Ethiopian Veterinary Journal

Year: 2022 Volume: 26, No. 2



2022

ISSN:1683-6324 (print) ISSN:2221-5034 (online)

Ethiop. Vet. J. is the Official Scientific Organ of the Ethiopian Veterinary Association

Ethiopian Veterinary Journal (*Ethiop. Vet. J.*)

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The Ethiopian Veterinary Journal (*Ethiop. Vet. J.*) is a multidisciplinary peer-reviewed journal intended to promote animal health and production of national and regional/international importance. The journal publishes review articles, original research articles, short communication as well as technical notes in English. Under special circumstances, articles in Amharic may be considered for publication.

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Ethiop. Vet. J., 2022, 26 (2)
ISSN: 1683-6324

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Cover drawing by Wossene Abay and Yilma Jobre

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Effects of supplementation of different indigenous species of browses to Arsi-Bale yearling goats on feed intake, growth performance, and helminthes loads

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Abstract

This study was conducted to evaluate the effects of browse tree leaves meals on growth performance and parasite load of naturally parasitized yearling goats. Thirty-six Arsi-Bale yearling bucks which were naturally parasitized with helminths were randomly allocated to one of the following feeding treatments; T1 = *Chloris gayana* grass hay ad lib + 100g concentrate, T2 = T1 + dried 100g of *Acacia tortilis* leaves, T3 = T1 + dried 100g of *Acacia seyal* leaves, T4 = T1 + dried 100g of *Acacia senegal* leaves, T5 = T1 + dried 100g of *Millettia ferruginea* leaves and T6 = T1 + dried 100g of *Vernonia amygadalina* leaves. Goats were fed on corresponding diets for 70 days. Fecal egg count and body weight changes were recorded every 14 days. Higher feed conversion efficiency was observed in goats supplemented with dried browse tree leaves of *Acacia seyal* (T3), *Millettia ferruginea* (T5), and *Vernonia amygadalina* (T6). All supplemented goats had significantly ($p<0.05$) lower fecal egg count (FEC) at day 70 and grew significantly ($p<0.05$) faster than the control group. Goats supplemented with dried browse tree leaves of *Acacia seyal*, *Millettia ferruginea,* and *Vernonia amygadalina* grew faster than goats supplemented with other dried browse tree leaves**.** A rapid and significant reduction of FEC was observed in goats supplemented with *Millettia ferruginea* starting from day 14 and the lowest ($p<0.05$) value was attained at day 70 after treatment. Similarly, goats supplemented with *Vernonia amygadalina* showed an accelerated reduction of FEC starting from day 28 and attained the lowest ($p<0.05$) value at day 70 after treatment. Goats supplemented with *Millettia ferruginea* (T5) and *Vernonia amygadalina* (T6) had the lowest ($p<0.05$) FEC at all times after supplementation and grew faster than the other groups. The rapid and accelerated reduction of FECs and fastest growth rate observed in goats supplemented with *Millettia ferruginea* and *Vernonia amygadalina* indicated that these

browse tree leaves could be effective to control helminths parasite and improve the growth performance of parasitized goats.

Keywords: Indigenous browses tree leaves; parasitized goats; initial parasite load; fecal egg count; average daily weight gain.

Introduction

Browse plants play a significant role in the nutrition of ruminants in tropical regions. Browse species, because of their resistance to heat, drought, salinity, alkalinity, and grazing, are the major feed resources during the dry season (Amsalu *et al.*, 2021). In addition, the major advantage of browse plants over herbaceous legumes and grasses is their higher crude protein content (Amsalu *et al.*, 2021). This advantage makes them a promising supplement to crop residues and poor-quality natural pasture-based diets. Tropical browse species have been also reported to reduce gastro-intestinal parasite (GIP) burden and increase parasitized animal performance (Tavendale *et al.,* 2005).

Gastrointestinal parasites are one of the factors that contribute to the low productivity of livestock in developing countries (Pathak, 2013). Synthetic anthelmintics have long been considered the most effective way of controlling parasitic infections. However, these drugs are expensive and sometimes unavailable to smallholder farmers and pastoralists in developing countries. There are also cases of increased resistance to anthelmintics worldwide in ruminants (Pathak, 2013). Similarly, other control options, such as rotational grazing, may not be readily practiced by many smallholder farmers due to limited land size, or by many pastoralists due to communal land ownership. It follows that a search for novel and more sustainable anthelmintics is the best approach to the control of helminthosis.

In developing nations, traditional methods of controlling nematodes, used by small farmers, remain largely dependent on medicinal plants. Because these plants potentially represent a sustainable alternative to conventional chemotherapy. Recent research results indicated that tannins-containing plants have reduced worm burden and increased animal performance (Athanasiadou *et al.*, 2000; Molan *et al*., 2000a, b,). Feeding forage legumes such as Sulla (*Hedysarum oronarium*) and Lotus (*Lotus pedunculatus*) significantly increased the growth of parasitized lambs (Niezen *et al.,* 1998; Robertson *et al.,* 1995).

The vast majority of the *in vivo* and *in vitro* studies examining the effects of tannin-rich plants against gastrointestinal nematodes have been performed on temperate legume forages (Hoste *et al.*, 2006). Although woody browses are largely distributed in the tropics, especially in East Africa, only a limited number of *in vitro* studies have addressed the question of their potential direct anthelmintic properties (Papachristou *et al.*, 2005). Particularly, indigenous browses from the Rift Valley of Ethiopia are not studied for their potential to control gastrointestinal parasites (GIP) in the ruminants. Hence, the current study was conducted to evaluate the effects of five indigenous browse species of tree leaves from the Rift Valley of Ethiopia on feed intake, growth performance, and reduction capacity of parasite load of naturally parasitized yearling goats.

Materials and methods

Study area

The experiment was conducted at Adami Tulu Agricultural Research Center, which is located 167 km south of Addis Ababa at an altitude of 1650 meters above sea level in the mid-rift valley. The agro-ecological zone of the area is semi-arid and sub-humid with acacia woodland vegetation type. The mean annual rainfall is 760mm. The mean minimum and maximum temperatures are 12.6°C and 27°C, respectively. The soil type is fine, sandy loam with sand: silt: clay in the ratio of 34: 38: 18, respectively (Worku *et al.*, 2019).

Collection and preparation of browse leaves

Leaves of *Acacia seyal, Acacia senegal, Acacia tortilis, Millettia ferruginea,* and *Vernonia amygadalina* were collected at the end of the rainy season (September 2017) for subsequent animal supplementation studies. The plants were chosen based on a recent questionnaire survey in the southern Rift Valley of Ethiopia which indicated that they were frequently used by small-scale farmers against parasitic infections or used to treat associated clinical signs (Belete *et al.,* 2012). Leafy twigs were sampled and shed-dried within 24 hours and placed in polyethylene bags and kept in a cool, dry room pending the animal studies.

Experimental design

Growing Arsi-Bale goats were purchased from small-scale farmers in the vicinity. The goats had been grazed on communal grazing land in such a way that they had acquired a natural worm burden (1300 – 3000 EPG). Thirty-six intact bucks aged 12 to 14 months (age determined by dentation) with an average body weight of 14±1.4 kg were selected and allowed to acclimatize to the experimental environment and diet for two weeks. After conducting the initial fecal egg count, goats were blocked based on their fecal egg counts (500 EPG as low parasite load and 3000 EPG as high parasite load) and randomly allocated to one of the following feeding treatments; Treatment one (T1) = *Chloris gayana* grass hay ad lib + 100g concentrate mixture/head/day (75% wheat bran, 24% maize, and 1% mineral mix), treatment two (T2) = T1 + 100g of *Acacia tortilis* leaves hay, treatment three (T3) = T1 + 100g of *Acacia seyal* leaves hay, treatment four (T4) = T1 + 100g of *Acacia senegal* leaves hay, treatment five (T5) = T1 + 100g of *Millettia ferruginea* leaves hay and treatment six (T6) = T1 + 100g of *Vernonia amygadalina* leaves hay.

Data collection

The animals were housed in wooden pens with raised floors and fed individually with their respective experimental feeds for 10 weeks and had free access to clean water throughout the experimental periods. Any feed refusals were collected and weighed early in the morning before the daily feed allowance was offered. Daily feed intake was calculated by deference from daily feed offered and refused. The body weight of the animals was taken and fecal samples were collected from the rectum of the animals for FEC analysis every 14-days. Fecal egg counts were assessed using the McMaster method (Zajac and Conboy, 2012). The feed conversion efficiency was calculated as a ratio of body weight gain to DM intake in g/day (Gulten *et al*., 2000)

Chemical analysis of experimental diets

Feed samples were analyzed for dry matter (DM), ether extract (EE), and ash according to AOAC (2005). The neutral detergent fiber (NDF) was determined according to Van Soest *et al*. (1991). Acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to AOAC (1995). Nitrogen was determined by the Kjeldahl procedure and CP was calculated as N x 6.25.

Metabolizable energy (ME) of diets was estimated according to the equation proposed by Donker (1989).

Statistical analysis

Data were analyzed using the general linear model (GLM) of SAS, Version 9.2. The FEC were square-root transformed to normalize the data and then analyzed as a randomized complete block design using two-way analysis of variance (ANOVA) with initial parasite load as blocks.

The statistical model used was $Y_{ij} = \mu + B_i + L_j + B * L_{ij} + e_{ij.}$ Where: Y_{ij} is an observation, μ is the overall mean, B_i is the effect of browses, L_j is the effect of initial parasite load, $B*L_{ij}$ is the interaction between species and initial parasite load and e_{ij} is the experimental error. The means were separated by Duncan multiple range test. Differences between means were considered statistically significant if $p < 0.05$.

Ethical consideration

The goats used for this experiment were purchased from the local market. During the experimental period, they were housed on a well-protected, cleaned, wooden bedded floor. They were not artificially parasitized, but they were naturally parasitized while they were grazing in their locality before the experiment. We used the common palatable browse species as a supplement to the normal feed to treat the natural parasite load. The experimental diets were formulated to fulfill the animal maintenance feed requirements. No feed additives were used. We took only fecal samples, no blood or serum sample were taken. During the experimental periods all animals were gaining live weight, there was no morbidity or mortality at all. All the above information can be cross-checked with the scientific results of the experiment itself.

Results

Chemical composition of the feed

The chemical compositions of feed ingredients used in the experiment are given in Table 1. The result indicated that the average dry matter contents of all feed ingredients were ($p>0.05$) similar. All browses used in the current study had CP content above 13% DM. *Acacia senegal* had the highest ($p<0.05$) CP content while *Chloris gayana* hay had the lowest ($p<0.05$). The NDF of feed

ingredients varied from 14 to 66% of DM. *Chloris gayana* hay had the highest (P<0.05) NDF concentration while *Acacia seyal* had the lowest (p<0.05). *Millettia ferruginea* had the highest (p<0.05) ADF concentration while *Acacia seyal* had the lowest (p<0.05). The highest (p<0.05) ash contents were observed in *Vernonia amygadalinan, Millettia ferruginea,* and *Acacia senegal,* and the lowest (p<0.05) was observed in maize grain.

Table 1. Chemical composition (%DM) and energy values (Mcal/Kg DM) of feed ingredients used in the study

	DM	Ash	CP	EE	NDF	ADF	ME
Chloris gayana hay	94.00	8.68^{b}	5.30^{d}	2.25^{b}	65.99^{a}	17.28^{b}	2.47^{g}
Wheat bran	91.00	2.83^{c}	12.20^{c}	3.21^{ab}	47.44^{c}	13.45^{c}	2.54^{e}
Maize grain	93.00	1.04^{c}	5.30^{d}	4.01^{a}	**27.74^{e}**	5.50^{f}	2.67^{a}
Acacia tortilis hay	94.00	7.22^{b}	21.76^{b}	3.09^{ab}	19.48^{f}	12.63^{d}	2.55^{d}
Acacia seyal hay	94.00	6.50^{b}	13.00^{c}	3.30^{ab}	14.00^{g}	9.40^{e}	2.60^{b}
Acacia Senegal hay	94.00	10.63^{a}	28.80^{a}	4.38^{a}	22.00^{e}	12.10^{d}	2.56^{c}
Millettia ferruginea hay	93.20	10.62^{a}	22.46^{b}	3.49^{ab}	40.77^{d}	22.42^{a}	2.39^{h}
Vernonia amygadalina hay	93.00	12.60^{a}	22.50^{b}	2.86^{b}	22.10^{e}	14.40^{c}	2.52^{f}
SE	0.51	0.50	0.25	0.29	0.31	0.29	0.01

Means in the same column with different superscripts differ significantly (p<0.05). DM = Dry Matter, EE = Ether Extract, CP = crud protein, NDF = Neutral detergent fiber, ADF = Acid detergent fiber, ME = metabolisable energy (Mcal/Kg DM).

Nutrient (% DM) and energy (Mcal/Kg DM) contents of experimental diets

The nutrient and energy contents of experimental diets are given in Table 2. The lowest (p<0.05) fiber (NDF and ADF) contents and highest ME value were observed in diet T3 and the highest fiber (NDF and ADF) and lowest ME in diet T1. Diet four (T4) had the highest (p<0.05) CP content while T1 had the lowest (p<0.05). The lowest (p<0.05) ash content was observed in T1 while the highest was in T6.

Table 2. Nutrient (% DM) and energy (Mcal/Kg DM) contents of experimental diets

Diets	DM	Ash	CP	EE	NDF	ADF	ME
T1	92.80	4.18^{e}	7.74^{e}	3.16^{d}	52.99^{a}	14.66^{a}	2.52^{d}
T2	93.00	4.94^{c}	11.14^{c}	3.14^{d}	47.66^{c}	12.22^{c}	2.56^{b}
T3	93.00	4.76^{d}	9.00^{d}	3.19^{c}	46.29^{d}	11.41^{d}	2.57^{a}
T4	93.00	5.80^{b}	12.90^{a}	3.46^{a}	48.29^{b}	12.10^{c}	2.56^{b}
T5	92.68	5.79^{b}	11.13^{c}	3.24^{b}	48.32^{b}	12.66^{b}	2.55^{c}
T6	92.75	6.29^{a}	11.33^{b}	3.08^{e}	48.32^{b}	12.66^{b}	2.55^{c}
SE	0.3	0.01	0.03	0.01	0.06	0.09	0.01

Means in the same column with different superscripts differ significantly ($p<0.05$). DM = Dry Matter, EE = Ether Extract, CP = Crude protein, NDF = Neutral detergent fiber, ADF = Acid detergent fiber, ME = metabolisable energy (Mcal/Kg DM), T1= Chloris gayana hay adlib + concentrate, T2 = T1 + Acacia tortilis, T3 = T1 + Acacia seyal, T4 = T1 + Acacia senegal, T5 = T1 + Millettia ferruginea, T6 = T1 + Vernonia amygadalina,

Feed and nutrient intake

The daily feed and nutrient intake of the experimental animals is presented in Table 3. The result indicated that all goats supplemented with browse species were significantly ($p<0.05$) higher in their total feed intake (TFI) and dry matter intake (DMI) than the control groups (T1). Total feed intake and DM intake were ($p<0.05$) the highest in goats supplemented with *Acacia tortilis* followed by goats supplemented with *Acacia seyal.* Among the supplemented groups, the lowest ($p<0.05$) TFI and DMI have been observed in goats supplemented with *Vernonia amygadalina* followed by goats supplemented with *Millettia ferruginea.* However, a higher feed conversion efficiency was observed in goats supplemented with *Vernonia amygadalina* and *Millettia ferruginea.* Crude protein intake was ($p<0.05$) the highest in goats supplemented with *Acacia senegal* followed by goats supplemented with *Acacia tortilis.* Goats supplemented with *Acacia tortilis* had the highest ($p<0.05$) NDFI while those supplemented with *Vernonia amygadalina* had the least. The intake of ADF was highest ($p<0.05$) in goats supplemented with *Acacia tortilis* and the control group.

Table 3. Average feed intake (g) of Arsi Bale goats fed *Chloris gayana* hay supplemented with different browse species.

	Intake (g/head/d)						
Diets	**TFI**	**DMI**	**CPI**	**NDFI**	**ADFI**	**ME**	**FCEf**
T 1	527.00^{e}	489.06^{e}	55.36^{d}	259.15^{c}	71.69^{a}	1.23^{f}	0.02
T 2	638.00^{a}	593.34^{a}	66.10^{b}	282.80^{a}	72.52^{a}	1.52^{a}	0.06
T 3	618.00^{b}	574.74^{b}	51.73^{e}	266.05^{b}	65.58^{b}	1.48^{b}	0.07
T 4	598.00^{c}	556.14^{c}	71.74^{a}	268.56^{b}	67.30^{b}	1.43^{c}	0.03
T 5	587.40^{c}	545.79^{c}	60.67^{c}	265.14^{d}	68.47^{b}	1.39^{d}	0.07
T 6	556.00^{d}	515.69^{d}	58.43^{c}	249.18^{e}	65.29^{b}	1.32^{e}	0.07
SE	0.41	2.2	0.4	1.3	0.6	0.01	0.01

Means in the same column with different superscripts differ significantly ($p<0.05$). TFI = Total feed intake, DMI = Dry matter intake, CPI = Crud protein intake, NDFI = Neutral detergent fiber intake, ADFI = Acid detergent fiber intake, ME = metabolisable energy (Mcal/d), T1= Chloris gayana hay adlib + concentrate, T2 = T1 + Acacia tortilis, T3 = T1 + Acacia seyal, T4 = T1 + Acacia senegal, T5 = T1 + Millettia ferruginea, T6 = T1 + Vernonia amygadalina, FCEf = Feed conversion efficiency (g gain/g intake)

Effects of browse supplementation on fecal egg count and average daily weight gain

Effects of browse supplementation on fecal egg count (FEC) and average daily weight gain (ADG) of Arsi Bale goats are presented in table 4. The result indicated that all supplemented goats gradually reduced their FEC with time attaining the lowest FEC at 70 days after treatment while the FEC in the control group (T1) progressively increased throughout the experimental period. Goats supplemented with *Acacia seyal*, *Millettia ferruginea,* and *Vernonia amygadalina* grew faster than goats supplemented with other browses. All supplemental goats had significantly ($p<0.05$) lower FCE and except for T4, grew significantly ($p<0.05$) faster than the control group. Goats in the control group had the highest ($p<0.05$) FEC and lowest ADG throughout the experimental period. Goats supplemented with *Millettia ferruginea* (T5) and *Vernonia amygadalina* (T6) had the lowest ($p<0.05$) FEC at all times after treatment and grew faster than the other groups. A rapid and significant reduction of FEC was observed in goats supplemented with *Millettia ferruginea* starting from day 14 and the lowest ($p<0.05$) value was attained at day 70 after treatment. Similarly, goats supplemented with *Vernonia amygadalina* showed an accelerated reduction of FEC starting from day 28 and attained the lowest ($p<0.05$) value at day 70 after treatment. Goats supplemented with *Millettia ferruginea* reduced their FEC by 57, 61, and 66% after 28, 42, and 56 days of treatment, respectively.

The FEC of goats supplemented with *Vernonia amygadalina* reduced by 40, 62, and 74% after 42, 56, and 70 days of treatment, respectively.

Table 4. Effects of browse supplementation on fecal egg count (eggs per gram feces) and ADG (g/d/h) of Arsi Bale goats

	Fecal egg count (eggs per gram feces)*						
Treatments	**Day 0**	**Day 14**	**Day 28**	**Day 42**	**Day 56**	**Day 70**	**ADG**
T 1	1550 (35.15)	3000 (54.68)^{a}	4000 (63.19)^{a}	3500 (59.09)^{a}	2966 (54.00)^{a}	3075 (55.00)^{a}	10.00^{b}
T 2	2262 (46.94)	2500 (49.87)^{ab}	3500 (59.09)^{a}	3000 (54.68)^{a}	2500 (49.87)^{ab}	1800 (42.40)^{b}	32.14^{a}
T 3	1550 (35.15)	2175 (43.44)^{ab}	2800 (49.73)^{ab}	1844 (40.23)^{b}	1500 (38.45)^{bc}	1500 (38.45)^{b}	39.29^{a}
T 4	2262 (46.94)	1944 (41.99)^{ab}	1625 (37.71)^{bc}	1406 (36.51)^{b}	1188 (32.68)^{cd}	1213 (32.83)^{bc}	14.29^{ab}
T 5	1150 (30.15)	825 (27.66)^{c}	500 (21.92)^{c}	444 (20.95)^{c}	388 (18.56)^{d}	588 (23.95)^{c}	35.72^{a}
T 6	2675 (49.87)	2425 (40.64)^{ab}	2175 (34.36)^{bc}	1600 (32.56)^{b}	1025 (25.44)^{cd}	688 (23.77)^{c}	35.72^{a}
SE	5.28	4.10	6.5	3.5	4.4	3.9	5.5

Means in the same column with different superscripts differ significantly ($p<0.05$). T1= *Chloris gayana* hay adlib + concentrate, T2 = T1 + *Acacia tortilis*, T3 = T1 + *Acacia seyal*, T4 = T1 + *Acacia senegal*, T5 = T1 + *Millettia ferruginea*, T6 = T1 + *Vernonia amygadalina*. *Data for FECs are shown as arithmetic means with the means of the square-root transformed FECs in parentheses. Statistical analysis was undertaken on square-root transformed data. S.E.S of FEC is for square-root transformed data

Effects of initial parasite load on FEC and ADG

Effects of initial parasite load on subsequent fecal egg counts and ADG of Arsi-Bale goats are indicated in Table 5. Initial parasite load had a significant ($p<0.05$) effect on subsequent fecal egg count and ADG of goats. Goats with a high initial parasite (HIP) load showed ($p<0.05$) higher FEC at all times until day 70, at which the effects become ($p>0.05$) non-significant. Goats with low initial parasite (LIP) load grew significantly ($p<0.05$) at a faster rate than the other groups.

Table 5. Effects of initial parasite load on subsequent fecal egg count (eggs per gram feces) and ADG (gm/d) of Arsi Bale goats.

	Fecal egg count (eggs per gram feces)*					
Initial load	**Day 14**	**Day 28**	**Day 42**	**Day 56**	**Day 70**	**ADG (g/d)**
HIP Load	3019 (53.54)[a]	3033 (50.59)[a]	2500 (47.87)[a]	2068 (43.45)[a]	1683 (39.05)	22.32
LIP Load	1271 (32.54)[b]	1833 (38.10)[b]	1431 (33.47)[b]	1121 (29.54)[b]	1271 (33.08)	33.39[a]
SE	2.4	3.8	2.0	2.5	2.2	3.1

Means in the same column with different superscripts differ significantly ($p<0.05$). *Data for FECs are shown as arithmetic means with the means of the square-root transformed FECs in parentheses. SE of FEC is for square-root transformed data. HIP = High initial parasite load (3000 EPG), LIP = Low initial parasite load (500 EPG)

Effects of initial parasite load and browse supplementation on FEC and ADG

The effects of initial parasite load and browse supplementation on fecal egg count and ADG is presented in table 6. Though not significant, goats with HIP load in all treatments had higher FEC than those with LIP load at all times after supplementation. The ADG of goats with LIP load in each of the supplemented treatments was higher than that of goats with the HIP load. The ADG of goats with LIP load was significantly higher ($p<0.05$) when supplemented with *Acacia seyal* than other browses. Goats with HIP load and supplemented with *Millettia ferruginea* and *Vernonia amygadalina* grew faster than goats with HIP load in other treatments. The effects of *Vernonia amygadalina* supplementation were significant and effective on goats with LIP loads. Fecal egg count of goats with LIP loads and supplemented with *Vernonia amygadalina* were ($p<0.05$) lowest of all other groups at all times after supplementation. The group of goats with HIP load and supplemented with *Millettia ferruginea* had the lowest ($p<0.05$) FEC at all times after supplementation as compared to all other groups of goats with HIP load in other treatments. *Vernonia amygadalina* supplementation reduced the FEC of goats with HIP load by 76% and that of goats with LIP load by 50% at day 70 after supplementation. *Millettia ferruginea* supplementation reduced the FEC of goats with HIP load by 68% and that of goats with LIP load by 51% at day 56 after supplementation.

Table 6. The interaction effects of parasite load and browse supplementation on fecal egg count (eggs per gram feces) and ADG (g/d/head) of Arsi Bale goats.

