated aerobically at 37 °C for 24 hours. Then colonies showing typical gram's reaction and cellular morphology were further sub-culture on both blood and MacConkey agar plates. The general appearance of colonies, presence and nature of hemolysis, and the ability to ferment lactose was recorded. The suspected pure colonies from both blood and MacConkey agars were transferred onto nutrient agar slants for further identification by biochemical tests. *M. haemolytica* was characterized as able to produce a narrow zone of hemolysis on blood agar, able to grow on MacConkey agar, but unable to produce indole, whereas *P. multocida* was characterized as unable to produce hemolysis on blood agar, unable to grow on MacConkey agar, and able to produce indole as described by [22].

# Data management and Statistical Analysis

Data was stored in Microsoft excel sheet for handling. After checking for its correctness, it was transferred to SPSS version 20.0 for analysis. Descriptive analysis, such as frequency and percentage were used to describe the proportion of calves affected by bacteria. Chi-square and regression analysis were employed to establish the association between cultured bacteria result and risk factors. Before regression analysis, the data was cheeked for fulfillments of assumptions, such as correlation of each variables (not more than 0.7), correlation of independent variables with dependent variable (minimum of 0.3), and multi-collinearity tests (VIF (> 10) and Tolerance (< 0.1)). Thus, Age, sex, and breed were used in the final model. The OR was computed at 95% CI and in all cases the difference between parameters were tested for significance at probability level of less than 0.05 ($P \leq 0.05$).

# Results

# Overall Prevalence of Isolates

Physical examination indicated that 47 (55.9%) and 37 (44.1%) animals were with signs of respiratory illness and with no symptoms of respiratory illness, respectively. Out of 84 calves examined, 64 (76.2%)

were found to harbor *Pasteurella* species and the prevalence was significantly higher ($p < 0.001$) in animals with symptoms of respiratory illness (Table 1). Meanwhile, the proportion of isolated *Mannheimia haemolytica* (46.4%) was higher than *Pasteurella multocida* (28.8%) (Table 2).

Table 1
Summary of total isolate with corresponding health status

| Health status | No. of positive samples (%) | 95% CI | χ2 value (p-value) |
|---|---|---|---|
| No-respiratory problems (n = 37) | 20 (54.1) | 36.9–70.5 | 17.86 (0.000) |
| With respiratory problems (n = 47) | 44 (93.6) | 82.5–98.7 | |
| Total (n = 84) | 64 (76.2) | 65.7–84.8 | |

Table 2
Distribution of *Pasteurella* species isolated from nasopharyngeal swabs of calves (n = 84)

| Species of *Pasteurella* | Number of positive samples | % of isolates |
|---|---|---|
| *M. haemolytica* | 39 | 46.4% |
| *P. multocida* | 257 | 28.8% |
| Over all | 64 | 76.2% |

The prevalence of pasteurellosis in relation to risk factors revealed a statistically significant association with age ($p = 0.05$), breed ($p = 0.05$), and livelihood ($p = 0.03$). The occurrence was higher in male (84.1%) than female calves (67.5%); in young (82.5%) than older calves (63%); in cross breed (82.5%) compared to local bred (63%); and urban (82.8%) than peri-urban (mixed crop productions) areas (61.5%) (Table 3). Based on logistic regression analysis, the odds of being affected by pasteurellosis was significantly higher in calves aged < 16 weeks (OR = 3.1; 95% CI = 1.0-9.6; P = 0.04) than the older category and cross-bred than local one (OR = 3.6; 95% CI = 1.1–11.5; P = 0.03) (Table 4).