Treatments		Fecal egg count (eggs per gram feces)*					ADG
		Day 0	Day 14	Day 42	Day 56	Day 70	
T 1	HIP	2675 (51.59)^{a}	3250 (56.96)^{a}	3750 (61.21)^{a}	3506 (59.00)^{a}	3625 (60.00)^{a}	12.50^{b}
	LIP	425 (18.69)^{b}	2750 (52.378)^{ab}	3250 (56.97)^{a}	2426 (49.00)^{ab}	2525 (50.00)^{ab}	7.50^{b}
T 2	HIP	2762 (52.34)^{a}	2750 (52.38)^{ab}	3250 (56.97)^{a}	2750 (52.39)^{a}	1900 (43.58)^{ab}	21.43^{ab}
	LIP	1762 (41.54)^{ab}	2250 (47.36)^{ab}	2750 (52.39)^{ab}	2250 (47.36)^{ab}	1700 (41.22)^{ab}	42.86^{ab}
T 3	HIP	2675 (51.59)^{a}	3625 (60.10)^{a}	2988 (54.04)^{ab}	1750 (41.73)^{ab}	1750 (41.733)^{ab}	28.58^{ab}
	LIP	425 (18.69)^{b}	725 (26.82)^{b}	700 (26.42)^{bc}	1250 (35.18)^{ab}	1250 (35.18)^{bc}	50.00^{a}
T 4	HIP	4375 (64.95)^{a}	2675 (50.72)^{ab}	1413 (37.45)^{b}	1850 (43.01)^{ab}	1025 (29.97)^{bc}	14.29^{b}
	LIP	150 (12.10)^{b}	1213 (33.25)^{b}	1400 (35.58)^{b}	525 (22.35)^{b}	1400 (35.70)^{bc}	14.29^{b}
T 5	HIP	1975 (42.61)^{ab}	1175 (33.54)^{b}	500 (22.36)^{bc}	625 (24.88)^{b}	600 (24.41)^{bc}	28.57^{ab}
	LIP	325 (17.67)^{b}	475 (21.78)^{b}	388 (19.55)^{bc}	150 (12.25)^{b}	575 (23.50)^{bc}	42.86^{ab}
T 6	HIP	5000 (70.45)^{a}	4638 (67.60)^{a}	3100 (55.21)^{ab}	1925 (39.76)^{ab}	1200 (34.64)^{bc}	28.57^{ab}
	LIP	350 (17.24)^{b}	213 (13.68)^{b}	100 (9.92)^{c}	125 (11.13)^{b}	175 (12.91)^{c}	42.86^{ab}
SE		7.4	5.7	4.9	6.2	5.4	7.8

Means in the same column with different superscripts differ significantly ($p<0.05$). T1= *Chloris gayana* hay adlib + concentrate, T2 = T1 + *Acacia tortilis*, T3 = T1 + *Acacia seyal*, T4 = T1 + *Acacia senegal*, T5 = T1 + *Millettia ferruginea*, T6 = T1 + *Vernonia amygadalina*. HIP = high initial parasite load, LIP = low initial parasite load. *Data for FECs are shown as arithmetic means with the means of the square-root transformed FECs in parentheses. Statistical analysis was undertaken on square-root transformed data. SE of FEC is for square-root transformed data

Discussion

Chemical composition of feed

The chemical composition of hay, wheat bran, and maize used in this experiment was comparable with the values reported by many workers (Awet, 2007; Sebsebe *et al.,* 2007; Tolera, 2008; Sisay *et al*., 2015). The range of CP contents

of the browses in the current study is in line with the findings of Njidda and Nasiru (2010) and Theart *et al*. (2015) who reported 14 to 21% and 13 to 25%, respectively. It has been indicated that most tropical browses are high in CP and can be used to supplement poor-quality roughages to increase the productivity of ruminant livestock in the tropics (Makkar and Becker, 1998; Njidda and Nasiru, 2010; Theart *et al*., 2015) which is consistent with the results obtained in the current experiment. In this study, the NDF and ADF of browses varied from 14 to 41 and 9 to 22% DM, respectively, which is comparable with the findings of Fadel Elseed *et al.* (2002) and Kaitho *et al.* (1997). The low NDF and ADF contents in *Acacia seysal* and *Acacia tortilis* in this study indicate that these browses have better dry matter intake and high digestibility. Schroeder (2004) reported the negative correlation of NDF and ADF with dry matter intake and digestibility of browses.

Feed and nutrient intake

The TF and DM intakes of supplemented goats in the current study ranged from 556 to 638 gm and from 515 to 593 gm, respectively. The current findings are in line with the findings of Sisay *et al*. (2015) who reported a range of 556 to 638 gm for TF and 524 to 601 gm for DM intakes of Arsi Bale goats supplemented with *Millettia ferruginea*. The highest TF and DM intake observed in goats supplemented with *Acacia tortilis* and *Acacia seyal* could be attributed to the lowest NDF and ADF values of *Acacia tortilis* and *Acacia seyal* (Table 1). It is well accepted that forage intake and degradation are mainly affected by the NDF and ADF contents (Njidda and Nasiru 2010). Schroeder (2004) reported the negative correlation of NDF and ADF with dry matter intake and digestibility of browses. Theart *et al*. (2015) reported a negative correlation between NDF and ADF with *in vitro* digestibility. The higher feed conversion efficiency observed in goats supplemented with *Millettia ferruginea* and *Vernonia amygadalina* could be attributed to the significant reduction effects of those browses on FECs throughout the experimental periods (Table 3).

Fecal egg count and weight gain

This experiment demonstrated that both browse supplementation and levels of initial parasite load significantly affected daily weight gain and FECs of goats. All supplemented goats had significantly ($p<0.05$) lower FEC at day 70 and grew significantly ($p<0.05$) faster than the control group. These results were in line with the earlier studies (Osoro *et al*., 2007a,b) which showed that grazing goats on a diet supplemented with tannin-containing *heather* had lower

FECs and better growth performance, in comparison with animals on non-supplemented diets. Similarly, Paolini *et al.* (2003) reported a reduction of FECs and improvement of ADG of naturally infected goats when supplemented with Sainfoin (*Onobrychis viciifoliae*) hay. Naturally, infected deer reduced FECs and increased ADG when supplemented with Chicory (*Cichorium intybus*) (Hoskin 2003). Significant reduction of FECs and improvement of ADG were observed on sheep supplemented with *Lotus pedunculatus* (Niezen *et al*., 1998) and on lambs grazed on Sulla (*Hedysarum coronarium*) pasture (Niezen *et al.,* 1995).

Goats with LIP loads grew faster than goats with HIP loads in all treatments and goats supplemented with *Acacia seyal*, *Millettia ferruginea* and *Vernonia amygadalina* grew faster than goats supplemented with other browses. Niezen *et al*. (1995) reported significantly higher ADG from all lambs with low initial parasite loads compared to those with high initial loads. The highest weight gain attained by goats supplemented with *Millettia ferruginea* and *Vernonia amygadalina* could be attributed to the significant reduction effects of those browses on FECs throughout the experimental periods (Table 3). The highest weight gain attained by goats supplemented with *Acacia seyal* could be attributed to the high nutritive values (lowest NDF and ADF) of *Acacia seyal* (Table 1). Although *Acacia seyal* had high nutritive values and the intake was adequate to ensure high ADG in goats with low initial parasite load, the ADG of goats with high initial load and supplemented by *Acacia seyal* was suffered by parasite load.

The significant and accelerated reduction of FECs obtained by supplementing *Vernonia amygadalina* in the current study agrees with the findings of Amsalu *et al.* (2021) who conducted an *in vitro* experiment and reported the highest larval migration inhibition rate and highest adult mortality rate of *H. Contortus* when treated with *Vernonia amygadalina* leaf extracts.

Conclusions

All browse species except *Acacia seyal,* used as a supplement had high CP contents, sufficient to be considered as high protein forage that can be used as supplements for low-quality roughages. The lowest NDF, ADF contents, and high DM intake of *Acacia seyal,* coupled with its high supplemental value, inducing a high growth rate of parasitized goats, would make the browse a potential supplement for parasitized goats. The lower FEC at the end of the

experimental period and faster growth rate observed in all supplemented goats indicate that these browses species could have the potential to reduce parasite load and improve growth rates of goats if they are supplemented for longer periods. There was a rapid and accelerated reduction of FECs and the fastest growth rate in goats supplemented with *Millettia ferruginea* and *Vernonia amygadalina,* which indicated that these browses species could have the potential to control helminths parasite and improve the growth performance of parasitized goats even with short term supplementation. Further research should focus on the analysis of the concentrations of Total phenol, Total Tannin, and Condensed Tannin of these promising browses species and their effects on the GIP of livestock.

Acknowledgments

This research project was fully supported by the research fund granted by the Vice President for Research and Technology Transfer of the Hawassa University (HU) for which the authors are highly grateful. The great cooperation and support of Adami Tulu Research Center, especially the goat and dairy research teams, in providing experimental goats, feeding pens, Lab facilities, and guesthouses are highly acknowledged.

References

Amsalu, S., Tegene, N. and Ajebu, N., 2021. Anthelminthic effects of extracts of indigenous browses from mid rift valley of Ethiopia. *Ethiop. Vet. J.,* 25, 132-143.

Athanasiadou, S., Kyriazakis, I., Jackson, F. and Coop, R.L., 2000. Effect of short-term exposure to condensed tannins on adult *Trichostrongylus colubriformis*. *Vet. Rec.*, 146, 728–732.

Awet, E., 2007. Feed utilization, body weight, and carcass parameters of intact and castrated Afar sheep fed on urea-treated teff straw supplemented with wheat bran. An MSc. Thesis presented to the School of Graduate Studies of Haramaya University. 70p.

Belete, S., Hassen, A., Assafa, T., Amen, N. and Ebro, A., 2012. Identification and nutritive value of potential fodder trees and shrubs in the mid-Rift Valley of Ethiopia. *J. Anim. Plant Sci.,* 22, 1126-1132.

Donker, J. D., 1989. Improved energy prediction equations for dairy cattle rations. *.J. Dairy Sci.*, 72, 2942-2948.

Fagg, C. W. and Stewart, J. L., 1994. The value of *Acacia* and *Prosopis* in arid and semi-arid Environments. *J. Arid. Environ.,* 27, 3–25.

Fadel Elseed, A.M.A, Amin, A.E, Khadiga, A., Abdel Ati, J., Sekine, M., Hishinuma, M. and

Hamana, K., 2002. Nutritive evaluation of some fodder tree species during the dry season in central Sudan. *Asian-australas. J. Anim. Sci*., 15, 844-850.

Gulten, K., Rad, F. and kinder, M., 2000. Growth Performance and feed conversion efficiency of Siberian sturgeon juveniles (*Acipenser baeri*) reared in concrete raceways. *Turk. J. Vet. Anim. Sci.,* 24, 435-442.

Hoskin, S.O., 2003. Effect of withholding anthelmintic treatment on autumn growth and internal parasitism of weaner deer grazing perennial ryegrass-based pasture or chicory. *Proc. N. Z. Soc. An. Prod*. 63, 269–273.

Hoste, H., Frank, Spiridoula, J., Stig, A., Thamsborg, M., Simone and Hoskin, O., 2006. The effects of tannin-rich plants on parasitic nematodes in ruminants. *Trends Parasitol*.. 22, 256-261.

Kaitho, R. J., Umunna, N. N., Nsahlai, I. V., Tamminga, S., van Bruchem, J. and Hanson, J., 1997. Palatability of wilted and dried multipurpose tree species fed to sheep and goats. *Anim. Feed Sci. Technol*., 65, 151-163.

Makkar, H.P.S., and Becker,K., 1998. *Jatropha curcas* Toxicity: Identification of Toxic Principle(s). In: Toxic Plants and Other Natural Toxicants, Garland, T., A.C. Barr, J.M. Betz, J.C. Reagor and E.M. Bailey (Eds.). CAB International, New York, USA, pp: 554-558.

Molan, A.L., Waghorn, G.C., Min, B.R. and McNabb, W.C., 2000a: The effect of condensed tannins from seven herbages on *Trichostrongylus colubriformis* larval migration in vitro. *Folia Parasitol.,* 47, 39–44.

Molan, A.L., Hoskin, S.O., Barry, T. N. and McNabb, W.C., 2000b.The effect of condensed tannins extracted from four forages on deer lungworm and gastrointestinal nematode larval viability. *Vet. Rec.,* 147, 44–48.

Niezen, J.H., Robertson, H.A., Waghorn, G.C. and Charleston, W.A.G., 1998. Production, faecal egg counts, and worm burdens of ewe lambs which grazed six contrasting forages. *Vet. Parasitol.,* 80, 15–27.

Niezen, J.H., Waghorn, T.S., Charleston, W.A.G. and Waghorn, G.C., 1995. Growth and gastrointestinal nematode parasitism in lambs grazing either lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*) which contains condensed tannins. *J. Agric. Sci.,* 125, 281–289.

Njidda, A .A. and Nasiru, A., 2010. In vitro gas production and dry matter digestibility of tannin-containing forages of the semi-arid region of North-eastern Nigeria. *Pak. J. Nutr.*, 9, 60-66.

Osoro, K., Benito-Peña, A., Frutos, P., García, U., Ortega-Mora, L.M., Celaya, R. and Ferre, I . 2007a. The effect of heather supplementation on gastrointestinal nematode infections and 23 Performance in Cashmere and local Celtiberic goats on pasture. *Small Rumin. Res.,* 67, 184-191.

Osoro, K., Mateos-Sanz, A., Frutos, P., García, U., Ortega-Mora, L.M., Ferreira, L.M.M., Celaya R. and Ferre, I., 2007b. Anthelmintic and nutritional effects of heather supplementation on Cashmere goats grazing perennial ryegrass-white clover pastures. *J. Anim. Sci.,* 85, 861-870.

Paolini,V., Dorchies, P.h. and Hoste, H. 2003. Effects of sainfoin hay on gastrointestinal infection with nematodes in goats. *Vet. Rec*., 152, 600–601.

Papachristou, T.G., Platis, P.D. and Nastis, A.S., 2005. Foraging behavior of cattle and goats in oak forest stands of varying coppicing age in Northern Greece. *Small Rumin. Res*., 59, 141-156

Pathak, A. K., 2013. Potential of using condensed tannins to control gastrointestinal nematodes and improve small ruminant performance. *Int. J. Mol. Vet. Res.,* 3, 36-50.

Robertson, H.A., Niezen, J.H., Waghorn, G.C., Charleston, W.A.G. and Jinlong, M., 1995. The effect of six herbages on live weight gain, wool growth, and faecal egg count of parasitized ewe lambs. *Proc. New Zealand Soc. Anim. Prod.,* 55, 99–201.

Schroeder, J.W., 2004. Forage Nutrition for Ruminants. North Dakota State University Cooperative Extension. Retrieved from http://www.ag.ndsu.edu/pubs/ansci/dairy/as1252w.html.

Sisay, T., Sandip, B., Ajebu, N. and Bangu, B., 2015. Studies on supplementation of graded levels of *Millitia Ferruginea* leaf meal on feed intake, growth performance, and carcass characters of Arsi Bale goats. *GJSFR D Agric. Vet.,* 15, 11-20

Tavendale, M.H., Meagher, L.P., Pacheco, D., Walker, N., Attwood, G.T. and Sivakumaran, S., 2005. Methane production from *in vitro* rumen incubations with *Lotus pedunculatus* and Medicago *sativa*, and effects of extractable condensed tannin fractions on methanogenesis. *Anim. Feed Sci. Technol.,* 124, 403–404.

Theart, J.J.F., Hassen, A., van Niekerk, W.A. and Gemeda, B.S., 2015. In vitro screening of Kalahari browse species for rumen methane mitigation. *Sci. Agric.* 72, 478-483.

Tolera, A., 2008. Feed resources and feeding management: A manual for feedlot operators and development workers. Ethiopian Sanitary and Phytosanitary Standards and Livestock and Meat Marketing Program (SPS-LMM), Addis Ababa, pp 43.

Worku, A., Alemu, T., Gurru, M., Gudeto, A., Messele, F., *et al.,* 2019. Evaluation of different feeding options on growth response and carcass characteristic of yearling Kereyu-Bulls to attain local/export market weight. *Int. J. Agric. Sci. Food Technol*., 5(2), 050-053.

Zajac, A.M. and Conboy, G.A., 2012. Veterinary Clinical Parasitology. 8th ed. John Wiley and Sons UK, P40-87.

Antimicrobial resistance status of selected bacteria isolated from animal source foods and feed in Ethiopia

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Abstract

Antimicrobial resistance (AMR) of major food-borne pathogens has become an increasing public health problem worldwide. A cross-sectional study was conducted from August 2019 to July 2021 in high-potential meat and dairy products and commercial animal feed supply chain areas of Ethiopia. The objectives of the study was assessing AMR profile of target bacterial pathogens isolated from animal sources foods (ASFs) and feed. A total of 642 ASFs and feed samples collected from selected sampling sites were examined at the microbiology laboratory of animal products, veterinary drugs, and feed quality assessment center.Bacterial identification and antimicrobial susceptibility test (AST) were conducted using an automated Vitek 2 XL compact system. Out of 642 investigated samples, 24 different genera and 59 species of bacteria were identified. A total of 185 samples were positive for target bacteria of *Staphylococcus aureus*, *Escherichia coli,* and *Salmonella* Species. The AST results showed AMR of target bacteria isolates against some of the tested antimicrobials. Of these, 83%, 55%, and 92% isolates of *Staphylococcus aureus, Escherichia coli,* and *Salmonella* Species, showed high level of AMR to Benzylpenicillin, Tetracycline, and Cefalexin/Gentamicin, respectively. The target bacteria isolated from ASFs and feed demonstrated multidrug resistance against some of the tested antimicrobials having public and veterinary importance. This reflects that ASFs and feed could serve as one of the sources for the spread and transmission of antimicrobial-resistant bacterial pathogens. Hence, there is a need for improv-

ing hygiene and sanitation practices along the ASFs and feed supply chains. Besides raising community awareness about the risks of AMR, emphasis on the rational use of antimicrobials in animal health practice and further investigations on AMR are recommended.
Keywords: Animal source foods; Antimicrobial resistance; Ethiopia; Feed; Target bacteria.

Introduction

The antimicrobial resistance (AMR) of major food-borne pathogens has become an increasing public health problem worldwide (Ferri *et al*., 2017). Though AMR is attributed to multiple factors, the contribution of the expanding use of antimicrobials in food animals has been considered the main reason for the worldwide rapid increase of AMR (WHO, 2015).

The growing worldwide phenomenon of AMR is generally associated with the improper use, overuse, or misuse of antimicrobials in humans and animals (Ferri *et al*., 2017) and agriculture (Martinez, 2009) which could enhance selective pressure for resistant strains. The resistant strains in the gut of animals and humans could horizontally transfer genes to similar or different species (e.g. *Salmonella* to *Escherichia coli*) (Martinez, 2009; Von Wintersdorff *et al*., 2016). Thus, humans can get infected by these resistant strains through the consumption of contaminated food of animal origin or through direct or indirect contact (Ghanbarpour *et al*., 2020).

Some reports indicate that major pathogens isolated from animal source food (ASF) and feed are resistant to antimicrobial agents such as quinolones, penicillin, aminoglycosides, macrolides, and tetracyclines (Mache, 2002; Ampaire *et al*., 2016; Mulu *et al*., 2017; Solomon *et al*., 2017; Waseem *et al*., 2019; Dahlin, 2020), but these antibiotics are still being used widely in the livestock sector for various purposes such as growth promotion (Tang *et al*., 2019). This increase in the use of antibiotics is mainly to satisfy the need for ASF attributed to the human population, income growth, and urbanization (Abegaz *et al*., 2018; Van *et al*., 2020).

In Ethiopia, farm hygiene, biosecurity, diagnostic capability, monitoring, tracing, and notification of pathogens, and AMR at the farm and national level are not well developed and uncontrolled antibiotic use and misuse are very com-

mon (Abdi *et al*., 2017). Thus, major pathogens such as *Escherichia coli*, *Salmonella* Species (Spps), *Staphylococcus aureus, Listeria monocytogene*s, and *Campylobacter* Spps could pose a public health problem (Molins *et al*., 2001). Therefore, AMR surveillance of pathogens in the ASFs and feed supply chain is crucial in combating AMR, guiding risk management and policy actions. Hence, in the present study, surveillance of the AMR profile of selected target bacterial pathogens isolated from ASFs and feed was conducted.

Materials and methods

Study design and study areas

A cross-sectional study was conducted from August 2019 to July 2021 in the potential meat and dairy products, and commercial animal feed supply chain areas of Ethiopia. Milk samples were collected from individual dairy cattle farms and milk collectors residing in Bishoftu, Sebeta, and Sululta towns, and meat samples were collected from separate butchers and/or municipal abattoirs in Addis Ababa, Bishoftu, Dukem, Modjo, and Sebeta towns while feed samples were collected from commercial feed manufacturing plants and distributors located in Addis Ababa, Adama, Bahirdar, Bishoftu, Burayu, Debratebor, Dessie, Injibara, Gelan and Sekota towns. The study areas were selected purposively based on the accessibility of sampling sites of commercial ASFs and feed having relatively high local market demand.

Sample size and sampling techniques

A total of 642 ASFs and feed samples were collected from sampling sites following Ethiopian Standard Agency (ESA) (ESA, 2016) and European Food Safety Authority (EFSA) standards (EFSA, 2010). The sample size was determined based on the testing capacity of the laboratory. In brief, 203 cow raw milk samples were collected from distinct dairy farms and milk selling points. Whereas, 256 meat/ carcass swab samples of cattle, sheep, goat, and chicken were obtained from selected butcher houses and municipal abattoirs. In addition, 174 feed samples were gathered from feed manufacturing plants and distributors in different parts of the country. Each sample was collected in a properly labeled sterile container following the standard sampling protocols and packed carefully to avoid leakage and cross-contamination (Roberts and Greenwood, 2008). The weight of samples collected was about 500g, 500 ml, and 1 Kg for meat, milk, and feed samples, respectively. The meat and milk are transported

under a cold chain and analyzed immediately. The samples were transported to the Ethiopia agriculture authority, microbiology laboratory of animal products, veterinary drug and feed quality assessment center and stored under a cold chain (EFSA, 2010; ESA, 2016).

Target bacterial culturing, isolation, and identification techniques

The techniques recommended by the International organization for standardization (ISO) (ISO, 2002) and ESA (ESA, 2012) were employed for the sample preparation and morphological characterization of target bacteria colonies. Further confirmation of the target bacterial isolation was performed by a fully automated Vitek 2XL compact system following the manufacturer method (BioMérieux, 2011; BioMérieux, 2020a; BioMérieux, 2020b).

Staphylococcus aureus (S. aureus) and *Escherichia coli* (*E. coli*) identification

About 10 g of feed and meat samples, 10 x 10 cm of meat carcass swab, and 10 ml of milk samples were pre-enriched in 90 ml of sterile buffered peptone water (BPW) to yield a 1/10 dilution, then homogenized using a smasher, and incubated at 37°C for 24 hrs (Sandel and McKillip, 2004; ESA, 2012). For *E. coli* isolation, a loop full of the incubated culture was streaked onto MacConkey agar and then incubated at 37°C for 24 hrs. A single pink colony was taken from cultured MacConkey agar and sub-cultured on Eosin Methylene Blue (EMB) agar and then incubated at 37°C for 24hrs. Colonies showing metallic sheen on EMB were sub-cultured on nutrient agar, incubated at 37°C for 24 hrs, and finally confirmed by Vitek 2XL compact system using Gram Negative (GN) cards having 47 test substrates. Similarly, for *S. aureus* isolation, a loop full of suspension was taken from incubated BPW culture and cultured on blood agar, and then incubated at 37°C for 24 hrs. A single pink colony was taken from incubated blood agar and sub-cultured on Mannitol Salt Agar (MSA) and then incubated at 37°C for 24 hrs. Colonies showing golden yellowish on MSA were sub-cultured on nutrient agar, incubated at 37°C for 24 hrs and confirmed by automated Vitek 2XL compact system using Gram-Positive (GP) cards (Sandel and McKillip, 2004; BioMérieux, 2011; ES ISO, 2012; BioMérieux, 2020a; BioMérieux, 2020b; BioMérieux, 2020c).

Salmonella Species *identification*

About 25 g of feed and meat samples, 25 ml of milk samples, and 10 x 10 cm meat carcasses swabs were pre-enriched in 225 ml of sterile BPW for feed and milk samples, and 90 ml of sterile BPW for swabs samples to yield a 1/10 dilution, then homogenized using smasher and incubated at 37°C for 24 hrs. A 0.1 ml and 1ml of the incubated BPW culture were transferred into 10ml of Rappaport Vassiliadis Medium with Soya (RVS) and Muller-Kauffmann Tetrathionate (MKTT) broth respectively as selective enrichment and inoculated at 41.5°C for RVS and 37 °C for MKTT for 24 hrs. A loop full of the inoculated RVS and MKTT broth was cultured onto Xylose Lycine Deoxycholate (XLD) agar which is selective media and incubated at 37°C for 24-48 hrs (ES ISO, 2002). Reddish/pink colonies of *Salmonella* on XLD plates were sub-cultured on Nutrient Agar, incubated at 37°C for 24 hrs and confirmed by using Vitek 2XL compact system according to the manufacturer method (Pincus, 2006; BioMérieux, 2011; BioMérieux, 2020a; BioMérieux, 2020b; BioMérieux, 2020c).

Confirmatory identification of target bacteria species by Vitek 2XL compact system

The Vitek 2 GN and Vitek 2 GP identification cards were used for confirmatory identification of GN and GP bacteria, respectively by Vitek 2XL compact system according to the manufacturer's recommendations (Pincus, 2006; BioMérieux, 2011; BioMérieux, 2020a). In brief, 2–3 pure fresh colonies taken from incubated nutrient agar were suspended in 3.0 ml of sterilized saline and thoroughly mixed to have 0.5 to 0.63McFarland turbidity (BioMérieux, 2020c). The bacteria in the suspension were identified using Vitek 2XL compact system (BioMérieux, 2011; BioMérieux, 2020a; BioMérieux, 2020b; BioMérieux, 2020c). The results were interpreted by the Vitek database at different confidence levels or probabilities as excellent (96-99%), very good (93-95%), good (89-92%), acceptable (85-88%), none or low reactive/discrimination biopattern and unidentified microorganisms. Bacteria identification results were considered acceptable when the confidence level is ≥85% probability. The final identification results were obtained automatically approximately 8 hrs or less for GP bacteria and 10 hrs or less for GN bacteria (Pincus, 2006; BioMérieux, 2011; BioMérieux, 2020a; BioMérieux, 2020b).

Antimicrobial susceptibility test (AST)

AST against selected antimicrobial agents were conducted for each of isolated target bacteria (*S.aureus, E.coli* and *Salmonella* Spps) according to the Vitek 2XL compact system protocol (BioMérieux, 2011; BioMérieux, 2022a; BioMérieux, 2020b). Eight antimicrobial classes containing a total of 23 antimicrobial agents coated with Vitek 2 AST-GN96 card and Vitek 2 AST-GP79 cards were used (Table 1). The selection criteria of the antimicrobials were based on the Office International des Epizooties (OIE) list of antimicrobial agents of veterinary importance (OIE, 2015).

The pure colonies of the identified *S. aureus, E.coli* and *Salmonella* Spps were suspended in 3.0 ml of sterilized saline using different sterile test tubes and thoroughly mixed (BioMérieux, 2011; BioMérieux, 2022a; BioMérieux, 2020b). The turbidity of the bacterial suspensions was adjusted with a DensiChekPlus meter to match that of a McFarland 0.5–0.63 standard (BioMérieux, 2020c). Then after, AST cards were selected based on the gram characteristics of the bacteria isolates (AST GP card for *S.aurues* and AST GN card for *E.coli* and *Salmonella*), then loaded into the Vitek XL compact system for automatic processing. The results were interpreted by the Vitek and obtained automatically. Susceptibility test results of the bacteria were supposed to be observed in less than 19 hrs (Pincus, 2006; BioMérieux, 2011; BioMérieux, 2020a).

The break-point of antimicrobial is expressed numerically in µg/ml as minimum inhibitory concentration (MIC) for AST/AMR interpretation. AST results in interpretations were based on the guidelines of the Clinical and Laboratory Standards Institute (CLS) (CLSI, 2017). The results interpretations for AST/AMR obtained from Vitek 2XL compact system were categorized as Susceptible (S), Intermediate (I) and Resistant (R) (BioMérieux, 2020a; BioMérieux, 2020b).

Table1. Target bacteria pathogens-antimicrobial agent combinations used for antimicrobial susceptibility test study.

Target bacteria	Antimicrobial class	Antimicrobial agents (AMA)	AST card coated with AMA	AMA importance in veterinary***
Salmonella and E.coli	1. Quinolones	Flumequine	AST-GN	VHIA
		Marbofloxacin	AST-GN	VCIA
		Enrofloxacin**	Both	
	2. Beta-lactams			
	2.1 Cephalosporins	Cefalexin	AST-GN	VHIA
		Cefalotin**	Both	
		Cefoperazone	AST-GN	VCIA
		Ceftiofur**	Both	
		Cefquinome**	Both	
	2.2 Penicillins	Amoxicillin/ Clavulanic acid/	AST-GN	VCIA
S.aureus		Ampicillin**	Both	
		Benzylpenicillin*	AST-GP	
		Oxacillin*	AST-GP	
	3. Aminoglycosides	Gentamycin**	Both	VCIA
		Neomycin**	Both	
		Amikacin	AST-GP	
		Kanamycin	AST-GP	
	4. Macrolides	Erythromycin	AST-GP	VCIA
		Tilmicosin	AST-GP	
		Tylosin	AST-GP	
	5. Lincosamides	Clindamycin	AST-GP	VHIA
	6. Tetracyclines	Tetracycline**	Both	VCIA
	7. Sulfonamide and Diaminopyrimidines/ Trimethoprim	Trimethoprim/ Sulfamethoxazole**	Both	VCIA
	8. Amphenicols	Florfenicol**	Both	VCIA

*Benzylpenicillin and *Oxacillin antimicrobial agents were used only for *S.aureus.*

**Antimicrobial agents coated with both AST-GP and AST-GN cards were used for *S.aureus, E.coli,* and *Salmonella* Spps.

***Importance of antimicrobial agents in veterinary (i) Veterinary critically important antimicrobial agents (VCIH) (ii) Veterinary highly important antimicrobial agents (VHIA) (iii)Veterinary important antimicrobial agents(VIA) (OIE, 2015).

Data management and analysis

Microsoft® Excel (2010) was used for data management and analysis. Data generated from laboratory investigations were coded, entered, and/ or calculated on an MS Excel spreadsheet. The percentage of occurrence of bacteria and /or target bacteria Spps isolated from the ASF and feed was calculated as the number of positive (confirmed) samples divided by the total number of samples investigated (processed) in the laboratory. Similarly, the percentage of AST profiles (AMR development) of target bacteria was calculated as the number of target bacteria isolates that showed resistance against tested AMA divided by the total number of target bacteria tested for AST against selected AMA.

Results

Out of 642 investigated ASFs and feed samples, 24 different genera and 59 species of bacteria were identified from morphologically known target bacteria colonies loaded into the Vitek 2XL compact system using GP and GN identification cards. All the identified GP and GN bacteria are depicted in Table 2.

Table 2. Bacteria genera and species identified from the test samples by gram positive and gram negative cards.

S/N	Identified bacteria genera and species types	No (%) of occurrence bacteria species			
		Milk (n=203)	Meat (n=265)	Feed (n=174)	Total (N=642)
I	Gram-negative bacteria				
1.0	***Escherichia coli***	24 (20.70)	77 (29.00)	0 (0.00)	101 (15.70)
2.0	***Salmonella*** **Group**				
2.1	*S.Typhi*	1 (0.49)	1(0.37)	0 (0.00)	2 (0.31)
2.2	*S.enterica diarizonae*	1 (0.49)	7(2.64)	2 (1.15)	10 (1.56)
3.0	***Klebsiella*** **Species**				
3.1	*K. oxytoca*	7(3.45)	5 (1.88)	0 (0.00)	12 (1.87)
3.2	*K. pneumoniae*	3 (1.47)	4 (1.50)	3 (1.72)	10 (1.56)
4.0	***Shigella*** **Group**	2 (0.98)	1 (0.37)	0 (0.00)	3 (0.47)
5.0	***Proteus*** **Species**				
5.1	*P.mirabilis*	4 (1.97)	12 (4.53)	2 (1.15)	18 (2.80)
5.2	*P.vulgaris*	0 (0.00)	3 (1.13)	0 (0.00)	3 (0.47)
5.3	*P.hauseri*	0 (0.00)	1 (0.37)	0 (0.00)	1(0.15)

S/N	Identified bacteria genera and species types	No (%) of occurrence bacteria species			
		Milk (n=203)	Meat (n=265)	Feed (n=174)	Total (N=642)
6.0	***Enterobacter* Species**				
6.1	*E.aerogenes*	15 (7.39)	15 (5.66)	8 (4.60)	38 (5.92)
6.2	*E.cloacae complex*	14 (6.90)	8 (3.02)	8 (4.60)	30 (4.67)
6.3	*E.cloacae dissolvents*	0 (0.00)	1 (0.37)	0 (0.00)	1 (0.15)
6.4	*E./Kluyveria /intermedia*	1 (0.49)	0 (0.00)	0 (0.00)	1 (0.15)
7.0	***Morganella* Species**				
7.1	*M. morgenisspmorgeni*	1 (0.49)	2 (0.75)	0 (0.00)	3 (0.47)
8.0	***Raultella* Species**				
8.1	*R.planticola*	4 (1.97)	0 (0.00)	0 (0.00)	4 (0.62)
8.2	*R.ornithinoltyica*	10 (4.92)	18 (6.79)	7 (4.02)	35 (5.45)
9.0	***Pantoe* Species**	4 (1.97)	1 (0.37)	1 (0.57)	6 (0.93)
10.0	***Pseudomonas* Species**				
10.1	*P.aeroginosa*	3 (1.47)	12 (4.52)	0 (0.00)	15 (2.33)
10.2	*P.putida*	0 (0.00)	1 (0.37)	0 (0.00)	1 (0.15)
11.0	***Serratia* Group**				
11.1	*S.fonticola*	0 (0.00)	4 (1.5)	0 (0.00)	4 (0.62)
11.2	*S.mercescense*	0 (0.00)	1 (0.37)	0 (0.00))	1 (0.15)
11.3	*S.liquefaciences*	0 (0.00)	2 (0.75)	0 (0.00)	2 (0.31)
11.4	*S.plymuthica*	0 (0.00)	1 (0.37)	0 (0.00)	1 (0.15)
11.5	*S.odorifera*	1 (0.49)	0 (0.00)	0 (0.00)	1 (0.15)
12.0	***Citrobacter* Species**				
12.1	*C.freundii*	2 (0.98)	16 (6.03)	1 (0.57)	19 (2.96)
12.2	*C.sedlaki*	0 (0.00)	0 (0.00)	1 (0.57)	1 (0.15)
12.3	*C.braakii*	0 (0.00)	3 (1.13)	0 (0.00)	3 (0.47)
12.4	*C.werkmanii*	1 (0.49)	1 (0.37)	0 (0.00)	2 (0.31)
13.0	***Providencia* Species**				
13.1	*P.stuartii*	0 (0.00)	1(0.37)	0 (0.00)	1 (0.15)
13.2	*P.rettgeri*	0 (0.00)	1 (0.37)	0 (0.00)	1 (0.15)
14.0	***Cedecea* Species**				
14.1	*C.davisae*	0 (0.00)	1(0.37)	0 (0.00)	1 (0.15)
15.0	***Burkholderia* Species**				
15.1	*B.cepacia group*	2 (0.98)	1 (0.37)	1 (0.57)	3 (0.47)
16.0	***Sphingomonadacea* Species**				

S/N	Identified bacteria genera and species types	No (%) of occurrence bacteria species			
		Milk (n=203)	Meat (n=265)	Feed (n=174)	Total (N=642)
16.1	*S.paucimobilis*	1 (0.49)	0 (0.00)	0 (0.00)	1 (0.15)
17.0	***Aeromonas* Species**				
17.1	A.hyrdophila/punctata	0 (0.00)	1 (0.37)	0 (0.00)	1 (0.15)
17.2	*A.salmonicida*	2 (0.98)	0 (0.00)	0 (0.00)	2 (0.31)
18.0	***Pasteurella* Species**				
18.1	*P. canis*	1 (0.49)	0 (0.00)	0 (0.00)	1 (0.15)
II	**Gram-positive bacteria**				
1.0	***Staphylococcus* Species**				
1.1	*S.aureus*	46 (22.66)	26 (9.81)	0 (0.00)	72 (11.21)
1.2	*S.saprophyticus*	1 (0.49)	2 (0.75)	0 (0.00)	3 (0.47)
1.3	*S.xylosus*	2 (0.98)	1 (0.37)	0 (0.00)	3 (0.47)
1.4	*S.sciuri*	5 (2.46)	23 (8.68)	0 (0.00)	28 (4.36)
1.5	*S.gallinarum*	0 (0.00)	2 (0.75)	0 (0.00)	2 (0.31)
1.6	*S.lentus*	7 (3.45)	7 (2.64)	0 (0.00)	14 (2.18)
1.7	*S.intermedius*	1 (0.49)	0 (0.00)	0 (0.00)	1 (0.15)
1.8	*S.vitulinus*	0 (0.00)	2 (0.75)	0 (0.00)	2 (0.31)
1.9	*S.hominis*	0 (0.00)	1 (0.37)	0 (0.00)	1 (0.15)
1.10	*S.warneri*	0 (0.00)	7 (2.64)	0 (0.00)	7 (1.09)
1.11	*S.chromogenes*	2 (0.98)	0 (0.00)	0 (0.00)	2 (0.31)
1.12	*S.haemolyticus*	1(0.49)	0 (0.00)	0 (0.00)	1 (0.15)
1.13	*S.cohnii spp cohnii*	1(0.49)	0 (0.00)	0 (0.00)	1 (0.15)
1.14	*S.simulans*	0 (0.00)	3(1.13)	0 (0.00)	3 (0.47)
2.0	***Enterococcus* Species**				
2.1	*E.faecium*	2 (0.98)	0 (0.00)	2 (1.15)	4 (0.62)
2.2	E.faecalis	1 (0.49)	12 (4.52)	0 (0.00)	13 (2.02)
2.3	*E.gallinarium*	0 (0.00)	4 (1.5)	0 (0.00)	4 (0.62)
3.0	***Aerococcus* Species**				
3.1	*A.viridans*	2 (0.98)	2 (0.75)	1 (0.57)	5 (0.77)
4.0	*Leuconostoc species*				
4.1	*L.mesentero*	9 (4.43)	3 (1.13)	2 (1.15)	14 (2.18)
5.0	***Streptococcus* Species**				
5.1	*S.pseudoporcinus*	0 (0.00)	1 (0.37)	0 (0.00)	1 (0.15)
5.2	*S.thoraltensis*	0 (0.00)	1 (0.37)	0 (0.00)	1 (0.15)

S/N	Identified bacteria genera and species types	No (%) of occurrence bacteria species			
		Milk (n=203)	Meat (n=265)	Feed (n=174)	Total (N=642)
6.0	*Kocuria species*				
6.1	*K.kristinae*	0 (0.00)	4 (1.5)	0 (0.00)	4 (0.62)
6.2	*K.rhizophila*	0 (0.00)	2 (0.75)	0 (0.00)	2 (0.31)
III	Mixed bacteria isolation	45 (22.17)	57 (21.50)	5 (3.40)	107 (16.6)

Of all the tested samples, 185 were positive for the three target bacterial pathogens; *S. aureus* 11.2% (72 out of 642), *E. coli* 15.7% (101 out of 642), and *Salmonella* Spps 1.9% (12 out of 642) (Table 3).

Table 3. Target bacteria species occurrence on a sample basis.

Sample type	Tested sample number	No (%) of the target bacteria isolates/species			
		S. aureus	***E. coli***	***Salmonella* Spps**	**Total**
Raw milk	203	46 (20.0)	24 (11.8)	2 (1.0)	72 (35.5)
Meat/carcass swabs	265	26 (9.8)	77 (29.1)	8 (3.0)	111 (41.9)
Feedstuffs	174	0 (0)	0 (0)	2 (1.1)	2 (1.1)
Total	642	72 (11.2)	101(15.7)	12 (1.9)	185 (28.8)

Antimicrobial susceptibility test (AST)

The AST was conducted on all the identified target bacterial species. The AST result of *S. aureus, E. coli,* and *Salmonella* bacteria isolates tested against sixteen (16), fourteen (14), and fifteen (15) AMA, respectively shows high resistance to some antibiotics and no resistance (susceptible) to some antibiotics. The AMR development levels of *S. aureus*, *E. coli,* and *Salmonella* Spps against the selected antimicrobials are summarized and presented in Figures 1, 2, and 3, respectively.

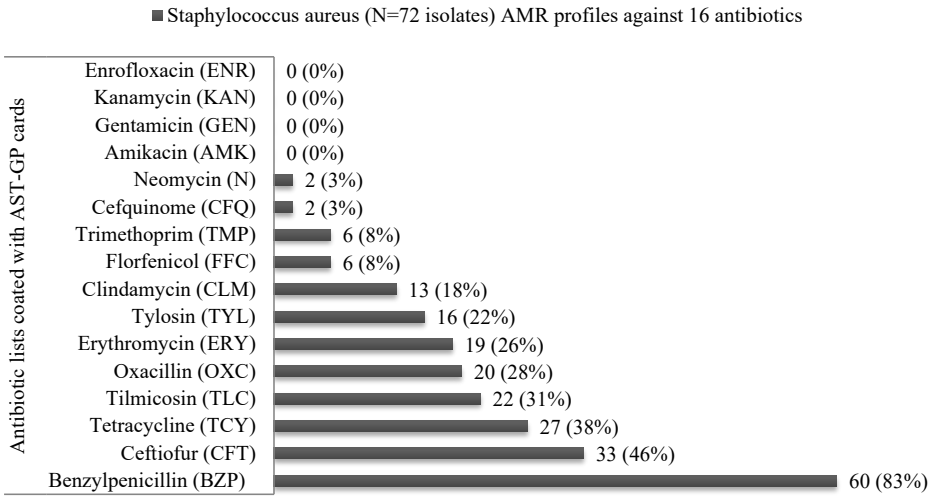


Figure 1. *Staphylococcus aureus* AMR development levels against selected antibiotics.

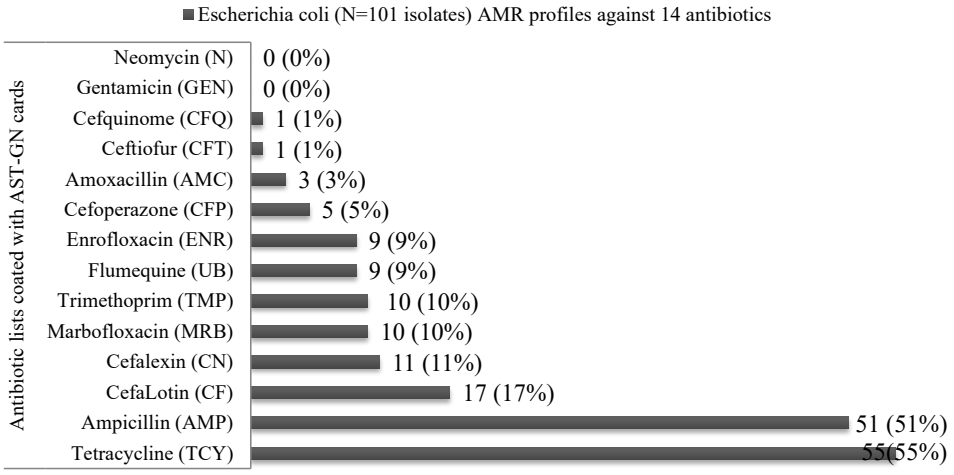


Figure 2. *Escherichia coli* AMR development levels against selected antibiotics.

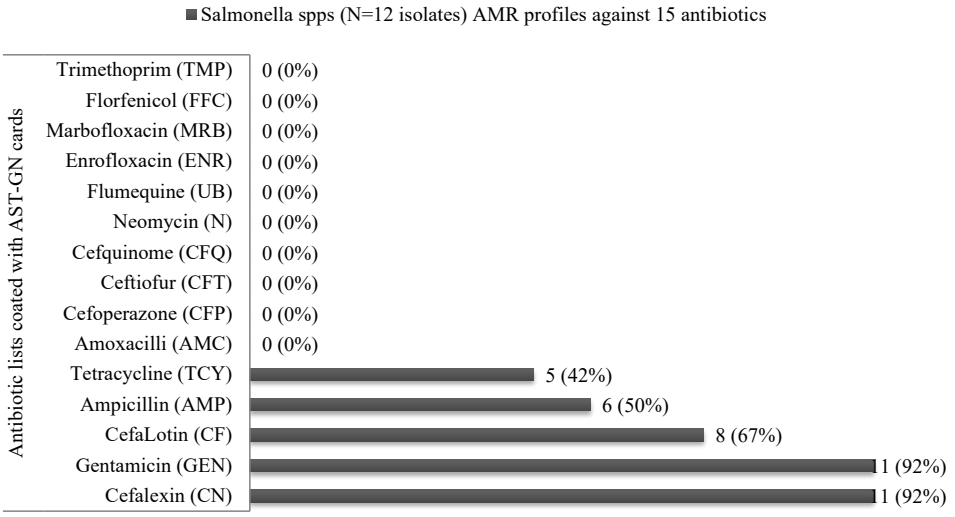


Figure 3. *Salmonella* Spps AMR development levels against selected antibiotics.

Discussion

In the present study, a total of 642 samples consisting of 203 raw milk, 265 meat/carcass swabs, and 174 feedstuffs were investigated for the presence of target bacteria and AST profiles. The findings proved the presence of 24 different genera and 59 species of bacteria in the investigated samples with the overall high prevalence of *E.coli* 15.7% (101 out of 642) followed by *S. aureus* 11.2% (72 out of 642) and *Salmonella* Spps 1.9% (12 out of 642). This implies that ASFs and feed samples obtained from the study areas could contribute as the reservoirs for strains of *S. aureus*, *E. coli,* and *Salmonella* Spps and point to the presence of poor food hygiene and sanitation management practices. All isolates of target bacterial Spps were tested for AST against the AMA having different levels of veterinary and public health importance.

The AST results observed in the present study suggest resistance of *S. aureus* for the majority (75%) of the tested antimicrobials except for Amikacin, Gentamycin, Kanamycin, and Enrofloxacin. The *S. aureus* bacteria recovered from milk and meat samples demonstrated a relatively high level of resistance to

Benzylpenicillin (83%), Tetracycline (38%), and Erythromycin (26%). Similar patterns of resistance were reported in a previous study conducted in Bishoftu, Ethiopia which indicates the resistance of 18 *S.aureus* isolated from 253 samples of meat and dairy milk to Penicillin (94.45%), Tetracycline (27.8 %), and Erythromycin (33.33%) (Matewos, 2020). Also, the resistance of *S. aureus* isolates to Oxacillin (28%) and Clindamycin (18%), observed in the current study was in accordance with the findings reported for Oxacillin (31%) and Clindamycin (13.8%) (Reta *et al*., 2016). The findings observed for *S. aureus* indicated that ASFs and feed being supplied to the market in the study areas could potentially be a threat to both public and animal health as well as the environment. The resistance of *S. aureus* to clindamycin; a drug that is not used in veterinary practice in Ethiopia suggests the transfer of genes of resistant strain among the environment, livestock, and human. All *S. aureus* isolates are found to be 100% susceptible to Amikacin, Gentamycin, and Kanamycin antimicrobials agents and suggesting that they are relatively the most effective drugs *in-vitro*.

E. coli isolates obtained from milk and meat samples are non-responsive to Tetracycline (55%), Ampicillin (51%), Cefalotin (17%), Cefalexin (11%), Marbofloxacin (10%), Trimethoprim (10%), Flumequine (9%) and Enrofloxacin (9%). In previous studies, a relatively low proportion of *E. coli* isolates show resistance against Tetracycline (8.1%), Ampicillin (21.5%), and Trimethoprim (4.6%) (Mwanyika *et al*., 2016) and relatively high resistance of *E. coli* O157:H7against Tetracycline (81.8%) is reported (Mohamed *et al*., 2020). This might be due to differences among different environments and hygiene practices.

In contrast to our finding, high resistance rates of *E. coli* were observed (>60%) for Flumequine, while resistance reported for Enrofloxacin and Marbofloxacin are almost similar (<40%) (Vanni *et al*., 2014).

In the current study, the AST results of *Salmonella* Spps isolated from milk, meat and feed samples conducted using fifteen (15) selected antimicrobials coated with GN cards indicated more resistance to Cefalexin (92%), Gentamicin (92%), Cefalotin (67%), Ampicillin (50%) and Tetracycline (42%) as compared to the findings reported in the previous study (Ejo *et al*., 2016; Okorie-Kanu *et al*., 2016) in which more than 90-100% of *Salmonella* Spps demonstrate resistance to Tetracycline. A study conducted in Addis Ababa (Alemu *et al*., 2011) showed that *Salmonella* Spps isolated from dairy lactating cows demonstrate

resistance to Ampicillin (100%) and Tetracycline (33.33%). The findings of the present study reveal that *Salmonella* Spps showed no resistance to Amoxicillin, Cefoperazone, Ceftiofur, cefquinome, Neomycin, Flumequine, Enrofloxacillin, Marbofloxacin, Florfenicol, and Trimethoprim. This might be due to the low frequency of use in the study area in veterinary services, and perhaps in human medicine.

The limitation of this study is that the knowledge, attitude, and practice (KAP) of personnel working in the municipal abattoir, dairy farm, and feed manufacturing plant were not captured during sample collection. Besides, due to the shortage of AMA-coated test cards, the AST tests were conducted for only prioritized target pathogenic bacteria.

Conclusions

Overall, the findings of the present study reveal a high level of AMR for some antimicrobials having different levels of public and veterinary importance against target bacteria isolated from ASFs and feed. This implies that ASFs and feedstuffs in the study areas could potentially be a reservoir of drug-resistant bacteria dissemination and transmission. Thus, consumption of ASFs and feedstuffs could present public and veterinary health risks. In addition, since a large proportion of the population in Ethiopia lives near animals, the findings suggest a high possibility of transmission of resistant microorganisms from animals to humans and vice versa. This reflects that there needs to be a strict medicine use regulatory activity and improved food hygiene and sanitation management practices along the ASFs and feed supply chains.

Acknowledgments

The authors would like to thank the Ethiopian Agriculture Authority (EAA), Microbiology Laboratory of Animal Products, and Veterinary Drug and Feed Quality Assessment Centre staff who contributed to the sample collection and analysis. We are grateful for the input of scholars from Jimma University, Ethiopia, and the Food and Agriculture Organization of the United Nation (FAO), Ethiopia. Ethiopia Agriculture Authority (EAA) and Ministry of Agriculture (MoA) financially supported the AMR surveillance activities and FAO in Ethiopia provided capacity-building training on AST and AMR surveillance and provided consumables.

References

Abdi, R.D., Mengstie, F., Beyi, A.F., Beyene, T., Waktole, H., Mammo, B., Ayana, D. and Abunna, F., 2017. Determination of the sources and antimicrobial resistance patterns of Salmonella isolated from the poultry industry in Southern Ethiopia. *BMC Infect Dis*., 17 (1), 1-2.

Abegaz, G.A., Hassen, I.W. and Minten, B., 2018. Consumption of animal-source foods in Ethiopia: Patterns, changes, and determinants. Strategy support program (ESSP) Working Paper 113. Washington, D.C. and Addis Ababa, Ethiopia: International food policy research institute (IFPRI) and Ethiopian development research institute (EDRI). http://ebrary.ifpri.org/cdm/ref/collection/p15738coll2/id/132252

Alemu, A., Atnafu, A., Addis, Z., Shiferaw, Y., Teklu, T., Mathewos, B., Birhan, W., Gebretsadik, S. and Gelaw, B., 2011. Soil-transmitted helminths and *Schistosoma mansoni* infections among school children in Zarima town, northwest Ethiopia. *BMC Infect Dis*., 11(1), 1-7.