Table 3
Pasteurellosis in calves and associated risk factors

| Variables | No. of Animals examined | No of Animals with Pathogens (%) | CI (95%) | χ2 analysis | |
|---|---|---|---|---|---|
| | | | | χ2 value | p-value |
| Sex | | | | | |
| Female | 40 | 27 (67.5) | 50.9–81.4 | 3.2 | 0.075 |
| Male | 44 | 37 (84.1) | 69.9–93.4 | | |
| Age | | | | | |
| < 16 weeks | 57 | 47 (82.5) | 70.1–91.3 | 3.8 | 0.05 |
| ≥ 16 weeks | 27 | 17 (63.0) | 42.4–80.6 | | |
| BCS | | | | | |
| Poor | 13 | 10 (76.9) | 46.2–94.9 | 0.005 | 0.99 |
| Medium | 50 | 38 (76.0) | 61.8–86.9 | | |
| Good | 21 | 16 (76.2) | 52.8–91.8 | | |
| Breed | | | | | |
| Local | 27 | 17 (63.0) | 42.7–80.6 | 3.8 | 0.05 |
| Cross-breed | 57 | 47 (82.5) | 70.1–91.3 | | |
| Livelihood | | | | | |
| Mixed crop | 26 | 16 (61.5) | 40.6–79.8 | 4.46 | 0.035 |
| Urban | 58 | 48 (82.8) | 70.6–91. 4 | | |
| Farming system | | | | | |
| Semi-intensive | 34 | 24 (70.6) | 52.5–84.9 | 0.98 | 0.32 |
| Intensive | 50 | 40 (80.0) | 66.3–89.9 | | |

| Variables | No. of Animals examined | No of Animals with Pathogens (%) | CI (95%) | χ2 analysis | |
|---|---|---|---|---|---|
| | | | | χ2 value | p-value |
| Herd size | | | | | |
| Small | 12 | 9 (75.0) | 42.8–94.5 | 0.46 | 0.79 |
| Medium | 59 | 46 (78.0) | 65.3–87.7 | | |
| Large | 13 | 9 (69.2) | 38.6–90.9 | | |
| Maternity pen | | | | | |
| Present | 27 | 18 (66.7) | 46.0–83.5 | 1.9 | 0.16 |
| Absent | 57 | 46 (80.7) | 69.6–91.1 | | |
| MCF | | | | | |
| Suckling | 66 | 50 (75.8) | 63.6–85.5 | 0.03 | 0.86 |
| Hand/bucket | 18 | 14 (77.8) | 52.4–93.6 | | |

*CI = Confidence interval; BCS = Body condition status; MCF = Method of colostrum feeding.*

Table 4
Regression analysis of pasteurellosis with associated risk factors

| Variables | No. of Animals examined | No of Animals with pathogens (%) | Multivariable LG analysis | |
|---|---|---|---|---|
| | | | Odds ratio (95% CI) | p-value |
| Sex | | | | |
| Female | 40 | 27 (67.5) | * | |
| Male | 44 | 37 (84.1) | 1.64 (0.52–5.2) | 0.4 |
| Age | | | | |
| ≥ 16 weeks | 27 | 17 (63.0) | * | |
| < 16 weeks | 57 | 47 (82.5) | 3.1 (1.0-9.6) | 0.04 |
| Breed | | | | |
| Cross-bred | 27 | 17 (63.0) | * | |
| Local | 57 | 47 (82.5) | 3.6 (1.1–11.5) | 0.03 |

**= explanatory variables; LG = Logistic regression; CI = Confidence interval.*

# Prevalence of Pasteurella species and Risk Factors

The result showed that *M. haemolytica* and *P. multocida* varied in proportion with different factors examined. Thus, sex and health status were significant associated (P < 0.05) with the occurrence of *M. haemolytica*, in that it was higher in male (56.8%) than female calves (35.0%) and animals with signs of respiratory illness (57.4%) than healthy one (32.4%). Moreover, the prevalence was relatively higher in calves of < 16 weeks old (50.9%) than older calves (37.0%) (Table 5). With regard to *P. multocida*, the occurrence was higher in cross bred (33.3%) than local (22.2%) and calves with signs respiratory illness (36.2%) than healthy one (21.6%) (Table 6).