Ampaire, L., Muhindo, A., Orikiriza, P., Mwanga Amumpaire, J., Boum, Y. and Bebell, L., 2016. A review of antimicrobial resistance in East Africa. *Afric. J. Lab. Med*., 5 (1), 1-6.

BioMérieux, 2011. BioMérieux university Vitek 2 compact industry user quick guide 2011/03/17 Page 1-15. http://www.biomerieux.com/.

BioMérieux, 2020a. Vitek 2 instrument user manual, 2020. http://www.biomerieux.com/.

BioMérieux , 2020b. VITEK 2 software user manual, 2020. http://www.biomerieux.com/.

BioMérieux, 2020c. DensiCheck plus instrument user manual, 2020. http://www.biomerieux.com/.

CLSI, 2017. Clinical and Laboratory Standard Institute. Performance standards for antimicrobial susceptibility testing: Tween-seventh informational supplement M100-S27. CLSI, Wayne, PA, USA, 2017. https://clsi.org/standards/products/microbiology/documents/

Dahlin, L., 2020. Occurrence of antibiotic-resistant *Staphylococcus aureus* in pigs in smallholder farms in Lira, Uganda. Second cycle, A2E. Uppsala: SLU, Dept. of Clinical Sciences. http://urn.kb.se/resolve?urn=urn:nbn:se:slu:epsilon-s-16337

Ejo, M., Garedew, L., Alebachew, Z. and Worku, W., 2016. Prevalence and antimicrobial resistance of *Salmonella* isolated from animal-origin food items in Gondar, Ethiopia. *BioMed. Res. Int.,* 2016 (8). https://doi.org/10.1155/2016/4290506

ESA, 2012. ES ISO 16654-2012. Microbiology of food and animal feed stuffs-Horizontal method for detection of *Escherichia coli* O157. Identical with ISO 16654-2001.

ESA, 2016. ES-ISO 17728:2016. Sampling techniques of food and feed samples.

EFSA, 2010. Standard sample description for food and feed. *EFSA J*., 8(1), 1457.

Ferri, M., Ranucci, E., Romagnoli, P. and Giaccone, V., 2017. Antimicrobial resistance: A global emerging threat to public health systems. *Crit. Rev. Food Sci. Nutr.,* 57 (13), 2857-76.

Ghanbarpour, R., Aflatoonian, M.R., Askari, A., Abiri, Z., Naderi, Z., Bagheri, M.,*et al.,* 2020. Domestic and game pigeons as reservoirs for *Escherichia coli* harbouring antimicrobial resistance genes. *J. Glob. Antimicrob. Res.,* 2020(22), 571-577.

ISO, 2002. Microbiology of food and animal feeding stuff-Horizontal method for the detection of *Salmonella* spp**.** ISO 6579:2002(E) 4th ed. Multiple distributed through the American national standards institute (ANSI) (August 23, 2007).

Mache, A., 2002. Salmonella serogroups and their antibiotic resistance patterns isolated from diarrhoeal stools of pediatric out-patients in Jimma Hospital and Jimma Health Center, South West Ethiopia. *Ethiop. J. Hlth. Sci*.,12 (1), 37-46.

Martinez, J.L., 2009. Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environment. Pol*., 157 (11), 2893-902.

Matewos, K. K., 2020. The prevalence, distribution of staphylococcus, and its antimicrobial susceptibility status of the isolates from meat and dairy milk of Bishoftu, Ethiopia. *Int. J. Adv. Res. Biol. Sci.,* 7(3), 152-167. DOI: http://dx.doi.org/10.22192/ijarbs.2020.07.03.018

Mohamed, F.A., Ramli, A.N. and Ahmad, N., 2020. Survival study and haemolysin activity of *Escherichia coli* in raw and pasteurized milk produced in Negeri Sembilan: In Production Stage. *J. Acad*., 8(1), 66-77.

Molins, R.A., Motarjemi, Y. and Käferstein, F.K., 2001. Irradiation: a critical control point in ensuring the microbiological safety of raw foods. *Food Control*., 12(6), 347-356.

Mulu, W., Abera, B., Yimer, M., Hailu, T., Ayele, H., and Abate, D., 2017. Bacterial agents and antibiotic resistance profiles of infections from different sites that occurred among patients at Debre Markos Referral Hospital, Ethiopia: a cross-sectional study. *BMC Res. Note.* 10 (1), 1-9.

Mwanyika, G., Call, D.R., Rugumisa, B., Luanda, C., Murutu, R., Subbiah, M. and Buza, J., 2016 Load and prevalence of antimicrobial-resistant *Escherichia coli* from fresh goat meat in Arusha, Tanzania. *J. food. Protect.,* 79(9), 1635-41.

OIE, 2015. OIE List of Antimicrobial Agents of veterinary importance. Criteria used for categorization.*https://www.oie.int/app/uploads/2021/03/eng-oie-list-antimicrobials-may2015.pdf*

Okorie-Kanu, O.J., Ezenduka, E.V., Okorie-Kanu ,C.O., Ugwu, L.C. and Nnamani, U.J., 2016. Occurrence and antimicrobial resistance of pathogenic *Escherichia coli* and *Salmonella* Spp in retail raw table eggs sold for human consumption in Enugu state, Nigeria. *Vet. World.*, 9(11), 1312

Pincus, D.H., 2006. Microbial identification using the bioMérieux Vitek® 2 system. Encyclopedia of Rapid Microbiological Methods. Bethesda, MD: Parenteral Drug Association. 2006:1-32.

Reta, M.A., Bereda, T.W. and Alemu, A.N., 2016. Bacterial contamination of raw cow's milk consumed at Jigjiga City of Somali Regional State, Eastern Ethiopia. *Int. J. Food Cont*., 3(1), 1-9.

Roberts, D. and Greenwood, M., 2008. Practical food microbiology.3rd edition. *Blackwell Science Ltd*, Oxford, UK.

Sandel, M. K., and McKillip, J.L., 2004. Virulence and recovery of *Staphylococcus aureus* relevant to the food industry using improvements on traditional approaches. *Food Control*, 15(1), 5-10.

Solomon, F.B., Wadilo, F.W., Arota, A.A. and Abraham, Y.L., 2017. Antibiotic-resistant airborne bacteria and their multidrug resistance pattern at University teaching referral Hospital in South Ethiopia. *Ann. Clin. Microbiol. Antimicrob.,* 16 (1), 1-7.

Tang, K.L., Caffrey, N.P., Nóbrega, D.B., Cork, S.C., Ronksley, P.E., Barkema, H.W., *et al.,* 2019. Comparison of different approaches to antibiotic restriction in food-producing animals: stratified results from a systematic review and meta-analysis. *BMJ Glob Hlth*., 4(4), e001710.

Van, T.T., Yidana, Z., Smooker, P.M. and Coloe, P.J., 2020. Antibiotic use in food animals worldwide, with a focus on Africa: Pluses and minuses. *J. Glob. Antimicrob. Res*., 1(20), 170-7.

Vanni ,M., Meucci ,V., Tognetti, R., Cagnardi, P., Montesissa, C., Piccirillo, A., Rossi, AM., Di Bello, D. and Intorre, L., 2014. Fluoroquinolone resistance and molecular characterization of gyrA and parC quinolone resistance-determining regions in Escherichia coli isolated from poultry. *Poult. Sci.,* 93(4), 856-63.

Von Wintersdorff, C.J., Penders, J., Van Niekerk, J.M., Mills ,N.D., Majumder, S., Van Alphen, *et al.,* 2016. Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Front. Microbiol.,* 2016 (7),173.

Waseem, H., Ali, J., Sarwar, F., Khan, A., Rehman, H.S., Choudri, M., *et al.,* 2019. Assessment of knowledge and attitude trends towards antimicrobial resistance (AMR) among the community members, pharmacists/pharmacy owners, and physicians in district Sialkot, Pakistan. *Antimicrobial. Resist. Infect. Control.* 8 (1), 1-7.

WHO, 2015. Report of the 6th meeting of the WHO advisory group on integrated surveillance of antimicrobial resistance with AGISAR 5-year strategic framework to support implementation of the global action plan on antimicrobial resistance (2015-2019), 10-12 June 2015, Seoul, Republic of Korea.

Prevalence and economic connotation of bovine and caprine hydatidosis at Abergele International Export Slaughterouse, Mekele, Tigray Region

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Abstract

A cross-sectional study was conducted from November 2017 to April 2018 to investigate the prevalence and economic significance of bovine and caprine hydatidosis at Abergelle international export slaughterhouse, Mekele, Tigray region. This study has been carried out based on antemortem and postmortem examinations. Among the 940 cattle and goats examined, 104 (11.06%) were found to be positive for hydatid cyst. Whereas, from 520 slaughtered cattle and 420 goats, 86 (16.54%) and18 (4.29%) were positive for hydatid cyst, respectively. There was a statistically significant difference ($p<0.05$) in the prevalence of hydatidosis in cattle with different body condition scores (55.34% in poor and 6.25% in good body condition). The same holds true for goats (14.28% in poor body condition and 0.97% in good body condition score). But the variation was not significant concerning the age and origin of the animals. From examined organs, 55 (10.57%) of the lung, and 31 (5.96%) of the liver of cattle were positive. However, in goats, 13 (3.09%) lungs and 5 (1.19%) livers were positive for the cyst. The cyst viability and fertility test indicated that 28 (32.56%) cysts were fertile in cattle while 53 (61.28%) were infertile and 5 (5.81%) were calcified. Of these 28 fertile cysts in cattle, 8 (28.57%) were viable and 20 (71.43%) were non-viable. In goats, 13 (72.2%) were fertile, 4 (22.2%) were infertile and 1 (5.6%) was calcified. Of these 13 fertile cysts in goats, 8(61.54%) were viable and 5(38.5%) were non-viable. The annual economic loss estimated for bovine and caprine hydatidosis was 2,101,540.2 and 65,897.9 ETB, respectively. The annual financial loss recorded altogether by hydatidosis of cattle and goats at Abergelle International Export Slaughter House was estimated to be 2,167,438.1 ETB. In conclusion, hydatidosis is a major cause of organ and carcass condemnation and financial loss at the Abergelle export slaughterhouse. The prevalence of hydatidosis was high in cattle compared to goats and in both

species; the lung was the most frequently affected organ by hydatid cyst followed by the liver.

Keywords: Bovine; Caprine; Economy; Hydatidosis; Mekele; Prevalence.

Introduction

Livestock production plays an important role in Ethiopia's economy. Ethiopia has the largest livestock population in Africa, having an estimated number of over 70 million heads of cattle, 42 million sheep, 52 million goats, 8 million camels, and 56 million, poultry (CSA, 2021). However the contribution of this huge livestock resource to the national income is not adequate and diseases are among the major factors responsible for poor production, productivity, and public health concerns (FAO, 1994; Eddi *et al*., 2004; Shapiro *et al*., 2017).

Hydatidosis is among the important diseases that cause organ condemnation leading to huge economic loss and public health problems in Ethiopia (Jobre *et al*., 1996; Eddi *et al*., 2004; Bekele and Butako, 2011). Hydatidosis is a term used to describe infection of animals and humans with larval (metacestode) stage of echinococcus species which are found in the small intestine of dogs and other carnivores (Parija, 2013). This parasite is found worldwide and causes economic losses from the condemnation of affected organs and serious public health problems in certain parts of the world (Schantz, 1990; Eddi *et al*., 2004).

Echinococcosis is a cyclozoonosis that requires two vertebrate hosts to uphold the life cycle (Eckert *et al*., 2002; Taylor *et al*., 2007). Humans can accidentally become intermediate hosts by ingesting the eggs of the tapeworm. While most cysts develop in the liver and lungs, other organs and tissue may become affected. *Echinococcus granulosus* is the species most widely distributed throughout the world and the primary concern in ruminants, including sheep and goats. *E. granulosus* has little host specificity concerning intermediate hosts. Hydatid cysts have been seen in a wide range of mammals including domestic ruminants, camels, giraffes, pigs, equines, elephants, marsupials, hippopotamuses, and different types of deer as well as humans (Raush, 1986; Eddi *et al*., 2004).

All disease-causing species of *Echinococcus* are transmitted to intermediate hosts via the ingestion of eggs and are transmitted to definitive hosts through eating infected, cyst-containing organs. Humans are accidental intermediate hosts that become infected by handling soil, dirt, or animal hair that contains eggs (Eckert *et al*., 2001).

Ethiopia has been well-known for its high prevalence of hydatid disease since the 1970s. Moreover, reports of findings from abattoirs in various locations revealed that hydatidosis is widespread in Ethiopia with great economic and public health significance (Kebede *et al.*, 2009; Bekele, 2010; Daniele *et al*., 2012., Getachew *et al*., 2012a, b). However, there were few studies on the prevalence of hydatidosis and its economic losses associated with organ condemnation in Abergelle export slaughterhouse (Gebremeskel and Kalayou, 2009; Teka *et al*., 2017). It would also be indispensable to update on the current status of hydatidosis to forward relevant suggestions on its control. Therefore, the objectives of the present study were to estimate the prevalence of bovine and caprine hydatidosis at Abergelle International export slaughterhouse and to estimate the economic losses of hydatidosis due to organ condemnation and carcass weight loss as well as to estimate cyst viability and fertility.

Materials and methods

The study area (abattoir)

The study was conducted at Abergelle International export slaughterhouse, Mekelle. The abattoir has the capacity of slaughtering 300 cattle and 1000 sheep and goats daily. But these days they have stopped exporting animal products for a while and supply the meat requirement of the people which were found in and around Mekelle. The livestock population of the region is 3,103,468 cattle; 1,376,961 sheep; 3,107,994 goats; 5,427 horses; 463,492 donkeys; 7,694 mules; 32,552 camels; 3,829,788 poultry and 255,607 beehives (CSA, 2021). Tigray has a subtropical climate and is among the driest regions of Ethiopia, receiving an average of 590 mm rainfall annually. While some precipitation occurs from February to May in the Belg season, most rain falls during the Kremti season (June-September), which is followed by the Bega dry season (October-January). Generally, the climate of the study area conforms to that of the Ethiopian highlands (TRADB, 2017).

Study animals

Study animals were indigenous East African zebu cattle and goats that were brought to be slaughtered at Abergelle International export slaughterhouse and kept under traditional extensive husbandry systems. They were transported to the abattoir using vehicles and on foot. All animals brought to the abattoir were male. The slaughtered animals were mainly from Haramaya and its surrounding districts and kept under traditional extensive husbandry systems with communal grazing practice.

Study design

A cross-sectional epidemiological study was conducted from November 2017 to April 2018. Three days regular visit a week was carried to the abattoir. Goats and cattle that have been slaughtered were selected randomly and examined for the presence of a hydatid cyst.

Data collection

Sample size and sampling procedure

A stratified random sampling method was employed to select study animals. This is a method of sampling that involves dividing a population into smaller groups–called strata. The groups or strata are organized based on the shared characteristics or attributes of the members in the group such as origin, age, and body condition. Because of variations in the reports by different authors including Getachew *et al.* (2012b), who reported 60% and 36% for lung and liver localizations respectively in Mojo Luna export abattoir and 17.55% reported prevalence in Mekele municipal abattoir by Gebremeskel and Kalayou (2009), the sample size was determined according to Thrusfield (2007) based on the expected prevalence of 50%, the 5% desired absolute precision (d=0.05) and 95% confidence interval (95% CI).

$$n = \frac{1.96^2 \, Pexp \, (1-Pexp)}{d^2}$$

$$n = \frac{1.96^2 * 0.5(1-0.5)}{0.05^2}$$

$$n = 384$$

Where:
n= is the required sample size
P = is expected prevalence
d= is the level of precision (5%).

Accordingly, the sample size was calculated as 384 for each species; however, to increase the precision, the sample size was made to 420 goats and 540 cattle.

Antemortem examination

During the antemortem examination the age, sex, origin, and body condition score (BCS) of the animals identified for post-mortem examination were recorded. The age was determined by the dentition formula according to the method described by Sanchez-Andrade *et al.* (2002), and two age groups were considered; less or equal to 2 years and above 2 years old. The body condition scoring was done according to Nicholson and Butteworth (1986) and classified into three categories: poor, medium, and good.-

Post mortem examination

Postmortem examination was carried out by visual inspection, palpation, and systematic incision of each visceral organ particularly the lung and liver according to procedures recommended by FAO (1994). The cysts collected from different organs were taken to the laboratory to conduct fertility and viability tests. All organs harboring hydatid cysts were partially or totally condemned and were judged according to guidelines on meat inspection for developing countries (Herenda, *et al.*, 1994).

After the postmortem results, cysts were collected from the infected organs, and cyst harbored by a particular organ was counted. Individual cysts were grossly examined for evidence of degeneration or calcification and were transported to Mekelle University Veterinary Laboratory by an ice box for fertility and viability tests. The content of the fluid was aspirated using an 18G needle and 20ml syringe into a sterile cylinder container to reduce pressure and risk of spilling over to the eye. After being punctured, the pressure was reduced and the cysts were incised using a scalpel blade and the whole content was transferred into a clean Petri dish and examined under a microscope of 40X magnification for the presence of protoscolices in the cyst. If the protoscolices are present as a white dot on the germinal epithelium or brood capsule or hydatid sand within the suspension, and the cysts were categorized as fertile then the fertile cysts were further subjected to a viability test.

The viability of the protoscolices was assessed by staining with 0.1% aqueous eosin solution and examined under a light microscope. Live protoscolices were not able to take up the dye whereas the dead ones were dyed (Moazeni and Nazer, 2010; Anne and Gary, 2012).

Cysts with no protoscolices were classified as infertile cysts (Daryani, *et al.,* 2007). Moreover, the infertile cysts were also classified as sterile or calcified. Sterile cysts are characterized by their smooth inner lining usually with slight turbid fluid in its content. Calcified cysts were having a gritty sound feeling upon incision (Taylor *et al.*, 2007).

Estimation of economic loss

Direct and indirect annual economic loss

The estimated direct annual economic loss due to hydatidosis in cattle is computed based on the measurement of the following parameters: level of organs (lungs, liver, spleen, kidney, and heart) condemnation, average local retail prices of healthy organs, and estimation of mean annual slaughter rate of cattle. Estimation of the indirect loss due to the decrease in carcass weight was made based on the reduction of 5% in meat production as previously established by Polydorous (1981). The average carcass weight of Ethiopian local breed cattle is estimated as 126 kg (WHO, 1983). The total economic loss was calculated as the summation of the cost of offal condemned plus the cost of carcass weight loss (ILCA, 1993).

- Annual cost of offal condemned = (CSR *PHLu*CPLu) + (CSR *PHLi * CPLi) + (CSR * PHHe * CPHe) + (CSR * PHKi * CPKi) + (CSR * PHSp * CPSp)
- Annual cost of carcass weight losses = 5% * CSR * PH* CPB *ACW

Where:

CSR= Average number of cattle and goats slaughtered per year =8,650 caprine and 12,312 bovine

PHLu = Percentage of lung condemned =10.57%

CPLu = Mean cost of one bovine and caprine lung in Mekelle = 24 birr for bovine and 35 birr for caprine

PHLi = Percentage of liver condemned = 5.96%

CPLi = Mean cost of one bovine and caprine liver in Mekelle =24 for bovine and 35 for caprine PHHe = Percentage of heart condemned = 0; CPHe = Mean cost of one bovine heart in Mekelle = 30Birr; PHKi = Percentage of kidney condemned = 0, CPHKi = Mean cost of one bovine kidney in Mekelle = 10birr; PHSp = Percentage of spleen condemned = 0%, CPSp = Mean cost of one bovine spleen in Mekele = 10Birr;

5% = Carcass weight losses in the individual animal due to hydatidosis
PH = Prevalence of hydatidosis in Abergelle abattoir = 4.29% for caprine and 16.54% for bovine
CPB = Average market price of one 1kg of beef and caprine in Mekele = 160 for bovine and 190 birrs for caprine.
ACW = average carcass weight of Ethiopia cattle = 126 kg and goat 15Kg (ILCA , 1979; WHO, 1983).

Data management analysis

The data obtained from antemortem and postmortem findings and characterization of cysts were coded and uploaded into the Microsoft excel 2016 spreadsheet computer program and analyzed using the Stata version 14 to compare the infection status with regard to the hypothesized risk factors like origin, age, body condition score, and cyst characteristics like cyst size (small, medium and large), cyst type (fertile, sterile, degenerated or calcified) and protoscolices viability (viable and nonviable). The prevalence of hydatidosis was calculated as the number of infected individuals divided by the number of individuals sampled x 100. The association of these variables with the prevalence of hydatidosis was assessed by logistic regression. A p-value less than 0.05 was considered significant.

Results

Overall prevalence

Of the total 940 slaughtered cattle and goats, the overall prevalence of hydatidosis was 11.06 % (104/940). The prevalence was 16.53% (86/500) in cattle and 4.29% (18/420) in goats (Table 1).

Table1: Overall prevalence of hydatidosis in Abergelle export slaughterhouse

No. of examined animal	No. of the infected animal	Prevalence (%)	X2	P- value
Bovine	86	16.54	28.47	0.054
Caprine	18	4.29		
Total	104	11.06		

Higher prevalence was found in cattle with poor body condition (55.34%) than in those with good body condition score (6.25%) and the difference was statistically significant ($p<0.05$). However, the prevalence of hydatidosis with age groups and origin of cattle showed no significant difference ($p>0.05$) (Table 2).

Table 2: Prevalence of bovine hydatidosis in relation to age, origin, and body condition score

Variables		No. of examined	No. of positive	Prevalence (%)	X^2	P- value
Origin	Mekelle	172	18	10.46	1.81	0.177
	Shire	159	32	20.12		
	Alamata	110	26	23.64		
	Abergelle	79	10	12.66		
Age	Young	109	18	16.51	0.00	0.994
	Adult	411	68	16.55		
Body condition	Poor	103	57	55.34	68.47	≤0.001
	Medium	161	24	14.91		
	Good	256	16	6.25		
	Total	520	86	16.53		

In both cattle and goats examined for the presence of cysts in different organs, the lung and the liver were the only infected organs. However, the recorded prevalence was significantly higher in the lungs (10.57%) than in the livers (5.96%) of infected cattle. On the other hand, a lower prevalence of 3.09% in lungs and 1.19% in livers was reported in goats (Table 3).

The fertility test indicated that 28 (32.56%) of the cysts in cattle were fertile, 53(61.28%) were infertile, and 5 (5.81%) were calcified cysts in the lung and liver. And from fertile cysts in cattle, 8 (28.57%) were viable and 20 (71.43%) were non-viable (Table 3).

Table 3: Classification of bovine hydatid cysts according to their fertility and viability status

Organ	No .examined	No. of cyst	% Prevalence	Fertile (%)	Infertile (%)	Calcified (%)	Viable (%)	Non-viable (%)
Lung	520	55	10.57	18(32.73)	35(63.63)	2(3.64)	5(27.8)	13 (72.2)
Liver	520	31	5.96	10(32.6)	18(58.06)	3(9.68)	3(30)	7(70)
Total	520	86	16.15	28 (32.56)	53(61.28)	5 (5.81)	8 (28.57)	20 (71.43)

Analysis of caprine hydatidosis showed no significant association ($p>0.05$) among age, body condition, and origin (Table 4).

Table 4: Prevalence of caprine hydatidosis in relation to body condition, age group, and origin

Variables		**No. of examined**	**No. of positive**	**Prevalence (%)**	**X2**	**p-value**
Body condition	Poor	84	12	14.28	21.32	≤0.001
	Medium	130	130	4	3.07	
	Good	206	206	2	0.97	
Origin	Mekelle	104	2	1.92	1.45	0.231
	Shirea	96	4	4.16		
	Alamata	75	5	6.67		
	Abergelle	63	2	3.17		
	Bati	82	5	6.09		
	Total	420	18	4.28		
Age	Young	111	4	3.6	2.83	0.084
	Adult	309	14	4.5		
	Total	420	18	4.28		

Of the total 420 caprines examined for hydatid cyst, 18(4.3%) were fertile, 4(22.2%) were infertile and 1(5.6%) were calcified. And from the fertile cysts, 8(61.54%), were viable and 5(38.5%) were non-viable (5.6%) (Table 5).

Table 5: Classification of cysts according to their fertility and viability status for caprine

Organ examined	No. examined	% Prevalence	No. of cyst (%)	Fertile	Non-fertile (%)	Calcified (%)	Viable (%)	Non-viable (%)
Lung	420	3.1	13 (3.1)	10 (76.9)	3 (23.1)	0.0	6 (60.0)	4 (40.0)
Liver	420	1.2	5 (1.2)	3 (23.1)	1 (20.0)	1 (5.6)	2 (66.7)	1 (33.3)
Total	420	4.3	18 (1.2)	13	4	1	8	5

The financial loss of hydatidosis

The financial loss of bovine and ovine hydatidosis was estimated by taking into account the market prices of the average condemned organs at the time of the study and the overall number of animals slaughtered per year in the abattoir and it was found to be about 48,844 ETB.