Table 5
M. haemolytica infection in calves and associated risk factors

| Variables | No. of Animals examined | No of Animals with *M. haemolytica* (%) | CI (95%) | χ2 analysis | |
|---|---|---|---|---|---|
| | | | | χ2 value | p-value |
| Sex | | | | | |
| Female | 40 | 14 (35.0) | 20.6–51.7 | 4.01 | 0.045 |
| Male | 44 | 25 (56.8) | 41.0–71.7 | | |
| Age | | | | | |
| < 16 weeks | 57 | 29 (50.9) | 37.3–64.4 | 1.4 | 0.23 |
| ≥ 16 weeks | 27 | 10 (37.0) | 19.4–57.6 | | |
| BCS | | | | | |
| Poor | 13 | 5 (38.5) | 13.9–68.4 | 0.64 | 0.73 |
| Medium | 50 | 23 (46.0) | 31.8–60.7 | | |
| Good | 21 | 11 (52.4) | 29.9–74.3 | | |
| Breed | | | | | |
| Local | 27 | 11 (40.7) | 22.4–61.2 | 0.52 | 0.47 |
| Cross-bred | 57 | 28 (49.1) | 35.6–62.7 | | |
| Respiratory illness | | | | | |
| Absent | 37 | 12 (32.4) | 18.0–49.8 | 5.2 | 0.02 |
| Present | 47 | 27 (57.4) | 42.2–71.7 | | |
| Livelihood | | | | | |
| Mixed crop | 26 | 11 (42.3) | 23.4–63.1 | 0.25 | 0.61 |

| Variables | No. of Animals examined | No of Animals with *M. haemolytica* (%) | CI (95%) | χ2 analysis | |
|---|---|---|---|---|---|
| | | | | χ2 value | p-value |
| Urban | 58 | 28 (48.3) | 34.9–61.8 | | |
| Farming system | | | | | |
| Semi-intensive | 34 | 17 (50.0) | 32.4–67.6 | 0.29 | 0.58 |
| Intensive | 50 | 22 (44.0) | 29.9–58.7 | | |
| Herd size | | | | | |
| Small | 12 | 5 (41.7) | 15.2–72.3 | 0.13 | 0.93 |
| Medium | 59 | 28 (47.5) | 34.3–60.9 | | |
| Large | 13 | 6 (46.2) | 19.2–74.9 | | |
| Maternity pen | | | | | |
| Present | 27 | 12 (44.4) | 25.5–64.7 | 0.06 | 0.80 |
| Absent | 57 | 27 (47.4) | 33.9–61.0 | | |
| MCF | | | | | |
| Suckling | 66 | 29 (43.9) | 31.7–56.7 | 0.76 | 0.38 |
| Hand/bucket | 18 | 10 (55.6) | 30.8–78.5 | | |

**= Health status is based on respiratory illness; CI = Confidence Interval; BCS = Body condition status; MCF = Method of colostrum feeding.*

Table 6
P. multocida infection in calves and associated risk factors

| Variables | No. of Animals examined | No of Animals with *P. multocida* (%) | CI (95%) | χ2 analysis | |
|---|---|---|---|---|---|
| | | | | χ2 value | p-value |
| Sex | | | | | |
| Female | 40 | 13 (32.5) | 18.6–49.1 | 0.27 | 0.61 |
| Male | 44 | 12 (27.3) | 14.9–42.8 | | |
| Age | | | | | |
| < 16 weeks | 57 | 18 (31.6) | 19.9–45.2 | 0.28 | 0.59 |
| ≥ 16 weeks | 27 | 7 (25.9) | 11.1–46.3 | | |
| BCS | | | | | |
| Poor | 13 | 5 (38.5) | 13.9–68.4 | 0.83 | 0.66 |
| Medium | 50 | 15 (30.0) | 17.9–44.6 | | |
| Good | 21 | 5 (23.8) | 8.2–47.2 | | |
| Breed | | | | | |
| Local | 27 | 6 (22.2) | 08.6–42.3 | 1.08 | 0.29 |
| Cross-bred | 57 | 19 (33.3) | 21.4–47.1 | | |
| Respiratory illness | | | | | |
| Absent | 37 | 8 (21.6) | 09.8–38.2 | 2.09 | 0.15 |
| Present | 47 | 17 (36.2) | 22.7–51.5 | | |
| Livelihood | | | | | |
| Mixed crop | 26 | 5 (19.2) | 6.6–39.4 | 1.99 | 0.16 |