Table 6: Financial loss hydatidosis for cattle and goats

Species	Organ examined	Average number animal slaughtered per year	Percent of condemned organs	Price of an organ in ETB	Condemned loss in ETB/ year
Cattle	Lung	12,312	10.57	24	31,233.1
	Liver	12,312	5.96	24	17,6115.1
	Total	12,312	16.54		48,844.2
Goats	Lung	8,650	3.1%	35	9,385.3
	Liver	8,650	1.2%	35	3,633
	Total	8650	4.29%		13,018.3

The overall economic loss due to organ condemnation and carcass weight loss was found to be 2,167,438.1 ETB/ year. This includes the combined loss due to organ condemned and carcass weight loss recorded for cattle (2,101,540.20= 48,844.2+2,052,696 ETB) and goat (65,897.9 =13,018.3+52, 879.6) hydatidosis at Abergelle abattoir, respectively.

Discussion

The study revealed the presence of bovine and caprine hydatidosis in different parts of Tigray. As an important disease for livestock and public health, detailed information on local epidemiology and the significance of hydatidosis

must be revealed. In this regard, the overall prevalence of hydatidosis for both cattle and goats was 11.06%. In the current study, the prevalence of hydatidosis in cattle was found to be 16.54% while in goats this was only 4.29%.

Meanwhile, the prevalence of bovine hydatidosis (16.54%) recorded in this study was comparable with previous studies conducted in Mekele (17.5%), Wolayita Soddo (16.85%), Gondar (17.3%), Addis Ababa (19.7%) and Wollo by Gebremeskel and Kalayau, (2009), Bekelle and Butako, (2011), Genet *et al.* (2012), Zelalam *et al.* (2012), and Alemu *et al* (2013), respectively. However, it was higher than the prevalence report of 7.5% revealed in the Shire abattoir (Kebede *et al*., 2009), and 6.8% in the Modjo Luna export abattoir (Daniel *et al*., 2012), and 11.26% in Mizan Teppi (Jemere *et al*., 2013). Similarly, it was higher in contrast to the reports of other authors elsewhere in different parts of the world like the reports of 2.1% from Zambia (Fredrick *et al*., 2012), 6.99% from Iran (Ahmadi and Meshkehkar, 2011), 2.8% from Sudan (Sahar Adam and Atif Elamin, 2011), 10.6% from Morocco (Azlaf and Dakkak, 2006) and Western Iran 16.4% (Hafez and Yamen, 2002).

The present result was relatively lower than the previous reports researched in different parts of the country through different periods by several authors. Accordingly, the reported prevalence indicated 34.05% in Bahr Dar abattoir (Kebebe *et al*., 2009), 19.9% in Addis Ababa abattoir (Kebebe *et al*., 2010a), 22.1% in Tigray (Kebebe *et al*., 2010b), 52.69% in Hawassa (Regassa *et al*., 2010), 46.5% in Debre Zeit, and 25.7% in South Omo (Jobre *et al*., 1996).

The present study has clearly shown the relatively similar prevalence of caprine hydatidosis in many parts of Africa and Asia. Accordingly, the prevalence of 6.56% from Saudi Arabia (Ismail *et al*., 2011), 7.1% from Ethiopia (Getaw *et al*., 2010), and 4.5% from Kenya (Njoroge *et al*., 2002) were reported. Likewise, for caprine hydatidosis, the 4.29% prevalence of the present study was relatively comparable with a report of 8.6% from Addis Ababa abattoir (Yitbarek *et al*., 2012) and 6.13% in Luna export abattoir, central Ethiopia (Getachew *et al*., 2012b).

These variations in the infection rates observed in cattle and goats from different parts of the country could be due to the variations in the climate and management practices of the animals. It could also be associated with different

factors like control measures put in place, the level of community awareness created about the disease, education, and the economic status of the population and the farming community. Factors such as differences in culture, social activity, animal husbandry systems, lack of proper removal of the infectious carcass, and attitude to dogs in different regions might have contributed to the variation in prevalence in different areas of a country (Arbabi and Hooshyr, 2006) and strain differences of *E. granulosus* that exists in different geographical location (McManus, 2006).

The significant difference between the rate of occurrence of hydatidosis and body condition of bovine and caprine might be due to the impact of *Echinococcus granulosus* larvae while it was alive. Cattle having poor and medium body conditions were found to have higher cysts burden which may be explained due to the retarded growth, weight loss, and moderate to severe infection in such animals as described by Polydorous (1981).

The majority of the cattle slaughtered in this abattoir were adults older than two years. Hence they were exposed to *E. granulosus* over a long period, with an increased possibility of acquiring the infection. Previous studies strongly suggested that the prevalence of bovine hydatidosis is profoundly influenced by age of the animal (Lahmar *et al*., 2001; Torgerson *et al*., 2005). Therefore, since the growth of the hydatid is gradual and maturity is reached in 6–12 months, cattle above the age of two have a higher risk of contracting *E. granulosus* (Urquhart *et al*., 1996).

Lack of variability concerning origin might be due to cattle movement between these topographical locations for the market, pasture, and exchange of animals for different purposes and also similarity in environmental factors, religious factors, and animal husbandry practices of the community of animal origin.

The current study indicated a high number of occurrence of hydatid cysts most predominantly in the lung of bovine (10.57%) than in caprine (3.09%) while in the liver this was only 5.96% and 1.19% in cattle and goats, respectively. Literature reveals that hydatid cysts are most commonly found in the lungs and liver of ungulates (Hubbert *et al*., 1975) and it is in agreement with the findings of Bekele and Butako (2011), Njoroge *et al*. (2002), and Eckert and Deplazes (2004), who showed that the lung and liver are the most common sites of hydatid cyst in domestic animals. This is explained by the fact that the lung

and liver possess the first great capillary sites encountered by the migrating *Echinococcus* oncospheres (hexacanth embryo) which adopt the portal route and primary negotiate hepatic and pulmonary filtering system sequentially before any other peripheral organs are involved (Urquhart *et al.,* 1996).

The higher prevalence in lungs is associated with the fact that cattle are slaughtered at an older age. During this period the capillaries of the liver are dilated and most cysts passed to the lungs. Besides this, the hexacanth embryo can enter the lymphatic circulation and be carried via the thoracic duct to the heart and lung in such case the lung will be infected before the liver. These were agreed with previous research (Urguhart *et al*., 1988; Getaw *et al*., 2010; Ibrahim, 2010). This finding is in agreement with the results of previous reports from different areas (Jobre *et al*., 1996; Kebede and Yamen 2002; Islam *et al*., 2003; Kebede *et al*., 2008; Kebede *et al*., 2009a).

Most of the hydatid cysts from cattle are considered to be sterile (Thompson *et al*., 1984). The fertility of hydatid cysts in the intermediate hosts is genotype-dependent. Cattle infected with G5 (genotype 5) had more than 90% fertile cysts. In contrast, cysts from cattle infected with G1, and G3 genotypes of *E. granulosus* were all sterile (Rinaldi *et al*., 2008). No data is available about the types of strains in the country so far. Information about strain differentiation is only available from Kenya and Sudan in the eastern part of Africa (Magambo, *et al*., 2006).

In the present study, a total annual economic loss of 65,897.9 ETB from organ rejection at postmortem (13,018.3 ETB) as well as the indirect loss as a result of the reduction in carcass weight (52, 879.6 ETB) due to caprine hydatidosis was reported the aggregated prevalence rate of cystic echinococcosis and the current retail market prices. The annual financial loss of 2,101,540.20 ETB, in our study due to bovine hydatidosis from offal condemnation (48,844.2 ETB) and carcass weight loss (2,052,696 ETB) was greater than the findings of Yilma (1984), 813,526.46 and Kebede *et al*. (2009a), 25,608.00 ETB from Debre Zeit abattoir, Hararge zone, and Tigray region and Regassa *et al*. (2010), 1791625.89 ETB from Hawassa municipal abattoir. And 51,883 USD by Kebede *et al*. (2009b) in cattle slaughtered at Debra Markos abattoir.
According to some authors (Alula, 2010; Kebede *et al*., 2009b; Regassa *et al*. 2010), the financial loss varied from region to region and even from abattoir to

abattoir based on the variation of prevalence of hydatidosis, the mean annual number of animals slaughtered at different abattoirs, and the retail market price of organs of cattle and goat hydatidosis at Abergelle abattoir.

Conclusions

Hydatidosis causes a considerable loss to the livestock industry. Hydatidosis is a major cause of organ and carcass condemnation and financial loss at the Abergelle export slaughterhouse. The prevalence of hydatidosis was high in cattle compared to goats and in both species, the lung was the most frequently affected organ by hydatid cyst followed by the liver. Though a large number of cysts in cattle was sterile, the presence of a higher proportion of viable cysts among the fertile cysts indicates the two species of animals are still important intermediate hosts in the region.

References

Ahmadi, N. and Meshkehkar M., 2011. An abattoir-based study on the prevalence and economic losses due to cystic echinococcosis in slaughtered herbivores in Ahwaz, southwestern Iran. *J. Helminthol.*, 85(1), 33-39.

Alemu, B., Nigatu, K., Tariku, T., Getachew, T. and Tesfu, K., 2013. Occurrences and financial significance of bovine cystic echinococcosis in Southern Wollo, Northeastern Ethiopia. *J. Vet. Med. Anim. Health,* 5(2), 51-56.

Alula, A., 2010. Major metacestodes in cattle slaughtered at Kombolcha Elfora abattoir, northeastern Ethiopia: Prevalence, cyst viability, organ distribution, and socio-economic implications. *Trop. Anim. Health. Prod.*, 17, 3-5.

Arbabi, M. and Hooshyr, H, 2006. Survey of echinococcosis and hydatidosis in Kashan region central Iran. *Iranian J. Public Health.*, 35(1),75-81.

Azlaf, R. and Dakkak, A., 2006. Epidemiological study of cystic echinococcosis in Morocco. *Vet. Parasitol.*, 137, 83-93.

Bekelle, J. and Butako B., 2011. Occurrence and financial loss assessment of echinococcosis in cattle slaughtered at Wolayita Soddo municipal abattoir, Southern Ethiopia. *Trop. Anim. Health Prod.*, 43, 221-228

Center for Disease Control, CDC., 2009. Parasites and Health: Echinococcosis. DPDx. CDC, https://www.cdc.gov/parasites/echinococcosis/index.html.

Central Statistical Agency, CSA, 2021. Agricultural sample survey. Volume II. Report on livestock and livestock characteristics (private peasant holdings). Statistical bulletin 3, Addis Ababa, Ethiopia. Pp. 188.

Daniel, G., Gizat A. and Getachew, T., 2012. Occurrence and fertility rates of hydatid cysts in sheep and goats slaughtered at Modjo Luna export slaughterhouse, Ethiopia. *Ethiop. Vet. J.,* 16(1), 83-91.

Daryani, A., Alaei, R., Arab, R., Sharif, M., Dehghan, M.H., and Ziae, H. 2007. The prevalence intensity and viability of hydatid cysts in slaughtered animals in the Arable province of North West Iran, *J. Helminthol*., 8, 13-17.

Eckert, J. and Deplazes, P., 2004. Biological, epidemiological, and clinical aspects of echinococcosis: a zoonosis of increasing concern. *Clin. Micro. Rev*., 17(1),107-135.

Eckert, J., Gemmell, M. A., Meslin, F.X. and Pawłowski, Z. S. 2001. WHO/OIE Manual on Echinococcosis in humans and animals: A public health problem of global concern. World Organization for Animal Health (Office International des Epizooties) and World Health Organization. https://apps.who.int/iris/handle/10665/42427

Eddi, C., Balogh, K., De Lubroth, J., Amanfu, W., Speedy, A. and Battaglia D., 2004. Veterinary Public Health activities at FAO: Echinococcosis/hydatid disease. Parassitologia, 46 (4), 381-2.

FAO, 1994. Guidelines for Echinococcosis surveillance, prevention, and control. FAO, Rome, Number, 29, 47.

Fredrick, B., King S., John B., Musso M. and Hetron M., 2013. A cross-sectional study Investigating Cystic hydatidosis in slaughtered cattle of Western Province in Zambia. ISRN *Parasitol.,* 1-9. Volume 2013, Article ID 468163, http://dx.doi.org/10.5402/2013/468163

Gebremeskel, B. and Kialayou, S., 2009. Prevalence, viability, and fertility study of bovine cystic echinococcosis in Mekelle city, Northern Ethiopia. *Rev. Méd. Vét.* 160 (2), 92-97.

Genet, M., Tadesse, G., and Basaznew, B. and Mersha, C., 2012. Pathological conditions causing organ and carcass condemnation and their financial losses in cattle slaughtered in Gondar, northwest Ethiopia. *Afr. J. Bas. Appl. Sci*., 4(6), 200-208.

Getachew, D., Almaw, G. and Terefe, G., 2012a. Occurrence and fertility rates of hydatid cysts in sheep and goats slaughtered at Modjo Luna export slaughterhouse, Ethiopia. *Ethiop. Vet. J*., 16, 83-91

Getachew, H., Guadu, T., Fentahun, T. and Chanie, M. (2012b): Small ruminant hydatidosis: Occurrence and economic importance in Addis Ababa abattoir. *Glob. Vet*., 8, 160-167.

Getaw, A., Beyene, D., Ayana, D., Megersa B. and Abunna, F., 2010. Hydatidosis: Prevalence and its economic importance in ruminants slaughtered at Adama municipal abattoir, Central Oromia, Ethiopia. *Acta Trop.,* 113, 221–225.

Hafez, A. and Yamen, A., 2002. Echinococcus hydatidosis in western Iran. *Vet. Parasitol.*, 30, 161-171.

Herenda, D., Chambers, P.G., Ettriqui, A., Seneviratna, P. and DaSilva, T.J.P., 1994. Manual on meat inspection for developing countries. FAO, Rome Pp. 1-2

Hubbert, W.T., Culloch., W.F and Sclnurredn Beger, A.A., 1975. Disease Transmitted from animal to man. 6th Edition, Choreler C. Thomas Publisher, Spring Filk Illinois.

Ibrahim, M.M., 2010. Study of cystic echinococcosis in slaughtered animals in Al Baha region, Saudi Arabia: Interaction between some biotic and abiotic factors. *Acta Trop.,* 113, 26–33.

ILCA (International Livestock Centre for Africa). 1979. Trypanotolerant livestock in West and Central Africa: Volume 1. General study. ILCA Monograph 2, ILCA, Addis Ababa, Ethiopia. pp 147.

ILCA (International Livestock Centre for Africa). 1993. Handbook of African livestock statistics. ILCA, Addis Ababa, Ethiopia. 66 pp.

Islam, M.K., Basaka, S.C., Majumder, S. and Sarder, S.A., 2003. Cystic echinococcosis in domestic ruminants in Cox's Bazar of Bangladesh. *Pak. J. Sci.*, 46, 251-254.

Jemere, B., Wosenyelesh, K, and Shishun, S. and Desie S., 2013. Prevalence and financial loss estimation of cystic echinococcosis in cattle slaughtered at Mizan Tefri and Teppi municipal abattoirs, Southwestern Ethiopia. *Europ. J. Appl. Sci.*, 5(1),12-18.

Jobre, Y., Labago, F., Tirunhe, R., Abebe G., and Dorchies, P., 1996. Hydatidosis in three selected regions in Ethiopia: an assessment trial on its prevalence, economic and public health importance. *Rev. Med. Vet.*, 147, 797-804.

Kebebe, E., Girma, Z. and Bersissa, K., 2010a. Hydatidosis of sheep and goats slaughtered at Addis Ababa abattoir: prevalence and risk factors. *Trop. Anim. Health Prod.*, 42, 803-805.

Kebede, N., Mitiku A. and Tilahun, G., 2009a. Hydatidosis of slaughtered animals in Bahir Dar abattoir, northwestern Ethiopia. *Trop. Anim. Health Prod.*, 41(1), 43-50.

Kebede W, Hagos A, Girma Z. and Lobago F. 2009a. Echinococcosis/ hydatidosis: its prevalence, economic and public health significance in Tigray region, Northern Ethiopia. *Trop. Anim. Health Prod.*, 41, 865-871.

Kebede, N., Gebre-Egziabher, Z., Tilahun G., and Wossene, A., 2009a. Prevalence and financial effects of hydatidosis in cattle slaughtered in Birre-Sheleko and Dangila abattoirs, northwestern Ethiopia. *Zoonoses Public Health*, 58(1), 41-46.

Lahmar, S., Kilami, M. and Torgerson, P.R., 2001. Frequency distributions of *Echinococcus granulosus* and other helminths in stray dogs in Tunisia. *Ann. Trop. Med. Parasitol.*, 95, 69-76.

Macpherson, C., Zeyhle, E. and Roming, T., 1985. An echinococcosis plot control program for northwest Turkana, Kenya. *Ann. Trop. Med. Parasitol.*, 78, 188-192.

Magambo, J, Njoroge E. and Zeyhle, E., 2006. Epidemiology and control of echinococcosis in sub-Saharan Africa. *Int. J. Parasitol.*, 155, 193-195.

McManus, DP, 2006. Molecular discrimination of taeniid cestodes. *Int. Parasitol.*, 55, S31-37.

Nicholson, M.J. and Butterworth, M.H., 1986. A Guide to Condition Scoring of Zebu Cattle. International Livestock Center (ILCA), Addis Ababa Ethiopia, Pp. 212-235.

Njoroge, E.M., Mbithi, P.M.F., Gathuma, J. M., Wachira, T.M., Gathura, T.M. and Magambo, J.K. 2002. A study of cystic echinococcosis in slaughtered animals in three selected areas of northern Turkana, Kenya. *Vet. Parasitol.*, 194, 85-91.

Parija, S.C. 2004. Textbook of Medical Parasitology, Protozoology, and Helminthology, 4th Edition, Karnataka, India, Pp360.

Polydorous, K., 1981. Animal health and economics case study: Echinococcosis with reference to Cyprus. *Bull. Int. Epizoot.*, 93 (5), 981-992.

Raush, R. L., 1986. Lifecycle patterns and geographic distribution of *Echinococcus* species. In R.C.A. Thompson, edition. The biology of echinococcus and hydatid disease. Pp. 44-80. London, UK.

Regassa, F., Molla, A. and Bekele J., 2010. Study on the prevalence of cystic hydatidosis and its economic significance in cattle slaughtered at Hawassa municipal abattoir. *Trop. Anim. Health Prod.*, 42(5), 977-984.

Rinaldi, E. M., Maurelli, M.P., Veneziano, Capuano, F., Perugini, A. G. and Cringoli, S., 2008. The role of cattle in the epidemiology of *Echinococcus granulosus* in an endemic area of southern Italy. *Parasitol. Res.*, 103, 175-179.

Sahar, A.M, and Atif E.A., 2011. Study on hydatid cyst infection in slaughterhouses in Khartoum state, Sudan. *Arch. Appl. Sci. Res.* 3 (6), 18-23.

Sanchez-Andrade, R., Paz-Silva, A., Sua´ rez, J.L., Panadero, R., Pedreira, J., Lo´ pez. C, Dı´ez-Banos, P. and Morrondo, P., 2002. Influence of age and breed on natural ovine fasciolosis in an endemic area (Galicia, NW Spain). *Vet. Res. Comm.*, 26,361-370

Schantz, P.M., 1990. Parasitic zoonosis in perspective. *Int. J. Parasitol.,* 21(2), 165-166.

Shalaby I. M. I., Banaja, A. A. and Jamoom, M. B., 2011. A comparative study on the prevalence of some parasites in animals slaughtered at New Taif abattoir. *Glob. Vet.*, 295-299.

Taylor, M. A., Coop, R. L. and Wall, R. L. 2007. Veterinary Parasitology, 3rd ed. Blackwell Sciences Limited, UK. Pp. 874.

Teka, G., 2017. Prevalence and economic significance of bovine hydatidosis and cysticercosis in Mekelle Municipality Abattoir, Northern Ethiopia. Open Access *J. Vet. Sci. Res.* ER, Volume - 2, DOI: 10.23880/oajvsr-16000135

Thompson, R.C.A, Kumaratilke L.M. and Eckert, J., 1984. Observations on *Echinococcus granulosus* of cattle origin in Switzerland. *Int. J. Parasitol.,* 14(3), 283-291.

Thrusfield, M., 2007. Veterinary epidemiology 3rd Edition, Blackwell sciences Ltd. United Kingdom, Pp. 626

Torgerson, P.R.B., Ogulzahan, A.E., Muminov, R.R., Karaeva, O.T., Kuttubaev, M., Aminzanov and Shakenov, B.S., 2005. Present situation of cystic echinococcosis in Central Asia. *Parasitol. Int*., 55, 207-212.

TRADB, 2017. Tigray Region, Agricultural Development Bureau. Agricultural Activities Report.

Urquhart, G., Armour J., Duncan, L., Dunn, A. and Jennings, F., 1996. Veterinary Parasitology 2ndedition. The Faculty of Veterinary Medicine. The University of Glasgow Scotland Blackwell science, pp126-129.

WHO, 1983. Guidelines for surveillance, prevention, and control of taeniasis or cysticercosis. Geneva, VPH, Pp. 83-149

Yitbarek, D, Mulugeta, T. and Mihreteab, B., 2012. Prevalence of hydatidosis in sheep slaughtered at Abergelle export abattoir, Mekelle, northern Ethiopia. *Glob.Vet.* 9(4), 490-496.

Epidemiology of camel contagious ecthyma and molecular detection of the pathogen in Arero district, Ethiopia

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Abstract

Even though camels (*Camelus dromedarius*) were traditionally believed to be resistant to most livestock diseases, research has demonstrated that they are susceptible to a large number of infectious agents. Based on the clinical appearance of typical lesions, camel contagious ecthyma (CCE), caused by a *Parapoxvirus* (PPV), is thought to be one of the most common viral diseases of camels in Ethiopia. A cross-sectional study was conducted from November 2013 to April 2014 in the Arero district of Borena Zone, Oromia Regional State of Ethiopia to investigate the epidemiological aspect of CCE and molecularly identify the causative agent. A polymerase chain reaction (PCR) based on B2L gene-specific primers of PPV was used for the confirmatory diagnosis of the CCE virus from the skin lesion of camels showing suspected clinical signs of CCE infection. Eighty-seven percent (87.0%) of camel owners reported the occurrence of CCE outbreaks in their herds in the past year (a year preceding the start of the study). The overall morbidity and mortality rates attributed to CCE were 20% (95% CI: 11– 36%) and 6.3% (95 % CI: 5.2 –7.6%), respectively. Younger camels had higher odds of becoming affected by CCE than adults [OR=3.44 (95 % CI: 2.29 –4.09)] and the difference was statistically significant. Confirmatory diagnosis of the suspected cases using conventional PCR generated the expected amplification product size of 1200bp for one of the samples. Therefore, the study confirms the presence and importance of CCE in Ethiopia and establishes the basis for further investigation.

Keywords: *Camelus dromedaries; Camel; Contagious Ecthyma; Epidemiology; PCR; Ethiopia.*

Introduction

The one-humped camel *(Camelus dromedarius)* is a crucial livestock species uniquely adapted to harsh environments (Mirkena *et al.*, 2018). Dromedaries provide a reliable source of livelihood, especially for some of the most food-insecure pastoral communities. In addition to providing milk, meat, and local transportation to households that keep them, camels are the source of cash income through the sale of live camels and their products (Asiimwe *et al.,* 2020; Salamula *et al.*, 2017).

The world's camel population has been estimated at almost 23 million, and more than 95% of camels are found in developing countries (Faye, 2015). Ethiopia possesses over 7 million dromedaries (CSA, 2020). Major camel-keeping societies in Ethiopia include Afar, Somali, Oromo (Karayu, Gabra, Boran, and Guji groups), Kunama, and Irob peoples, among others. Camel in these areas is becoming a leading animal because of the multipurpose role it has in the provision of milk, meat, social and cultural importance in addition to unpaid transport service (Mirkena *et al*., 2018). Despite all the benefits associated with camel production in the pastoral areas of Ethiopia, camels still face several challenges in their natural environment, the most important of which are camel diseases (Seifu, 2009).

Camel contagious ecthyma (CCE), also known as Orf, is a contagious skin disease of camelids caused by a poxvirus of the genus *Parapoxvirus* (PPV), subfamily *Chordopoxvirinae* of the family *Poxviridae* (Khalafalla *et al*., 2020; Oryan *et al*., 2017; Khalafalla *et al*., 2015). The disease has a worldwide distribution (Khalafalla *et al*., 2015). CCE is clinically recognized by the appearance of papules, vesicles, pustules and rapidly growing scabs confined to the lips and muzzle of the affected animals (Khalafalla *et al.*, 2020; Oryan *et al.*, 2017; Gelaye *et al.*, 2016b; Khalafalla *et al*., 2015). Infected animals are weak, fail to thrive, and are more susceptible to other bacterial infections (Zhu *et al*., 2019). The morbidity rate of CCE was reported as 100% while mortality reached up to 9% in young camels in Arabian Peninsula (Abubakr *et al*., 2007). Molecular technique, namely, PCR based on B2L gene-specific primers of PPV was exten-

sively used for the confirmatory diagnosis of contagious ecthyma in infected animals (Khalafalla *et al.*, 2020; Oryan *et al.*, 2017).

Although the increased occurrence of pox-like diseases in camels has been reported from major camel-keeping areas of Ethiopia, there is only one report on the identification of camelpox virus (CMLV) in the Chifra district of Afar and Jigjiga Zone of Somali Regional States of Ethiopia (Ayelet *et al.*, 2013), with no data available on the existence of camel contagious ecthyma or the identification of the causative agent. Therefore, this study aimed to determine the epidemiology of CCE infection and molecularly identify the causative agent in Arero district, Ethiopia.

Materials and methods

Study area

The study was conducted in the Arero district of Borana zone, Oromia Regional State of Ethiopia. Arero district is geographically located at 4°45'0'N and 38°49'0'E at a distance of 650 km south of Addis Ababa. The area is bordered on the southwest by Dire, on the West by Yabello, on the North by Bule Hora, on the northeast by the Guji Zone, on the east by the Somali Region, and on the South by Moyale (Olani *et al.*, 2016). The annual average temperature and rainfall are 19 °C and 716 mm, respectively. Animal husbandry in the area is characterized by an extensive pastoral production system with seasonal migration. Camels and cattle are the key livestock species in the area (Mirkena *et al.*, 2018; Faye, 2015). As aridity gradually increases and drought is a recurrent phenomenon in the area, the principal stock is shifting from cattle to camels (Dawo, 2010).

Study methods and sample size determination

The study employed a cross-sectional study design (November 2013 to April 2014). Arero district was selected because of its camel production potential and easy access to a major road. Three pastoral associations (PAs) were randomly included from the sampled district (i.e., Haro-Dimtu, Kaarra-Gumaata, and Silala PAs). A total of 129 volunteer households participated in this study. The sample size for respondents in the house-to-house interview was determined

using the formula ($n = 0.25/SE^2$) proposed by Arsham (Arsham *et al*., 2007), at the standard error (SE) of 0.044 and 95% confidence level.

A standardized, structured questionnaire was used to collect information relevant to the study objectives, such as age structure of the respondents, herd size and herding experience, CCE incidences in the past year (a year preceding the start of the study), age-wise morbidity and mortality rates attributed to CCE, seasonality of the disease, and opinion of camel owners on plant browse that are potentially associated with CCE occurrence. Herders' ability to identify CCE infection from other diseases with similar clinical signs and symptoms was cross-checked by enquiring about the clinical signs of the diseases. For those who mentioned clinical signs shared by the diseases easily confused with CCE such as Warts, the interviewers reviewed the clinical signs of CCE with camel owners to verify that the respondent had understood the disease correctly. One animal health assistant and a traditional healer were interviewed regarding the local name of CCE in each of the selected PAs. Some of the information collected during interviews was supported by field observation.

Sample collection and sampling procedures

The protocol for field studies and collection of animal samples was carried out following the ethical guideline of Jimma University, College of Agriculture and Veterinary Medicine (JUCAVM).

Fourteen (14) skin scrapings were collected from camels showing suspected clinical signs of PPV infection. Samples were immediately transferred into a cold box and transported to the National Veterinary Institute (NVI) of Ethiopia under the cold-chain system. The samples were then kept at -20 ^{0}C until laboratory analysis.

Viral isolation on cell culture

Skin scraping samples were washed three times with sterile phosphate buffer saline (PBS) containing antibiotics and antifungals, and ground using a sterile pestle and mortar. The supernatant (0.5 mL) was inoculated onto a confluent monolayer of Vero cells grown in a 25 cm^2 tissue culture flask containing 10 mL

Glasgow Minimum Essential Medium (Sigma-Aldrich) supplemented with 2% fetal calf serum (Gibco). The inoculated cultures were incubated at 37 ^{0}C, 5% CO_2, and observed daily for the appearance of virus-induced cytopathic effects (CPEs). Samples were considered negative when no CPE was observed following three blind passages (Gelaye *et al.*, 2016a; Gelaye *et al*., 2016b; Khalafalla *et al.,* 2015).

Polymerase chain reaction (PCR)

After isolating genomic DNA from the virus, the B2L gene was amplified using forward primers (5′-TGA GCT GGT TGG CGC TGT CCT-3′) and reverse primers (5'-CGC AGA CGT GGC TCA GTA CGT-3′). The reaction setup was prepared as follows: 5X standard reaction buffer (5 µl), 2 mM dNTPs (0.5 µl), 500 nM forward primer (1.25 µl), 500 nM reverse primer (1.25 µl), template DNA (5 µl), 2.5 U Taq DNA Polymerase (0.25 µl), nuclease-free water (to 25 µl). The thermal profile was set as follows: Initial denaturation (94 °C, 5 min, 1X cycle), Denaturation (94 °C, 1 min), Annealing (55 °C, 60 s, 35X), Extension (68 °C, 70 s), Final extension (68 °C, 5 min, 1X) (Khalafalla *et al*., 2020; Tedla *et al*., 2018).

Data analysis

A database was constructed in a Microsoft Excel to store the data. Analysis was performed using Statistical Package for Social Science (SPSS 2007 version 20) software. Descriptive (proportion) and inferential (logistic regression model) statistics were used to analyze the data. Potential risk factors associated with CCE occurrence were assessed by using a logistic regression model and an odds ratio (OR) estimate was used to determine the strength of association between the risk factors (independent variables) and disease (dependent variable). In all the analyses, confidence levels of 95 % and a $p < 0.05$ were used for the statistical significance test.

Ethical consideration

Ethical clearance was obtained from the Ethics Committee of the College of Agriculture and Veterinary Medicine, Jimma University (ethical code R/GS /217/2007). Written and oral consent was obtained from camel owners before the questionnaire survey and sample collection.

Results

Questionnaire survey results

Herd profile of the respondents was described in Table 1. Herd size was used to evaluate the relative contribution of camels to pastoralists and herding years were used to estimate their practices in camel husbandry. Out of the total interviewed respondents (n = 129), the majority (48%) were in the age range of 42-61years. The average herd size of the respondents was 13 camels. Over 40% (46.3%) of the respondents had been herding camels for more than ten years.

Table 1. Herd profile of the respondents

No	Characteristic	Categories	N	Percentage
1	Age of the respondents	25-41	27	21.0
		42-61	62	48.0
		62-81	40	31.0
2	Herd size of the respondents	1-7	41	32.3
		8-17	50	38.7
		≥18	38	29.0
3	Herding experience (years)	0-10	33	25.4
		11-21	60	46.3
		22-31	36	28.3

N=Number of respondents

Herders' knowledge of the diagnosis of CCE is described in Table 2. The result indicated that over 90% of the respondents were aware of CCE, and 62.0% (76/129) mentioned clinical signs suggestive of CCE such as sores and blisters on the lips, nose, and ears. The majority (87%) of the participants reported occurrence of CCE outbreaks in their herds in the past year (a year preceding the start of the study) with the overall morbidity and mortality rates of 20% (95% CI: 11.0– 36.0%) and 6.3% (95 % CI: 5.2 –7.6%), respectively.

Table 2. Selected variables of herders' knowledge and attitude on CCE

Variables	N	Percentage
Heard about CCE		
Yes	123	95.4
No	6	4.6
Major clinical signs mentioned		
Animals fail to grow	14	11.0
Mortality	11	9.0
Sores & blisters on the lips, nose, and ears	76	62.0
Abortion	2	1.6
Loss of appetite	9	7.4
Mentioned 1, 3, & 5	11	9.0
Diarrhea	0	0.0
Experienced CCE outbreaks in their herds in the past year?		
Yes	107	87.0
No	16	13.0

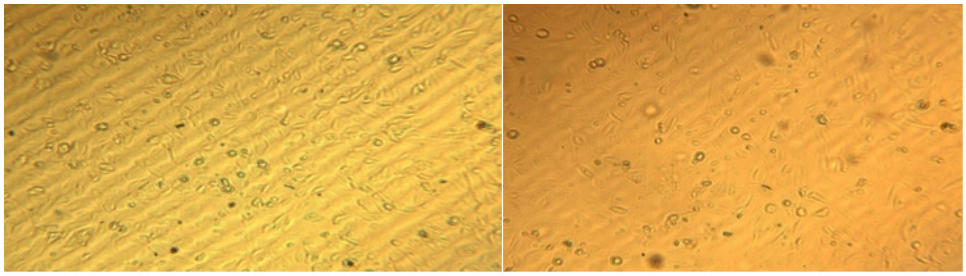
Table 3 describes epidemiological information related to CCE infection. The results indicated that camel calves (age less than two years) had higher odds of becoming affected by CCE than adults (OR=3.44; 95% CI: 1.75-8.80%) and the difference was statistically significant ($p < 0.05$). CCE outbreaks were nearly six times more severe (OR=5.8; 95% CI: 3.34 - 10.52%) in the rainy season than in the dry season. Camels that browsed at Acacia-dominated trees were nearly ten times (OR=9.6; 95% CI: 0.42 - 17.83%) more at risk of CCE infection than camels that browsed at low Acacia-dominated trees.

Table 3. The epidemiological aspect of CCE outbreaks in the study district

Major risk factors	Number Affected	Percentage	OR	95 % CI	P-value
Morbidity					
Calves affected	269	77.5	3.44	1.75 - 8.80	0.012
Adults affected	78	22.5			
Mortality					
Calves died	89	84.0	7.2	4.22 - 11.55	0.002
Adults died	17	16.0			
Seasonal occurrence of CCE as reported by camel owners					
Rainy season	219	63.0	5.8	3.34 - 10.52	0.006
Dry season	128	37.0			
Plant brows and CCE outbreaks as reported by camel owners					
During acaria trees abundance	293	84.4	9.6	5.42 – 17.83	0.024
At low acacia trees abundance	54	15.6			

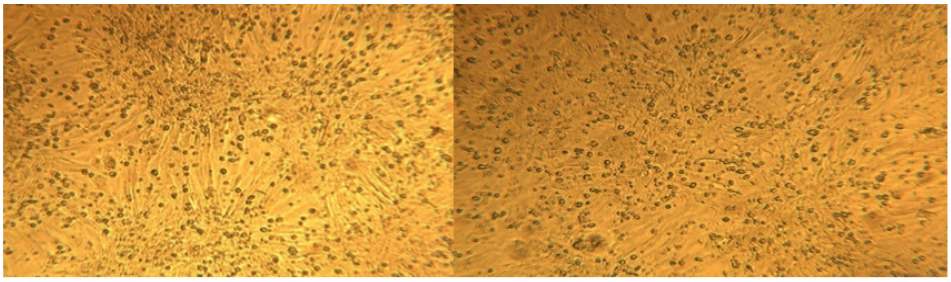
CI= Confidence interval; OR= Odds ratio

Virus isolation results



Normal Vero cell (unaffected)

A.



B.

Fig. 1. African green monkey Vero cell cultures before (top left) or non-inoculated normal monolayers (top right) (A), and CPE observed four days post-infection (B). The figure demonstrates a monolayer of African green monkey Vero cells cultures after infection with CCEV. The inoculated CCEV produces a CPE characterized by cell rounding and enlargement, pyknosis, granularity of the cytoplasm, and cell detachment.

PCR result

PPV-specific amplification by PCR confirmed that the disease was associated with **CCEV** infection showing bands with 1200 bp in one of the culture-positive samples (Figure 2).

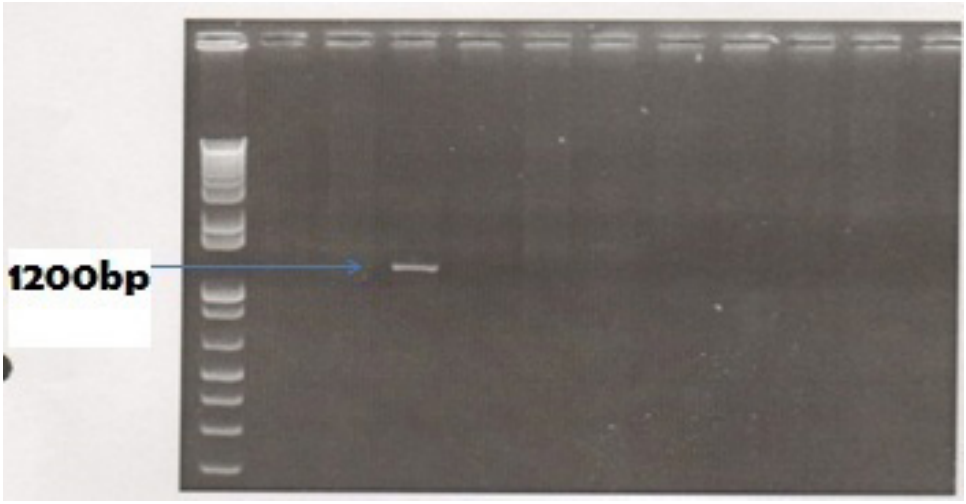


Figure 2. Molecular detection of CCEV. The figure shows gel electrophoretic separation of PCR products (read from left to right). Lane 1=100bp DNA ladder. Lane 2 is a negative control whereas all the rest are CCEV suspected tissue samples. Lane 4 is positive for CCEV around 1200bp, and no amplification was observed in all of the rest.

Discussion

Contagious ecthyma infection in camels is generally neglected worldwide. In Ethiopia, despite frequent outbreaks, there hasn't been any attempts to investigate the disease in the country where camels are important assets to the local community. This study investigated morbidity and mortality rates of the disease consistent with CCE, molecularly identified the causative agent, and determined the potential risk factors in the Arero district of Borana zone, Oromia Regional State of Ethiopia. Camel owners were interviewed to get some epidemiological information on the CCE infection. Their knowledge in describing CCE and ability to recognize its effect indicate their expertise in distinguishing camel health problems in their vicinities, which is in line with the results reported in Sudan (Khalafalla *et al*., 2020). Camel owners considered clinical signs of CCE consistently with the descriptions of the disease signs in the standard veterinary literature (Khalafalla *et al*., 2015; Radostits *et al.,* 2007; Munz *et al*., 1986).

The overall morbidity and mortality rates attributed to CCE in the present study were comparable with the reports from Sudan (Khalafalla *et al*., 2020; Khalafalla *et al*., 2015; Khalafalla, 1998) and Somalia (Moallin and Zessin, 1988), but lower when compared to the findings of Iran (Oryan *et al*., 2017; Mombeni *et al*., 2013), and Sudan (Khalafalla, 2000). The variation could be related to the age structure of animals included in the studies, and/or differences in the husbandry and health management systems of the countries.

During field clinical investigation, authors noticed that the majority of the animals showing clinical signs of suspected poxvirus infection were younger camels. In addition, the morbidity and mortality rates attributed to CCE were higher in younger than in adults. Our result is in agreement with the reports of different researchers (Khalafalla *et al*., 2020; Khalafalla *et al*., 2015; Mombeni *et al*., 2013; Khalafalla, 1998). The severity of the disease in young animals

might be due to a lack of prior exposure to infecting pathogens (Radostits *et al.*, 2007), and/or due to the absence of a fully developed immune system (Oryan *et al.*, 2017; Hosamani, 2006).

The present study identified that CCE had a marked seasonality, being associated with the rainy season, and seemed to occur at this particular time every year. These findings substantiate the previous reports on the seasonality of CCE and its association with the rainy season (Khalafalla *et al.*, 2020; Mombeni *et al.*, 2013; Khalafalla *et al.*, 1994; Buchnev *et al.*, 1987). Factors responsible for this epizootiological feature seem to be the abrasion of the skin of the lips, resulting from eating thorny acacia plants at this time of the year when no other source of food was available (Khalafalla, 2000). The same view was reflected by a researcher from the former *Union of Soviet Socialist Republics* (*USSR*), who argued that thorny plants damaged the lips allowing transmission of parapox virus through skin abrasions caused by browsing thorny trees (Buchnev *et al.*, 1987).

Inoculation of scabs supernatants on Vero cell cultures revealed a CPE in the form of cell rounding and enlargement, pyknosis, and granularity of the cytoplasm and cell detachment four days post-infection. This can be considered the first step in the screening of PPV infections from suspected samples. Furthermore, PPV-specific amplification by PCR revealed an amplification product of 1200 bp size, confirming that the disease in camels was associated with CCEV. This finding substantiates reports from different countries including Ethiopia (Gelaye *et al.*, 2016b); Iran (Oryan *et al.*, 2017; Mombeni *et al.*, 2013); and Sudan (Khalafalla *et al.*, 2015).

Given the increased incidences and economic importance of PPV infections in the camel population in Ethiopia, it will be worthwhile to obtain more epidemiological information about this disease for effective surveillance and to carefully monitor and handle disease outbreaks.

Conclusions

The present study confirmed the existence of CCE in the Arero district of Borena zone, Ethiopia. Younger camels accounted for the highest morbidity and

mortality rates compared with adults. Confirmatory diagnosis of the suspected cases using conventional PCR techniques generated the expected amplification product size of 1200bp. However, due to scarcity in the laboratory facility, the amplicon with the compatible size was not sequenced to confirm the specificity. Overall, the information obtained in this study would be worthwhile to improve the farmers' livelihood and may open new research avenues for the control and eradication of the disease at the local and national levels.

Acknowledgments

The authors are very much grateful to the National Veterinary Institute for providing space and laboratory inputs to carry out the laboratory work.

Abbreviations

BA: Birhanu Ayele; BD: Bareda Diba; BdA: Bedane Adane; BdG: Benti Deressa Gelalcha; bp: Base pair; CCE: Camel contagious ecthyma; CCEV: Camel contagious ecthyma virus; CMLV: camelpox virus; CPE: Cytopathic effect; JUCAVM: Jimma University College of Agriculture and Veterinary Medicine; NVI: National Veterinary Institute; OR: Odds ratio; PAs: Pastoral associations; PBS: Phosphate buffered saline; PCR: Polymerase chain reaction; PPV: Parapoxvirus; SPSS: Statistical Packages for Social Sciences; UV: Ultra violate.

Conflicts of Interest

The authors would like to declare that they have no financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

Funding

The present study received financial support from Jimma University, the Vice president of the research and publication office.

Author contributions

BA involved in manuscript formatting, data analysis, and final write-up. BD conceived the research idea, collected the data, and drafted the manuscript. BdA supervised the sample collection and was involved in data analysis. BdG conceived the research idea, supervised field and laboratory works, analyzed the data, and drafted and edited the manuscript. All authors read and approved the final manuscript.

References

Abubakr, M.I., Abu-Elzein, E.M., Housawi, F.M., Abdelrahman, A.O., Fadlallah, M.E., Nayel, M.N., *et al.,* 2007. Pseudocowpox virus: the etiological agent of contagious ecthyma (Auzdyk) in camels (*Camelus dromedarius*) in the Arabian peninsula. *Vector Borne Zoonotic Dis*. 7, 257-260.

Arsham, H., Ford, D., Morse, J. and Pitta, D., 2007. The rate decision: adjustable vs fixed rate mortgages. *JBER*. 5, 31-42.

Asiimwe, R., Ainembabazi, J.H., Egeru, A., Isoto, R., Aleper, D.K., Namaalwa, J. and Diiro, G.M., 2020. The role of camel production on household resilience to droughts in pastoral and agro-pastoral households in Uganda. *Pastoralism* 10, 5.

Buchnev, K.N., Tulepbaev, S.Z. and Sanzyzbaev, A.R., 1987. Infectious diseases of camels in the USSR. *Rev. Sci. Tech.* 6, 487-495.

CSA, 2020. Agricultural Sample Survey, 2019/20 (2012EC,), Volume II: Report on Livestock and livestock characteristics (Private peasant holdings) (Central Statistical Agency (CSA), Federal Democratic Republic of Ethiopia, Addis Ababa, 2020).

Dawo, F., 2010. Mysterious mortality in camels (*Camelus dromedarius*) in Borana, Ethiopia: evidence of its association with reproductive age groups. *Rev. Sci. Tech.* 29, 621-628.

Faye, B., 2015. Role, distribution, and perspective of camel breeding in the third-millennium economies. *Emir. J. Food Agric*, 27 (4), 318-327.

Gelaye, E., Achenbach, J.E., Ayelet, G., Jenberie, S., Yami, M., Grabherr, R., *et al.,* 2016a. Genetic characterization of poxviruses in *Camelus dromedarius* in Ethiopia, 2011-2014. *Antiviral Res.*,134, 17-25.

Gelaye, E., Achenbach, J.E., Jenberie, S., Ayelet, G., Belay, A., Yami, M., *et al.,* 2016b. Molecular characterization of orf virus from sheep and goats in Ethiopia, 2008-2013. *Virol. J.*, 13, 34.

Hosamani, M., Bhanuprakash, V., Scagliarini, A. and Singh, R. K, 2006. Comparative sequence analysis of major envelop protein gene (B2L) of Indian Orf viruses isolated from sheep and goats, *Vet. Microbiol.*, 116, 317–324.

Khalafalla, A.I., 1998. Epizootiology of Camel Pox, Camel Contagious Ecthyma and Camel Papillomatosis in Sudan. In: Proceedings of the Third Annual Meeting for Animal Production Under Arid Conditions, United Arab Emirates University pp. 115-131

Khalafalla, A.I., 2000. Camel contagious ecthyma: Risks in young calves. *Revue Elev. Med. Vet. Pays Trop.,* 53 (2), 173-176.

Khalafalla, A.I., Agab, H. and Abbas, B., 1994. An outbreak of contagious ecthyma in camels (*Camelus dromedarius*) in eastern Sudan. *Trop. Anim. Health Prod.*, 26, 253-254.

Khalafalla, A.I., El-Sabagh, I.M., Al-Busada, K.A., Al-Mubarak, A.I. and Ali, Y.H., 2015. Phylogenetic analysis of eight Sudanese camel contagious ecthyma viruses based on B2L gene sequence. *Virol. J.*, 12, 124.

Khalafalla, A.I., Elhag, A.E. and Ishag, H.Z.A., 2020. Field investigation and phylogenetic characterization of orf virus (ORFV) circulating in small ruminants and Pseudocowpoxvirus (PCPV) in dromedary camels of eastern Sudan. *Heliyon,* 6, e03595.

Mirkena, T., Walelign, E., Tewolde, N., Gari, G., Abebe, G. and Newman, S., 2018. Camel production systems in Ethiopia: a review of literature with notes on MERS-CoV risk factors. *Pastoralism,* 8, 30.

Moallin, A.S. and Zessin, K.H., 1988. Outbreak of camel contagious ecthyma in central Somalia. *Trop. Anim. Health Prod.,* 20, 185-186.

Mombeni, E.G., Mombeini, M.G., Varshovi, H., Khalaj, M., Kenarkohi, M., Goudarzi, M., *et al.*, 2013. Outbreak of contagious ecthyma in camels (*Camelus dromedarius* and *Camelus bactrianus*) in Southwest Iran. *Rev. Elev. Med. Vet. Pays Trop.,* 66, 113-115.

Munz, E., Schillinger, D., Reimann, M. and Mahnel, H., 1986. Electron microscopical diagnosis of Ecthyma contagiosum in camels (*Camelus dromedarius*). First report of the disease in Kenya. *Zentralbl Veterinarmed* B, 33, 73-77.

Olani, A., Habtamu, Y., Wegayehu, T. and Anberber, M., 2016. Prevalence of camel trypanosomosis (surra) and associated risk factors in Borena zone, southern Ethiopia. *Parasitol. Res.,* 115, 1141-1147.

Oryan, A., Mosadeghhesari, M., Zibaee, S. and Mohammadi, A., 2017. Identification and phylogenetic analysis of contagious ecthyma virus from camels (*Camelus dromedarius*) in Iran. *Onderstepoort J. Vet. Re*s., 84, e1-e5.

Radostits, O.M., Gay, C., Hinchcliff, K.W. and Constable, P.D., 2007. A textbook of the diseases of cattle, horses, sheep, pigs, and goats. 9th Edition, W.B. Saunders Company Ltd., London, 2045-2050 pp.

Salamula, J.B., Egeru, A., Asiimwe, R., Aleper, D.K. and Namaalwa, J.J., 2017. Socio-economic determinants of pastoralists' choice of camel production in Karamoja sub-region, Uganda. *Pastoralism,* 7, 26.

Seifu, E., 2009. Analysis on the contributions of and constraints to camel production in Shinile and Jijiga zones, eastern Ethiopia. *J. Agric. Environ. Int. Dev*., 103, 213-224.

Tedla, M., Berhan, N., Molla, W., Temesgen, W. and Alemu, S., 2018. Molecular identification and investigations of contagious ecthyma (Orf virus) in small ruminants, Northwest Ethiopia. *BMC Vet. Res.,* 14, 13.

Zhu, S., Zimmerman, D. and Deem, S.L., 2019. A Review of zoonotic pathogens of dromedary camels. *EcoHealth,* 16, 356-377.

Megenas and Taera, Ethiop. Vet. J., 2022, 26 (2), 72-92
DOI https://dx.doi.org/10.4314/evj.v26i2.5 *Ethiopian Veterinary Journal*

A comprehensive study on the cattle production situation owned by refugees and hosting communities in the Gambella region, southwestern Ethiopia

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Abstract

This study was designed to assess the state of cattle production and the contributions that cattle production makes to enhance the livelihoods, food security, and nutrition of refugees and the host community in the Gambella region in southwest Ethiopia. Two hundred fifty-five cattle owners were purposefully chosen for the study, and information was gathered through questionnaires, focus group discussions, and key informant interviews. The average number of animals per household (sd; ±0.002 range: 1 to 490) was 32.37. The study observed a total of 8,258 cattle of which 8,146 belonged to the Nuer breed and 112 to the Felata breed. The reason for maintaining cattle varies statistically across the host community, re-settlers, and refugee communities (x2=50.358; p-value =0.000). According to the current study, 92.5% (236/255) of cattle owners used a free-grazing system, and the mean daily milk yield per cow during the dry and rainy seasons, respectively, was 1.35L and 2.09L. All respondents (100%) agree that there exist cattle diseases in the research area, and 79.6% of cattle owners employed traditional medicine to prevent and treat cattle diseases. Some of the potentials for cattle production in the study areas were cultural value, the existence of disease-tolerant breeds, the availability of manpower, and local expertise. Therefore, it is highly advised that a thorough analysis of the various agro-ecologies in the area be necessary to understand the reproductive and productive performance of cattle.