| Variables | No. of Animals examined | No of Animals with *P. multocida* (%) | CI (95%) | χ2 analysis | |
|---|---|---|---|---|---|
| | | | | χ2 value | p-value |
| Urban | 58 | 20 (34.5) | 22.5–48.1 | | |
| Farming system | | | | | |
| Semi-intensive | 34 | 7 (20.6) | 08.7–37.9 | 2.3 | 0.14 |
| Intensive | 50 | 18 (36.0) | 22.9–50.8 | | |
| Herd size | | | | | |
| Small | 12 | 4 (33.3) | 9.9–65.1 | 0.37 | 0.83 |
| Medium | 59 | 18 (30.5) | 19.2–43.9 | | |
| Large | 13 | 3 (23.1) | 05.0 -53.8 | | |
| Maternity pen | | | | | |
| Present | 27 | 6 (22.2) | 8.6–42.3 | 1.08 | 0.29 |
| Absent | 57 | 19 (33.3) | 21.4–47.1 | | |
| MCF | | | | | |
| Suckling | 66 | 21 (31.8) | 20.9–44.4 | 0.62 | 0.43 |
| Hand/bucket | 18 | 4 (22.2) | 6.4–47.6 | | |

*CI = Confidence interval; BCS = Body condition status; MCF = Method of colostrum feeding.*

# Cultural and Biochemical Characteristics of Isolated Pasteurella Species

*Mannheimia haemolytica* were able to grow as small red colony on MacConkey agar and show β-hemolysis on blood agar, while *Pasteurella multocida* were unable to grow on MacConkey agar and non-hemolytic (Table 7), but had mucoid colony and gram negative on gram staining. Different biochemical tests performed to identify the isolated pathogen and all the isolates were positive for oxidase, catalase, nitrate and phosphate and able to ferment sucrose, glucose and mannose. *P. multocida* isolates were positive for indole test but unable to ferment lactose and maltose (Table 7).

Table 7
Results of biochemical characteristics of bacterial species isolated from calves

| Tests | Species | |
|---|---|---|
| | *P. multocida* | *M. haemolytica* |
| Growth on MacConkey agar | - | + |
| Hemolysis on sheep blood agar | - | + |
| Indole production | + | - |
| Oxidase | + | + |
| Catalase | + | + |
| Nitrate | + | + |
| Phosphatase | + | + |
| Urease | - | - |
| Acid from: | | |
| Lactose | - | + |
| Maltose | - | + |
| Sucrose | + | + |
| Mannose | + | + |

# Discussion

In the present study the overall isolation rate of pasteurellosis was found to be 76.2% (95% CI = 65.7–84.8). The current finding in the prevalence of pasteurellosis is relatively higher than reports of previous studies in the country. Thus, [23], [24], [25] [26], and [27], who 50.2, 40.8, 39.2, 13, and 8.7%, respectively. This might be due to the difference in sampling that this study was conducted on cases and suspected of pneumonia, study area, time of sampling and farm management [23].

Analysis on sex related susceptibility showed that pasteurellosis was higher in male (84.1%) than female (67.5%) calves, in that the odd of being positive was 1.64 times higher in the former (OR = 1.64; 95% CI = 0.52–5.2). The possible explanation for sex related susceptibility is that less colostral immunoglobulin absorbed in male than female during neonatal life which leads to disease in male calves [28]. It is also worth mentioning that male calves are not as valuable to the dairy operation as females and therefore may not receive an attention the heifers do have, possibly accounting for the higher infection in males [29, 30]. Meanwhile, the prevalence was significantly higher in younger calves (< 16 weeks) (82.5%) than older one (≥ 16 weeks) (63.0%) with younger calves have 3.1 times the chances of being affected than

older (OR = 3.1; 95% CI = 1.0-9.6; p = 0.04). The possible explanation for the age-related susceptibility might be due to failure of passive immunity in hand feeding practices of young calves being able to predispose for bacterial infection and other predisposing etiological agents [21]. For instance, calves less than one month of age lack sufficient postruminal digestive enzymes to break down most sugars and are limited in their ability to utilize starch, maltose, sucrose, or dextran [31]. Partial or complete failure of passive transfer of maternal antibodies is an important host factor related to development of pneumonia in young calves [7, 32].There was significant association ($p < 0.05$) between pasteurellosis and breed of calves, with higher prevalence in cross breed (82.5%) than local (63%) calves. This deference might be linked to variation in environment adaptability. Therefore, local breed has high disease resistant capability. Similarly, the difference in the isolation rate of the two breeds might be due to the difference in feed access of the calves. Local breeds feed relatively less in amount and quality than cross breed calves which consume much amount and quality feed. So local breeds being less exposed to infection and therefore, have lower isolation rate than cross breed [33].