Keywords: Cattle Production; Gambella; Host Community; Refugees; Southwestern Ethiopia.

Introduction

About 60% of rural households in developing countries are estimated to fully or partly depend on livestock for their livelihoods. However, there is no sufficient and appreciable data that entails the contribution of livestock to the livelihoods of the households including both the monetary and the non-monetary benefits. This poses challenges to the design and implementation of effective investments in the sector (Ugo *et al.,* 2011)

Ethiopia has the largest livestock population in Africa and has an estimated population of 57.829 million heads of cattle, 28.89 million sheep, 29.7 million goats, 2.08 million horses, 0.405 million mules, 7.88 million donkeys, 1.22 camels, and 60.50 million poultry (CSA, 2016). The livestock that constitutes Ethiopian farm animals, ruminants comprising of cattle, sheep, and goats are among the main source of draft power (cattle), wealth accumulation purposes, and income generation and are distributed within the different agro-ecological zones of the nation; about 99% of cattle populations are of local Zebu breed. The remaining 1% of exotic breeds is kept mainly for dairy production in urban and peri-urban areas to fulfill local market milk consumption demands (Welay *et al.,* 2018).

Literature indicates that livestock production and productivity are hindered by the poor nutritional value of the forage both in quantity and quality. It is also affected by poor livestock husbandry practices, animal health ailments, and husbandry constraints. The aforementioned constraints resulted from subsistence-oriented livestock production in Ethiopia in particular. The constraints also arise from epidemics and pandemics of animal diseases which have contributed to livestock morbidity and mortality. Consequently, it causes reduced livestock production and productivity oftentimes. In central Ethiopia, livestock husbandry practices are extensively managed on free grazing range/within the extensive system. Specifically, crop-producing farmers keep an average of five to 15 cows/shoats/household (HH) under the free-grazing range system, the animals move about freely to feed on forages/grasses, which are abundantly available during the rainy season. Hardly are the animals provided supplementary feeds (personal observation) and despite poorly designed housing shelter is provided by their owners around their homestead areas to protect them from predators (Steinfeld *et al.,* 2006).

In Ethiopia, where livestock agriculture is merely practiced; cattle are raised extensively in small-holder production systems (Metaferia *et al.*, 2011). As a result of extensive livestock production, productivity is hindered by anthrax, blackleg, lumpy skin disease (LSD), trypanosomiasis, and other major health problems experienced are parasites, with liver fluke being the major internal parasites, followed by ticks and biting flies. Animal health is recognized to be a significant source of production losses such as low weight gain, draught, fertility, and lactation performances (Tadesse, 2014). Factors like low feed supply to livestock, occupation of grazing lands by humans, and the conversion of grazing land into farmlands pose a challenge to the sector. The use of improved forages by smallholder farmers is not common and utilization of agro-industrial by-products is limited to urban and peri-urban areas (Bizelew *et al.*, 2016). Farmers have used traditional medicines and practices to treat their animals for different kinds of livestock diseases. Major animal health constraints and efficient livestock husbandry practices are not yet been identified and developed, respectively (Alemu *et al.*, 2019).

Improvement in cattle productivity can be achieved through the identification of production constraints and by the introduction of improved technologies or by refining existing practices in the system. There are limitations in identifying data on the existing breed type, cattle diseases, sources of cattle production inputs, the production potential of study areas, quality of livestock production, and like. More importantly, there was no study, especially in the refugee camps. Therefore, the research is designed to assess the existing cattle production challenges, opportunities, breed improvement, production system trends, roles of cattle production in improving livelihoods food security, and nutrition of refugee and host communities.

Materials and methods

Study area description

The study was conducted within the refugee hosting communities in Gambella Regional State (Lare woreda, Itang special woreda, and Gambella zuriya woreda) and in Refugee Camps (Kule, Jewi, Teirkidi, and Nyuengyiel) Refugee camps of Gambella Regional State, South Western Ethiopia from February to April 2020.

The Gambella People's Regional State is located in the South Western part of Ethiopia between the geographical coordinates of $6^0$28'38" to $8^0$34' North Latitude and 33^0 to $35^0$11'11" East Longitude. The region is situated 766 km away from the Ethiopian capital Addis Ababa. The region covers an area of about 34,063 km^2. It is bounded to the North, North East, and East by Oromia National Regional State, to the South and Southeast by the Southern Nations and Nationalities and People's Regional State, and to the Southwest, West, and Northwest by the Republic of South Sudan (CSA, 2016).

Most of the Gambella Region is flat and its climate is hot and humid. The mean annual temperature of the Region varies from 17.3^0C to 28.3^0C and the absolute maximum temperature occurs in mid-March and is about 45^0C. The absolute minimum temperature occurs in December and is 10.3^0C. The annual rainfall of the Region in the lower altitudes varies from 900-1,500mm; at higher altitudes, it ranges from 1,900-2,100mm. The annual evapotranspiration in the Gambella reaches about 1,612mm and the maximum value occurs in March and is about 212 mm (CSA, 2016). Based on the 2013/2014 Census conducted by the Central Statistical Agency of Ethiopia (CSA), the Gambella Region has a total population estimation of 406,000 and livestock population of Gambella 253,389 cattle, 39,564 sheep, and 83,897 goats (CSA, 2016).

The population of the refugee camps was coming from South Sudan at various times due to instability in their country. The refugee camps were established at different times depending on the influx of refugees to the region. In the refugee area where the camps are established, there is natural grass and the people had to practice rearing animals in their country the presence of a conducive environment around the refugee camps leads them to keep different farm animals such as cattle, goats, sheep, and fishing using artificial ponds in harmony with the host communities.

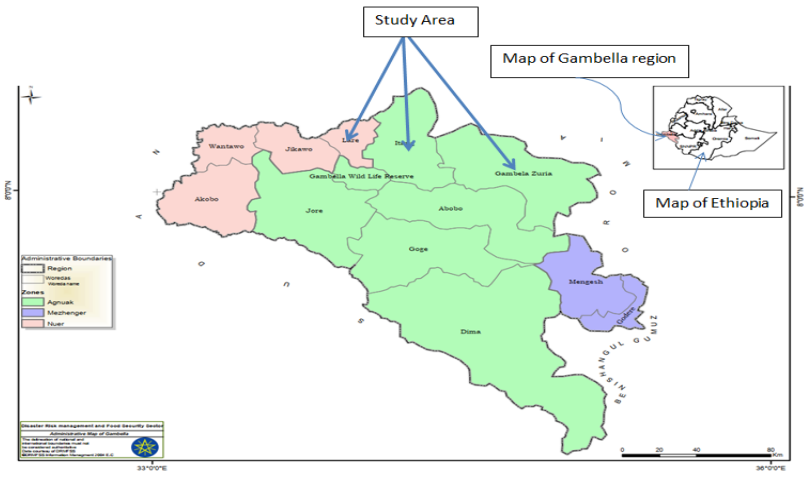


Figure1. Map of the study area; Source: Bureau of Finance and Economic Development, Gambella, Ethiopia (2017)

Study methods

The study was conducted within the refugee Hosting communities and in refugee camps found in Gambella Regional State, southwest Ethiopia from February to April 2020. A cross-sectional study design was employed in the study areas to determine the production situation, the animal health and disease problems, the existing challenges of cattle production, and potential opportunities for the region in general and the study area in particular.

Seven (three woredas and four refugee camps) were selected purposively based on their livestock population and milk shed status. The Source of the study population was both the host communities in different districts of the Gambella Region such as Lare woreda, Itang special woreda, and Gambella zuriya woreda, and refugee communities living in Kule, Jewi, Teirkidi, and Nyuengyiel refugee camps in the Region. The study population constitutes cattle owners from three refugee hosting woredas and the refugee camps that are mainly under the agro-pastoral production and Pastoral production system of the area.

Sample size determination

The sample size was determined based on Yamanes' (1967) sample size determination formula for finite population and a proportional number of respondents were collected from each study site, based on prior information on cattle ownership, farmers' cooperation, logistics, and accessibility.

$$n = \frac{N}{1+N(d^2)}$$

N= populations of cattle owners in the host community and in the refugee, which is 374,214.

d = desired absolute precision which is 94% (95%) confidence level was used due to shortage of time and budget constraints. Therefore, the sample size calculated was 278 but, 255 of the questionnaires were collected appropriately and the remaining 23 were not collected due to quality cases and the absence of farmers in the stated villages.

Sampling procedure and data collection

The mixed (quantitative and qualitative) research approach was used for data collection and a Stratified sampling technique was employed the study population was classified into the refugee hosting communities based on their administrational units and for the refugee employ the refugee camps were used as a stratum. In the process of the questionnaire development, due emphasis was given to the following core subjects: socio-economic characteristics of the household, livestock production and associate constraints, types of feed resources, and time of availability. Enumerators were recruited and trained to administer the pretested questionnaires which were structured and semi-structured. All Cattle owners staying more than 5 years in the study area were included in the present study and experts were selected according to their proximity to cattle and cattle owners.

Different data collection tools such as questionnaires, focus group discussions, and key informant interviews. A structured and semi-structured questionnaire was designed to collect both qualitative and quantitative data from the sample population. The questionnaire was designed and inscribed in the English language. It consisted of four sections. The first section is designed to capture general background information of the respondent while section two is intended

to collect information on cattle production; Section three is for capturing information on milk and meat consumption trend, and Section four is aimed at gathering information on animal health/ diseases problems.

Focus group discussion was employed with developmental agents, community leaders/elders, cattle owners, Community animal health workers, and *Kebele* Administrators on selected issues. Six to eight discussants were incorporated and their views of the agreement were analyzed. Key informants' interview was undergone with the regional and woreda level Livestock and fishery experts in the area of breeding practice, cattle health problems, cattle forage, vaccination practice, and the plan of future cattle production in the study areas.

Source of data

Primary data collection

Primary data were gathered through a combination of techniques; key informant interviews, focused group discussion, and personal farm visits and discussions with farmers using structured and semi-structured questionnaires. Before the actual implementation of the field survey, pretesting of the structured and semi-structured questionnaire survey was carried out by trained data collectors on smallholder farmers in agro-pastoral areas of the Gambella region. Accordingly, the prepared structured and semi-structured questionnaire survey was modified and an interview was conducted to collect the required data. The case study was conducted to collect relevant information on the existing cattle production conditions of refugees and the host communities.

Secondary data collection

Secondary sources of information relevant to the subject were collected from various published papers such as those published on the production system, cattle breed, and system of animal feed conservation and grey literature (reports from woreda and the refugee camp) and were reviewed to extract additional information to complete the primary data. Secondary data were collected to enhance the understanding of the cattle production conditions in the study areas. The main sources for secondary data were reports and documents from district livestock offices. Secondary data collected included information and findings from past studies on the sector

Pilot survey

The pilot survey was conducted in March 2020 before the actual data collection. It was designed to collect data and information that were used to identify the problems facing the cattle owners. Discussions were employed with officials from the regional office of Agriculture, Cooperatives (districts), and office of Livestock and Fishery Development and Refugee representatives to obtain their views on the general status of cattle production in the study areas. Depending on the availability and willingness of respondents to participate in the pilot survey; the selection of members of focus group discussions followed purposive sampling where members were selected from a population of actors who kept cattle. During the pilot study, several issues on cattle production were discussed including, the cattle breed improvement trends, forage availability, seasonality and volumes of milk production, meat production and consumption, cattle health problems/health, and constraints faced so far.

Data management and analysis

Data needed for the study was recorded on a data sheet during data collection. Data recorded were managed using the Microsoft Excel computer program, and entered and stored by SPSS IBM 20 data analysis software. Pretesting of the structured data extraction tool took place on 6% of the sample before the full course of data collection. After minor correction was made to the extraction tool data collection was restarted. The data collected were also checked for completeness and consistency.

To control data quality data collectors were recruited for all the required data. One day of training was provided to them with the aim of the study and the data collection tools. Supervision of data collection was done by the researchers. Data analysis involved both qualitative and quantitative techniques and questionnaires were administered with SPSS IBM v20 software. Chi-square ($\chi 2$) was used for the association of different factors such as host community with refugee, and sex with cattle production. A P-value <0.05 was considered statistically significant at a 95% confidence interval. Qualitative data were transcribed, consolidated, and narrated. Then the data was coded; categorized and finally analyzed using open code 3.6.2.0windows.

Results

Socio-demographic characteristics of the study participants

The study revealed that the overall mean of family size per household was 8.04. Specifically, family size per household in the Host community is 9, in re-settlers 6, and refugee 11. Re-settlers were people who were displaced by a disaster like a flood and re-located by the regional government. Refugee communities are people who came from South Sudan through the border and live in the refugee camps. The majorities 81.6% (208/255) of the total respondents were from male-headed households and the remaining 18.4% (47/255) were from female-headed households. The overall mean age of cattle owners was 43.02 $\pm$ 9 (range=24-70). The overall mean cattle number in the study area was 32.37$\pm$ 0.002 (range=1 to 490) animals per household.

Existing breed type, production potential, production system, and breed improvement of the study area

Dominantly Nuer breeds (disease tolerant cattle) and Felata breeds (which came from central Africa) are kept by the host, the re-settler, and the refugee communities.

Table 1. Herd composition of cattle in the host and refugee community

Herd composition	Nuer breed (n=8146)	Felata breed (n=112)	Total
Ox	703	40	743
Bull	707	21	728
Cow	3850	38	3888
Heifer	1332	7	1339
Calves	1554	6	1560
Total	8146	112	8258

The study revealed that 92.5% (236/255) of respondents are married. From the study areas, 49.8% (127/255) of cattle owners were not educated and only a limited number attained primary education 73.7% (188/255) of the respondent witnessed that their primary occupation is keeping cattle/ livestock.

Table 2. Profiles of the respondents

Parameters	Variables	Frequency (%)
Status of the owner	Refugee	59.6 (152/255)
	Host community	38.8 (99/255)
	Re-settler	1.6 (4/255)
Sex	Male	81.6 (208/255)
	Female	18.4 (47/255)
Marital status	Single	1.6 (4/255)
	Married	92.5 (236/255)
	Divorced	1.2 (3/255)
	Widowed	4.7 (12/255)
Level of education	Primary	29.4 (75/255)
	Secondary	12.2 (31/255)
	Post-secondary	8.6 (22/255)
	Non- educated	49.8 (127/255)
Primary occupation	Waged employee	3.9 (10/255)
	Livestock keeping	58.8 (150/255)
	Business	6.7 (17/255)
	Crop production	30.6 (78/255)

The result showed that keeping cows is higher (2459) in host communities followed by refugee (1279) communities.

Table 3. Information on cattle production and management

Cattle composition	Host community	Re-settler	Refugee	Total
Oxen	514	12	217	743
Bull	533	15	180	728
Cow	2459	150	1279	3888
Heifers	887	80	372	1339
Calves	990	73	497	1560
Total	**5383**	**330**	**2545**	**8258**
Percentage	65.2% (5383/8258)	4% (330/8258)	30.8% (2545/8258)	100%

As shown in Table 3, the majority 98.6% (8146/8258) of the owner in the study area reared Nuer breed cattle than Felata breed 1.4% (112/8258). The refugee

and re-settler come to the region with their cattle from the beginning when they migrate.

Table 4. Information on the breeds of cattle (n) in the study area

Cattle	Host community	Re-settler	Refugee	Total	%
Nuer breed	5346	330	2470	8146	98.6 (8146/8258)
Felatta/Fulani breed	37	-	75	112	1.4 (112/8258)
Total	5383	330	2545	8258	100%

As the above Table indicated re-settler has not kept Felatta breed because of that they prefer the Nuer breed to Felata/Fulani breed. From the focus group discussion, especially in the Nuer community cattle, are everything for life, and marriage is impossible or very difficult without cattle dowry. As respondents stated that "they keep cattle for dowry and it is considered as wealth in the community."

Table 5. Purpose of keeping cattle

Parameters	Source of meet	For income	Dowry
Host community	47	48	4
Re-settlers	0	4	0
Refugee	19	93	40
Frequency (%)	25.9 (66/255)	56.9(145/255)	17.3 (44/255)

The majority 56.9% (145/255) of the community kept cattle as a source of income. Most 59.6% (152/255) of the respondents provide water for their animals twice a day and 33.7% (86/255) of the cattle owners stated that water is freely available for their animals. But, less number 6.7% (17/255) of respondents witnessed that water is available for their animals only once a day. About 74.1% (189/255) of the respondents indicated that cattle owners have shade or a house for their animals and, 25.9% (66/255) did not. The study found that the owners have sheds for their animals 47.8% (122/255) keep their cattle in a separate house with a roof and 16.9% (43/255) of the farmers indicated that separate house without a roof and the remaining respondents (24 in number) are keeping their animals within the family house in the host community.

During the present study 1602 lactating cows were registered in the study area and a total of 1036 from the host community, 488 from the refugee area, and 78

lactating cows from displaced/ re-settled communities. Most 94.9% (242/255) of the respondents are applying twice a day milking frequency and 3.5% (9/255) and 4 (1.6%) of the respondents practice once and three times a day for milking frequency.

Table 6. The average milk production per day per cow in the litter

Seasonal variation of milk production (l)	Mean (±sd)	Range
Dry season	1.35±0.75	0.5-2 L
Wet season	2.09±0.89	0.75-3.5 L

As the above Table indicated, milk production in the study area was reported high in the wet season due to the availability of animal feed. Most 62.7% (160/255) of the respondents stated that they process milk into other milk products like yogurt, butter, and cheese locally. But, 36.9% (94/255) of cattle owners have no idea about milk processing rather than they consume in the form of raw milk.

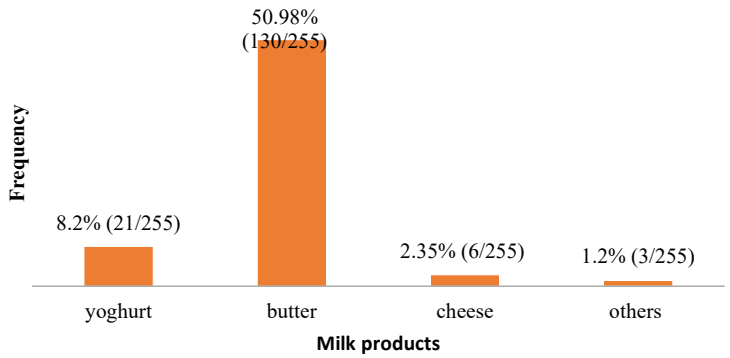


Figure 2. Milk products from the study area

From the total respondents, the result revealed that 52.9% (135/255) of them intended to purchase dairy products from local markets and 47.1% (120/255) are not buying any dairy products from the market.

The produced milk from home is consumed by all members is rated 85.1% (217/255) but, 14.1% (36/255) and 0.8% (2/255) of the respondents stated that the milk is given to infants and the sick person at home respectively. Most of the time milk is consumed after every meal which is witnessed by 46.7% (119/255) of the respondents. Whereas, 37.6% (96/255) and 13.7% (35/255) of

respondents responded that milk is consumed once a day and a few times a week, respectively. Most 66.3% (169/255) of respondents responded that they boil milk before consumption and 33.7% (86/255) of them are not boil milk and consume it in the form of raw milk. In the study area milk is preserved by refrigerator 7.8% (20/255), 61.1% (157/255) by traditional means, 1.2% (3/255) by processing, 28.2% (72/255) by boiling, and 0.8% (2/255) of the respondents use other preservation methods. 41.6% (106/255) of respondents used Plastics, 6.3% (16/255) aluminum cans, 22.4% (57/255) stainless steel, and 29% (74/25) of the respondents used locally accessible materials for milk storage and handling.

The majority 92.5% (236/255) of respondents from the study area had a habit of meat consumption and only 7.5% (19/255) of the farmers respond no. The time when meat consumption is also analyzed and found 51.8% (132/255), 9% (23/255), 14.1% (36/255), 20.4% (52/255) during the holiday, every day, when there is food shortage and others, respectively.

Most 63.9% (163/255) of the respondents stated that there are butchers in their area. Whereas 34.9% (89/255) of the respondents witnessed no butchers in their residents and 40% (102/255) of the respondents suggested that butchers have necessary tools 93.7% (239/255) and 1.6% (4/255) of the respondents consume meat in the form of cooked and fried respectively.

The challenge constraints and opportunities

All 100% (255/255) of the respondents confirm the occurrence of cattle disease in the study area. The majority 91.8% (234/255) of the respondents know the name of the disease locally (Lumpy skin disease, contagious bovine pleuropneumonia, pasteurellosis, foot and mouth disease, Ticks, and trypanosomiasis) and 79.6% (203/255) of cattle owners use traditional medicine for their cattle disease prevention and treatment. In the study area, only 20.4% (52/255) of the respondents did not use traditional ways for the prevention and treatment of their animals. The level of disease severity trend is medium according to responses from the cattle owners at 48.2% (123/255), higher 32.9% (84/255), and lower 18.9% (48/255).

The result revealed that 48.2% (123/255) of the respondents indicated that they buy a drug from a drug shop and administered it by themselves and only 18.8% (48/255) of the respondents took their animals to animal clinics. Biting flies are a major problem in the study area and 94.5% (241/255) of the respon-

dents witnessed that flies are affecting/disturbing their cattle during the night time and the fly population is difficult during the wet 85.9% (219/255) and at the dry season 12.4% (31/255). Farmers were awarded about treatment heal their animals and 64.3% (164/255) of the respondents confirmed that they had a habit of treating animals. From the study area, bacterial, parasitic, and viral disease are the major causes of death for their cattle and accounts for 28.2% (72/255), 20.4% (52/255), and 7.8% (20/255) respectively. More than half 58% (148/255) of the respondents witnessed that their animals are not vaccinated in the past five years and 42% (107/255) of the respondents responded that they got two vaccines in the past five years for their cattle.

Most of the owners (61.2% (156/255) responded that there were no government or non-government organizations' interventions about their animal diseases. But, 38.8% (99/255) of the respondents witnessed that there is government intervention against animal diseases. From the result, poor extension service is done so far by the government and 39.6% (101/255) stated that they got different pieces of training from the government and non-governmental organizations.

Technical constraints

Key informant interview indicated, "There was an effort in the past to identify and eliminate [minimize] constraints we have on animal health and disease aspect. Logically, the disease can be controlled and even prevented before it turns into damaging the more productivity-and health management. Concern has, however, been expressed that in Africa this "preservationist" approach may have been over-emphasized at the expense of "husbandry" constraints. Management constraints relating both to technical problems (e.g., health, nutrition) and resource utilization may have been unduly neglected.

The components of these technical constraints are very well known to veterinarians and animal scientists and are therefore not discussed any further. However, the complex interactions among these factors could represent an important set of constraints. The identification of these interactions is one of the challenges to the regional livestock and fishery services of Gambella. Another problem dealt with here involves the input side of livestock production, particularly animal feed, but the principles mentioned are equally applicable.

Table 7. Major animal health problems in the study area (n=34)

Major Animal Health Problems Identified	Major Symptoms	Mean rank	Overall Rank
Contagious bovine pleuropneumonia (CBPP)	Chest pain and nasal discharge	4.96	1st
Trypanosomosis	Emaciation	5.59	5th
Foot and mouth disease (FMD)	Lameness	5.61	6th
Bovine pasteurellosis	Depression & coughing	5.05	2nd
Anthrax	Sudden death and bleeding	6.65	9th
Blackleg	Depression and lameness	7.78	10th
Lumpy skin diseases (LSD)	Skin granules and swelling	5.09	3rd
Fasciolosis	Emaciation and diarrhea	6.63	8th
Mastitis	Blind teat and pain during touch	5.68	7th
Neonatal diarrhea	Severe diarrhea and death	7.88	11th
Ectoparasites (ticks)	Emaciation/weight loss/	5.52	4th

Table 8. Production challenges

Challenges	Indicators	Consequences
Seasonal occurrence of diseases	At the entry and end of the rainy season, animals get sickness	Reduce production and animals death
Breeding	Only local breeds are available and no breeding strategy naturally or artificially	No improvement in production and productivity
Management	Very poor cattle management practice followed by owners	Animals suffer and reduce their production and productivity
Technology	No new technologies so far	No improvement in production
Theft	Stealing cattle in the nighttime and at grazing areas	Cattle owners are disappointed and stop rearing cattle
Awareness about production	Lacks training on the area cattle production in the study areas	No production improvement
Seasonal fluctuation of forage	Shortage of feed in the dry season and lack of forage preservation	Production reduces
Medication	Due to the absence of drugs, sick animals die in the host and refugee communities	Loss of cattle
Vaccination	Vaccination is very limited in the host community and no vaccination campaign in the refugee community so far	Animals get sick and production reduction
Flooding	During the wet season, the flood covers the grazing land, and biting insects affect the animals during night time	Animals feel discomfort, lose production, and become sick
Lack of supervision and absence of a skilled veterinarian	Cattle owners administer different drugs to their animals by their selves	Creates drug resistance microorganisms in the area
Absence of infrastructures	for instance, cattle pen/shade (Barn or stall especially refugee camps), veterinary clinics, installed vaccination and breeding crushes	Animals are easily affected by sunlight, rain, and disease

The key informant interviewee indicated that

"Theft is the major challenge in the camp animals/ cattle are stolen at grazing area during day time and at the shed in the evening by the refugees and the refugees had linkages with the host community and sell the stolen cattle to the communities. But, due to the creation of strong communication with the woreda and kebele administration, most of the stolen animals returned to their owner".