This study compares the level of isolation rate between animals kept under intensive and semi intensive management systems. The infection rate was higher in calves kept under intensive system (80%) compared to semi intensive one (70.6%). An intensive management system is mainly associated with confinements and predisposing calves are frequently contact to accumulations of urine and other wastes. This situation is likely to favors the spread of *Pasteurella* species among animals [29]. Similar observation was reported previously by [34; 35]. Moreover, the occurrence of pasteurellosis varied significantly ($p = 0.035$) among livelihood, in that it was higher in urban (82.8%) than mixed crop (61.5%) production system.

The overall proportion of *Mannheimia haemolytica* and *Pasteurella multocida* was 46.4% and 28.8%, respectively, indicating *M. haemolytica* was the major causative agent involved in calve pneumonic pasteurellosis in the study area. Although the infection rate varies, this finding is consistent with previous reports of [23]. Though *Mannheimia haemolytica* is a normal flora of the upper respiratory tract, suppressors of the host immune system favor the multiplication of *Pasteurella* species, leading to bronchopneumonia in purely pneumonic animals [36].The reasons for increased susceptibility to *M. haemolytica* infection in stressed animals are primarily attributed to the breakdown of innate pulmonary immune barriers by stressors [37, 38]. Although the percentage of isolation was relatively low (28.8%) the possible role of *P. multocida* in the etiology and pathogenesis of calve pneumonia should not be under estimated [23].

Concerning the isolation rate of *Pasteurella* species in relation to the health status, it was higher in animals with respiratory illness (93.6%) than no respiratory illness (54.1%), among which *Mannheimia haemolytica* and *Pasteurella multocida* were isolated from 57.4% and 36.2% of calves with respiratory disease symptoms, while they were isolated from 32.4% and 21.6% of calves with no respiratory illness, respectively. In this study significant variation was observed in prevalence of *Pasteurella* species in calves with and without respiratory illness. This suggests the possible involvement of these bacteria in the genesis of pneumonia.

# Conclusion

This present finding revealed that pasteurellosis is one of the major diseases of calves in the study area in which *M. haemolytica* and *P. multocida* were found to be commonly involved in respiratory infections. Age, breed, sex, and livelihood were the risk factors associated with occurrence of respiratory infection. The study focuses on *M. haemolytica* and *P. multocida*. Therefore, further epidemiological investigation is needed to rule out the role of concurrent microbial causes of calf pneumonia, so as to design appropriate control and preventive measures at farm as well as national level.

# Abbreviations

BAL: brocho alveolar lavage; BCS: body condition scores; BRD: bovine respiratory disease; CI: confidence interval; DNS: deep nasopharyngeal swabs; LG: logistic regression; MCF: methods of colostrum feeding; TTA: transtracheal aspiration

# Declarations

### Ethics approval and consent to participate

The investigators treated animals with kindness and took proper care by minimizing discomfort, distress or pain. Basically, the study was conducted by taking swab samples from the nasal cavity of animals. In addition, there were no human subjects and no private data was taken except history related with management of animals. Nevertheless, Verbal consent was obtained from calves owners for inclusion of their calves in the study. Confidentiality of the data obtained was strictly followed in the study periods as well as they were told that the data is not to be used for any other purpose that was not intention of the study. Finally, ethical clearance was obtained from Haramaya University ethical review board.

### Consent for publication

Not applicable

### Availability of data and materials

The datasets used and/or analyzed during the study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that none of them have financial or personal relationships with individuals or organizations that may have inappropriately influenced them in writing this paper and, therefore, declare that there is no competing interest.

### Funding

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### Author contributions

TM participated in the study design, field work and conducted the work. PW, AA and TF designed the study; provision test material, analyzed the statistics, performed data management and interpretation, prepared and wrote the manuscript. All authors read, evaluated and approved the final manuscript.

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### Author details

[1]Field Veterinarian, Amhara Livestock and Fishery Development Office of Sede woreda, East Gojam, Ethiopia. [2]College of Veterinary Medicine, Haramaya University, P.O. Box 138, Dire Dawa, Ethiopia. [3]College of Veterinary Medicine and Animal Sciences, University of Gondor, P.O. Box 196, Gondor, Ethiopia.

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# Figures

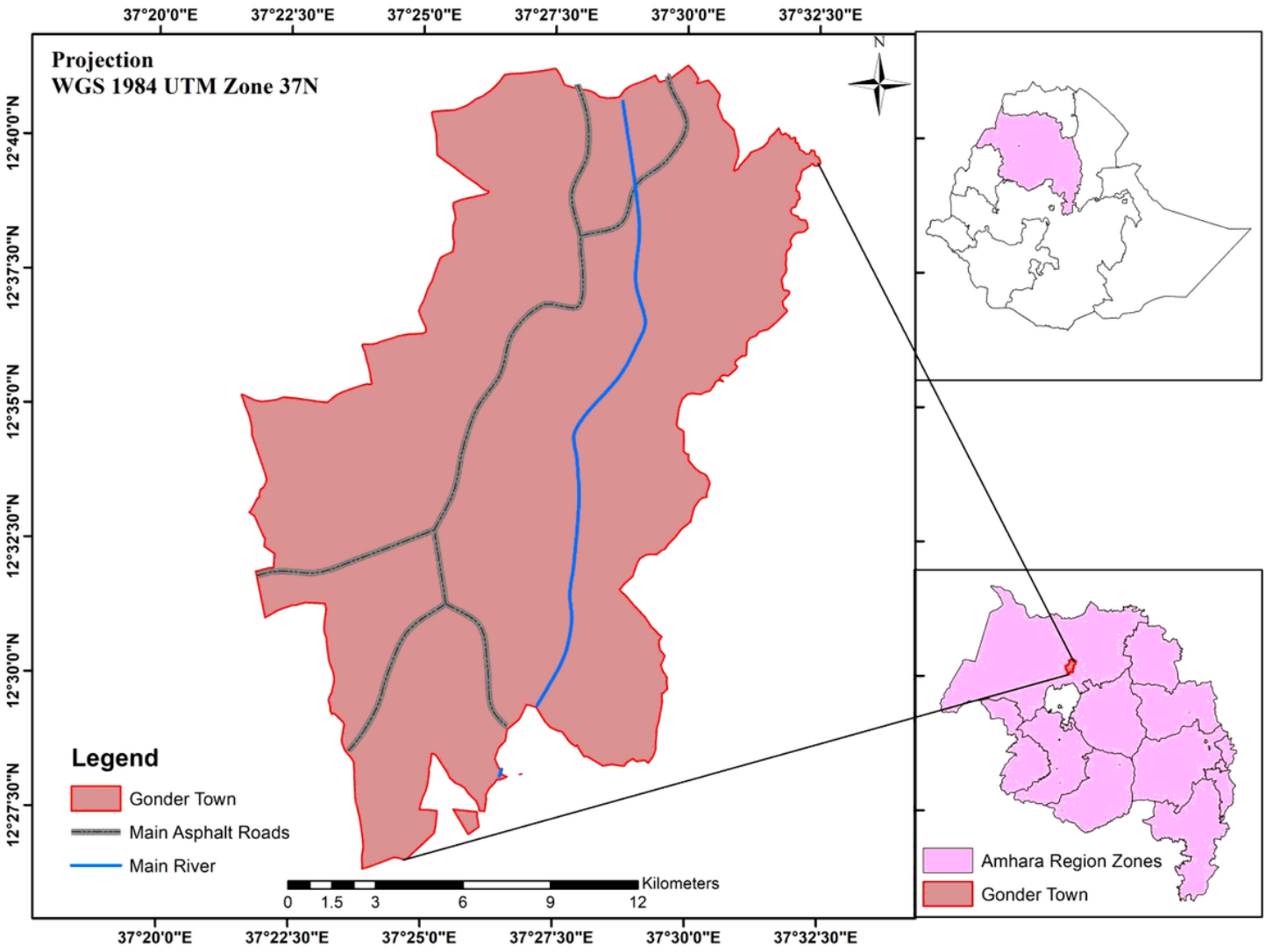


**Figure 1**

Map showing the Study area (Q-GIS version 3.10, http://osgeo4w-oslandia.com)