Opportunities

The opportunities available to the livestock and veterinary services in the study area to increase livestock output through the removal of constraints (or their adverse effects) form part of the current challenge. The magnitude of additional products which could be expected from preventing or reducing loss and wastage, from disease control or eradication, or productivity-enhancing husbandry could well serve as an impetus to the livestock and veterinary services. Let us look at a few quantified examples from recent studies on different sites. In summary, we should seize every opportunity to increase production by:

- Eliminating waste
- Minimizing losses
- Enhancing productivity.

Table 9. Cattle production opportunities in the study areas

Opportunities	Indicators
Cultural value	Marriage in the Nuer community
Disease tolerant breeds	Cattle are trypanosomosis tolerant
Presence of the Baro river	The yearly flowing of the river in the region
Presence of grazing land	Moving from place to place in different seasons
Manpower	No problem for keeping animals from the householder
Productivity	Compared with other local breeds
Market demand	The need for the animal product is high
Indigenous knowledge	Traditional knowledge for disease treatment and management

Discussion

From the study, it was observed that a total of 8258 cattle of which 8146 were Nuer breed cattle and 112 were Felata breed and from the total cattle population, 743 are oxen, 728 bulls, 3,888 cows, 1339 heifers, and 1,560 calves. An-

other study by GebreMariam *et al.* (2013) indicated that Ethiopia's cattle herd structure features relatively high male representation (44.5% of the population), and the largest proportions for both sexes fall into the 3–10-year age category. This is an indication of the uses to which the animals are put: oxen for draft power and cows for milk production. But, in the study area, bulls/ males are kept for reproduction, meat, and dowry purpose.

A study by Alemayehu (2005) showed that forage crops are commonly grown to feed dairy cattle with oats and vetch mixtures, fodder beet, elephant grass mixed with siratro and desmodium species, Rhodes/ lucerne mixture, phalaris/ Trifolium mixture, hedgerows of sesbania, Leucaena, and tree-lucerne being common ones. Whereas, the present study indicated that 92.5% of cattle owners practiced a free grazing system followed by 5.1% tethering and 1.6% semi-grazing, 0.8% zero grazing, and 62.4% (159/255) of the respondents indicated that they didn't grow feed, crops or pasture for their livestock by their own. But, 37.6% of the cattle owners practiced providing crop residues to their cattle as feed during the shortage of pasture.

The study had similar nature to that of Bizelew *et al.* (2016) in which Seasonal fluctuations in the availability and quality of feed inflect serious changes in livestock production. Most 86.3% (220/255) of the respondents did not purchase feed for their animals. 77.3% (197/255) of the respondents indicated that there is a feed shortage in the dry season than in the wet season of the study area and only 22.7% (58/255) of the cattle owners witnessed there is no seasonal feed shortage in their localities. Only 3.1% of the respondents indicated the purchase of feed supplements and feed additives. Whereas, the majority (96.9%) of the respondents witnessed that no purchase of feed supplements and additives was practiced for their animals.

The above result is supported by studies from other parts of Ethiopia and Most (81%) of farmers feed their animals free grazing from the open environment traveling from highlands and midlands to lowlands in search of adequate feed (different species of grasses) and crop residues during the wet season up to the beginning of the dry season (Welay *et al.,* 2018).

One of the key informants stated that 'Availability, quality, and quantity of feeds vary among various production systems. Cattle largely depend on range-

land grazing or crop residues that are of poor nutritive value. Feed is not uniformly supplied and the quality is poor. Natural pasture browses, and bushes account for the major food sources of livestock owned by agro-pastoralists. Of the total respondents, 18% (46/255) of them replied that they practice feed storage to preserve feed for their animals for the dry season. But, the majority (82%) of the respondents did not have feed storage for their animals or feed preservation experience. The remaining (6.3%) and (93.7%) respondents indicated that on-site and river/pond/spring were the source of water for their animals respectively.

In sub-Saharan Africa, technical constraints remain a major impediment to livestock development. The main constraints identified from the study can be grouped in the following broad categories: Animal feed and nutrition; genetic factors; animal health and disease problems; and inadequate management practices. A similar study by Tadesse (2014), Andualem (2016), and Beyene *et al.* (2017) indicated that animal healthcare and improved health management is also one of the major constraints of dairy development in Ethiopia, which caused poor performance across the production system. Many of the problems resulted from the interaction among the technical and non-technical constraints themselves. For instance, poorly fed animals have low disease resistance, and fertility problems, partly because the animal healthcare system relays heavily on veterinary measures.

Conclusions

Results of the study indicated that cattle play a significant socio-economic role in the study area, including the generation of income for households, food, and dowry. The potential for cattle production and productivity are proportionally hampered by various cattle management problems including the prevalence of major endemic diseases, poor feeding, high stocking rate on grazing lands, lack of support services such as extension services, veterinary services, insufficient data to plan improved services and inadequate information on how to improve animal breeding, marketing, and processing. The majority of the respondents used traditional medicine to treat their sick animals.

Therefore, based on the conclusion, it is strongly recommended that a detailed study of different agro-ecologies of the region is imperative to investigate the productive and reproductive performance of cattle, characterization of existing breeds to ascertain the different traits that will give a better performance

which will help in developing future intervention, research on cattle marketing system play a vital role in helping farmers and provision of strong extension services and training on improved forage cultivation, cattle production and management practices in the different parts of the region.

The distribution of livestock diseases is substantially higher. Management practices in livestock production were poorly practiced. This is the greatest threat to livestock production and productivity. More fundamentally, investment in animal infections and infestations control and management practice strategies is necessary to reduce the multiple impacts of livestock diseases and deaths on animal health and production through effective and timely vaccinations. The poor fertility and poor livestock performance can be addressed through better management and crossbreeding with improved breeds.

Acknowledgments

We are highly indebted to give our thanks to the woreda level Livestock and Fishery Bureau, woreda experts, the RCC members from refugee camps, and the regional livestock and fishery office.

References

Alemayehu, M., 2005. Feed resources base of Ethiopia: Status and opportunities for integrated development. Proceedings of the 12th Annual Conference of the Ethiopian Society of Animal Production (ESAP). Addis Ababa, Ethiopia. Pp. 377-386.

Alemu, J., Tilahun, K. and Kumar, P., 2019. Indigenous Knowledge and Practices on Medicinal Plants used by Local Communities of Gambella Region, South West Ethiopia. IJTDH. 39, 1-14.

Andualem, T., 2016. A review on cattle husbandry practices in Ethiopia. Int. J. Livest. Prod. 7, 5-11.

Beyene, T.J., Asfaw, F., Getachew, Y., Beyene, T., Collins, I., Beyi, A.F. and Revie, C.W., 2017. Improving cattle disease reporting and surveillance in Ethiopia using the smartphone-based application. Proceedings of the 3rd International Conference on Animal Health Surveillance, Rotorua, New Zealand. Pp.1-4.

Bizelew, G., Ajebu, N., Getnet, A. and Getahun, A., 2016. Assessment of Livestock Feed Resources in the Farming Systems of Mixed and Shifting Cultivation, Gambella Regional State, Southwestern Ethiopia. GJSFR D Agric. Vet., 16, 1-10.

CSA (Central Statistical Agency), 2013/2014. Population Projection of Ethiopia for the Year 2014. Federal Democratic Republic of Ethiopia, Central Statistical Agency, Addis Ababa, Pp4-38.

CSA (Central Statistical Agency), 2016. Agricultural Sample Survey Report on Livestock and Livestock Characteristics. Volume II, Addis Ababa, Ethiopia. Pp.190.

GebreMariam, S., Amare, S., Baker, D., Solomon, A. and Davies, R. 2013. Study of the Ethiopian live cattle and beef value chain. ILRI Discussion Paper 23. Nairobi: International Livestock Research Institute. Pp. 1-48.

Metaferia, F., Cherenet, T., Gelan, A., Abnet, F., Tesfay, A., Ali, J.A. and Gulilat, W., 2011. A Review to Improve Estimation of Livestock Contribution to the National GDP. Ministry of Finance and Economic Development and Ministry of Agriculture. Addis Ababa, Ethiopia. Pp. 1-43.

Steinfeld, H., Wassenaar, T. and Jutzi, S., 2006. Livestock production systems in developing countries: status, drivers, trends. Rev. Sci. Tech. Off. Int. Epiz. 25, 505-516.

Tadesse, B.T., 2014. Prevalence of the major infectious animal diseases affecting livestock trade industry in Ethiopia. JBAH, 4, 1-8.

Ugo, P.C., Derek, B., Nancy, M. and Alberto, Z., 2011. Measuring the contribution of livestock to household livelihoods. A livestock module for multi-topic household surveys. Joint paper of the World Bank, FAO, AU-IBAR, ILRI with support from the Gates Foundation. Uganda, Pp. 1-28.

Welay, G., M., Dawit, G. T., Gebreyohans, G. T., and Shishay, K. W., 2018. A preliminary survey of major diseases of ruminants and management practices in Western Tigray province, northern Ethiopia. BMC Vet. Res. 14, 293.

Yamane, T., 1967. Statistics: An Introductory Analysis, 2nd edition, New York: Harper and Row. Pp. 178-184.

Participatory epidemiological study on the burden of rabies in animals and humans in three districts of Buno Bedele Zone, West Ethiopia

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Abstract

Rabies is one of the priority zoonotic diseases and a major public health challenge in Ethiopia. Dog plays an important role in the transmission of the disease to humans. With this point in mind, this study was conducted in selected districts of Buno Bedele Zone namely Bedele, Gechi, and Dabo Hana districts, Western Oromia regional state from December 2019 to April 2020. The objective of the study was to know the status and burden of rabies in the community using the participatory approach and retrospective record assessment from animal and human health facilities. Twelve interview meetings were conducted in 12 *Kebeles*, which involved 156 informants. In this survey authors learned that dogs were the species most affected by rabies followed by cattle, human, cat, equine, and shoats. Accordingly, rabies in dog had an average score of 64 out of 100 with a range of 50-80. Besides, rabies outbreak was frequently noted between June to October in the study areas. Slaughter and sharing of the meat for household consumption was the most common practice taken to salvage bitten cattle. As per the available record in the study area, the estimated rabies cases incidence was 1.75 bovines, 18 dogs, 2.37 equines, 2.28 cats, and 0.37 shoats per 10,000 animals annually. The annual average post-exposure rabies vaccinations records were 75, 39, and 63 in Bedele, Dabo Hana, and Gechi districts, respectively. On the other hand, the average annual rabies death in humans was 2.2, 1.4, and 1.8 in Bedele, Dabo Hana, and Gechi districts, respectively. In general, this study shows that rabies is a disease that worth serious attention in the study areas.

Keywords: Burden; Ethiopia; Participatory epidemiology; Rabies.

Introduction

Rabies is a viral disease that affects warm-blooded mammals. Globally, human mortality from endemic canine rabies was estimated to be 55,000 deaths per year and 56% of the estimated deaths occur in Asia and 44% in Africa (WHO, 2007). In developing countries, about 98% of rabies human cases occur from dogs, many of which are stray dogs (Ali *et al*., 2013). Domestic dogs are the main cause of (>90%) human rabies in Africa. Once the symptoms start to appear, the disease ends almost all the time fatally (Tschopp *et al.*, 2015). It is a major public health problem in most parts of the developing world, where dogs play the main role as reservoirs and transmitters of the disease to humans. Traditionally dogs and to a minor extent cats are considered the main source of rabies but also wild animals are so (Esayas *et al.,* 2012).

The *Lyssavirus* (bullet-shaped RNA virus) causes rabies. It has two forms; furious and paralytic. Lipid solvents, low pH, and sunlight destroys the virus but it is resistant to some disinfectants. Rabies is transmitted only when the virus is introduced into a bite wound or mucus membranes. The transmission is based on the magnitude of the bite and the amount of virus in the saliva. The susceptibility to rabies infection depends on animal species, age, the strain of the virus, dose of the virus, and routes of exposure (Hemachudha *et al*., 2002).

In Ethiopia, rabies is a well-known and serious illness that has been around for generations (Fekadu, 1982). Nationwide data on rabies are not available to reveal the actual magnitude of the problem. However, the distribution of vaccines to the various regions and the fragmented reports on human and animal rabies cases are strong indicators of the widespread nature of the disease in the country (Deresa *et al*., 2010). Annually around 76 persons per million of the population have been getting anti-rabies post-exposure treatment in Ethiopia (Ramos *et al.,* 2015). An examination of the rabies situation in Ethiopia indicated that between 1990 and 2000, there were 2172 verified instances of animal rabies in and around Addis Ababa, and 73.5% of respondents said that dogs constitute a major source of the disease's spread among people (Ali *et al*., 2010).

Rabies disease is becoming a big problem and social issue in Buno Bedele Zone, where yet there is no study conducted on the burden of rabies in the Zone. Be-

dele Regional Veterinary Laboratory Center received 2-3 rabies outbreaks reports annually. At the national level, the multi-sectoral Dog-mediated Rabies Prevention and Elimination strategy plan 2018-2030 has been developed and endorsed for implementation. This study aimed to assess the burden of rabies in animals and humans in study areas using the participatory epidemiology method and provide baseline information for the implementation of a rabies control and prevention strategy plan.

Materials and methods

Study areas

The study was conducted from December 2019 to April 2020 in selected districts of Buno Bedele Zone namely Bedele, Gechi, and Dabo Hana, Oromia regional state, Ethiopia. Bruno Bedele Zone is located in the western part of Ethiopia at a 483 km distance from Addis Ababa found on the main highway to Gambella Regional state (Figure 1).

The livestock population of Bedele district is estimated to be 172,272 cattle, 27,931 sheep, 29,734 goats, 12,441 equines 97,279 chickens, 8,181 dogs, 7,667 cats. Participatory Disease Surveillance (PDS) study was done in Bedele town, Shobe, Yeballa, and Mirga Mute *Kebeles (Kebele* is a small administrative unit in Ethiopia*)* The livestock population of Gechi district is estimated to be 78,576 cattle, 22,428 sheep, 25,516 goats, 9,966 equines 44,962 chickens, 3,418 dogs, 1,852 cats. The livestock population of Dabo Hana district is estimated to be 78,576 cattle, 22,428 sheep, 25,516 goats, 9,966 equines 44,962 chickens, 3,418 dogs, 1,852 cats. A Participatory Disease Surveillance study was conducted in Kone town, Dahe, Lillo, and G/Amuma *Kebeles* (LRDO, 2019).

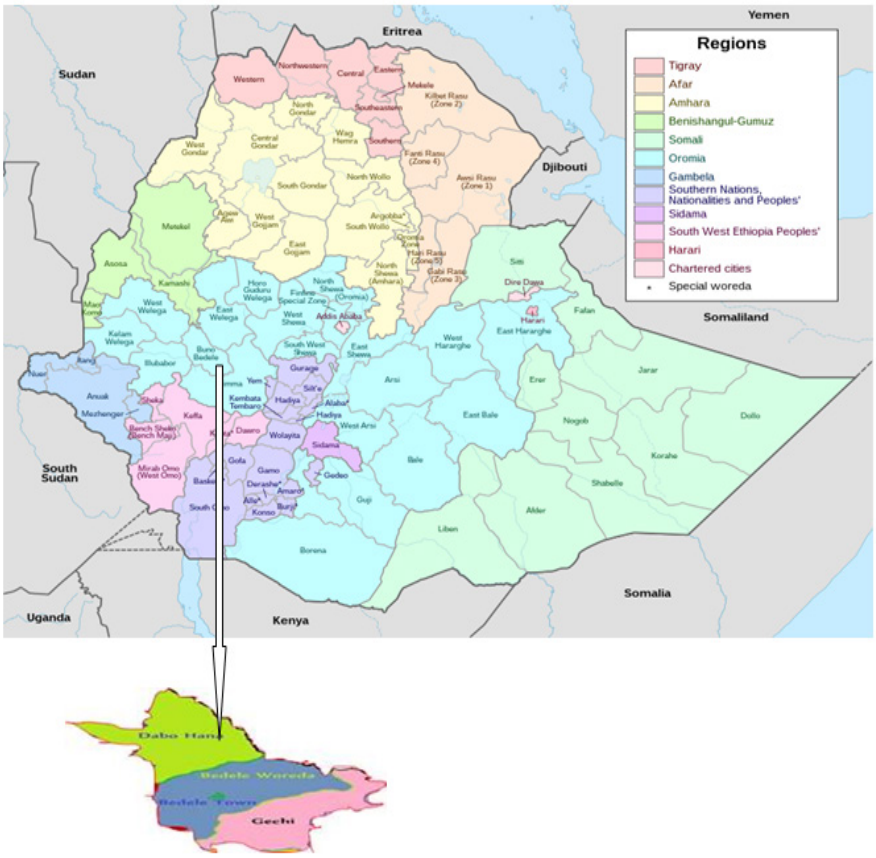


Figure 1: Map of study districts

Study populations

All dog and cat owners found in twelve *Kebeles* distributed in Bedele, Gachi, and Dabo Hana districts were considered as the study population. In all study areas, most dogs were roaming during the day and came back home at night. The most benefit obtained from dog keeping is guardian for crop fields and for homesteads from monkeys and baboons, which are the most predominant threat to their crops.

Study design

Standard Participatory Diseases Surveillance tools such as simple ranking, proportional piling, and matrix scoring were applied to generate evidence during facilitated discussion. The simple ranking was used to identify animal susceptibility and assess awareness of society on rabies by writing variables on a card, proportional piling to identify and rank major dog diseases using 100 beans, and matrix scoring using 30 beans to rank seasonal occurrence of rabies. The variation in scoring proportional piling was also captured by recording the difference between the highest and lowest score ranges. Data were collected from case books of veterinary clinics and human health centers to triangulate the information generated via participatory epidemiology.

Methodology

A total of 12 PDS interview meetings each containing 10-15 informant members were conducted in three districts and a total of 156 participants were involved in three districts. Four *Kebeles* (3 rural *Kebeles* and one town *Kebele*) were identified in each district to conduct PDS interviews. Community informants purposively identified those residents who own dogs. Each interview meeting lasted 2-3 hours. The PDS exercises were facilitated by two experts (one moderator and one record-keeping person).Variables: in the simple ranking (susceptibility of animals, comparison of cultural and vaccine (post-exposure prophylaxis), decision practice of rabid animal), proportional piling (major dog diseases), and matrix scoring (seasonal occurrence of rabies).

Data analysis

Collected data were managed in Microsoft Office Excel, summarized in tables, percentages, and descriptive analysis to describe central tendency (average) and ranges (Catley, 2005). In the simple ranking, the most frequently occurring variable (Mode of central tendency) was used and ranked 1st, 2nd, 3$^{rd.}$ and 4th. For proportional pilling 100 beans were used, and the scores for each variable were added up and divided by the number of interviews (PDS) to obtain the average score. In matrix scoring, 30 beans were used in each Peasant association to score the seasonal occurrence of rabies. In each Peasant association, 30 beans were divided and scored by farmers based on the incidence of rabies cases in four seasons a year- spring, winter, autumn, and summer. Accordingly, from four Peasant associations of each district 120 scores of beans were recorded.

Results

Participatory interview outputs

The number of informants in the 12 PDS interview meetings was 156 (29 females and 127 males) inhabitants in 12 *Kebeles* (9 rural and 3 town *Kebeles*). Informants responded that dogs are the species most affected by rabies followed by bovine, human, cat, equine, and shoat (Table 1).

Table 1: Simple ranking of livestock susceptibility to rabies

Affected Species	PDS interview													
	Dabo Hana District (PA)				Gechi district (PA)				Bedelle district (PA)					
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	Mode	Rank
Dog	1	1	1	1	2	2	1	2	2	2	2	1	1	1
Bovine	2	2	2	2	3	1	3	3	3	1	1	2	2	2
Human	3	4	4	3	1	3	2	1	1	3	3	3	3	3
Cat	4	3	3	4	5	4	5	4	4	4	4	4	4	4
Equine	6	5	5	5	4	5	4	5	5	6	5	5	5	5
Shoat	5	6	6	6	6	6	6	6	6	5	6	6	6	6

Informants indicated that food animals suspected of rabies infection were slaughtered and the meat was distributed among the community for consumption (Vernacular name called Kircha). When high-value farm animals were suspected of rabies case, informants responded that treating with cultural medicine ranked the second alternative because they believe that cultural medicine can cure even after the animal start to show clinical disease. Restraining suspected animals under a tree or around the homestead and taking them to a veterinary clinic were ranked third and the fourth decision that the community practice (Table 2). They prefer to give cultural medicine to humans bitten by a rabid animal because most informants believe that cultural medicine has a better remedy than post-exposure prophylactic vaccine (PEP).

Table 2: Simple ranking of informants' practices against suspected rabies-infected humans and food animals

Informants' awareness of rabies	PDS interview													
	Dabo Hana District(PA)				Gechi district (PA)				Bedelle district (PA)					
Human PEP Vaccine vs cultural medicine	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	Mode	Rank
Cultural medicine	2	2	1	2	2	1	1	1	1	1	1	1	1	1
PEP Vaccine	1	1	2	1	1	2	2	2	2	2	2	1	2	2
The decision against suspected rabid food animals														
Slaughter and eat meat	1	1	2	1	2	2	1	1	2	1	2	1	1	1
Give culural medicine	2	2	1	3	3	1	3	2	3	2	1	2	2	2
Restraining in one place	3	4	4	2	1	3	2	3	1	3	3	3	3	3
Take to the veterinary clinic	4	3	3	4	4	4	4	4	4	4	4	4	4	4

List of dog diseases and severity score

Informants identified dog diseases in their area and they scored rabies the top disease followed by mange mites, endoparasite, and fleas. The average score for Rabies was 64 with a range from 50-80 piles. The second dog disease was "Mange mites" with an average score of 20 and the range was from 0-40. Ticks and fleas were the least important among listed dog diseases (Table 3).

Table 3: List of major dog diseases and severity scores using proportional piling scores

Dog diseases	PDS interview													
	Vernacular name	Dabo Hana District (PA)				Gechi district (PA)				Bedele district (PA)				
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	Ave	Range
Rabies	Dhukuba Saree	71	75	65	70	80	60	50	80	53	50	50	60	64	50-80
Endo parasite	Qooqsaa	7	15	20	18	0	5	0	5	30	24	0	25	12	0-30
Mange	luqqisaa	16	10	0	12	15	25	30	0	0	16	40	0	20	0-40
Flea	Tafkii	6	0	15	0	5	10	20	15	17	10	10	15	10	0-20

Seasonal occurrence

All focused group discussion teams scored seasonal occurrence of rabies using matrix scoring. Accordingly, rabies outbreak occurrences from higher to lower order were: Summer 156 (43.3%), Spring 107 (29.7%), Autumn 55 (15.3%), and Winter 42 (11.7%) (Table 4).

Table 4: Seasonal occurrence of rabies outbreaks using matrix scoring

Districts	Seasons			
	Winter (Bona)	Autumn(Arfaasaa)	Summer(Ganna)	Spring (Birraa)
Bedele	13	21	51	35
Dabo	17	15	47	41
Gechi	12	19	58	31
Total	42	55	156	107

The PDS teams responded that rabies outbreak occurrence was commonly in the big rainy season from June to October and less common in the dry season from December to March (Figure 2).

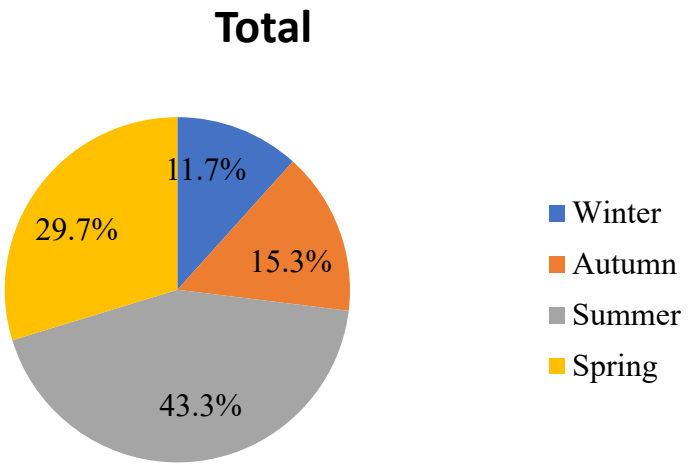


Figure 2: Seasonal rabies occurrence scores

Recorded data from veterinary clinics

Recorded data of suspected rabies cases from the year 2015-2019 indicated that estimated rabies cases of 1.75 bovines, 18 dogs, 2.37 equines, 2.28 cats, and 0.37 shoats had been recorded per 10,000 animals annually (Table 5). However, there was no laboratory confirmation for any of the recorded cases.

Table 5: Suspected rabid animal cases data from veterinary clinics from 2015-2019

District	Five years of suspected rabid animal cases					
	Bovine	Shoat	Equine	Dog	Cat	Total
Bedele	136	5	19	65	7	**232**
Dabo	97	3	14	51	4	**169**
Gechi	89	6	11	44	5	**155**
Total	**323**	**14**	**45**	**162**	**12**	556

Recorded data from human health centers

Annually 75, 39, and 63 post-exposure rabies vaccines were provided for dog-bitten humans in Bedele, Dabo Hana, and Gechi districts, respectively. Of

the 892 humans vaccinated with the post-exposure prophylactic vaccine, 62% (553/892) were male and 38% (339/892) were females (Table 6). On average annually 4.33, 5.27, and 3.50 human rabies deaths per 100,000 people were recorded in Bedele, Dabo Hana, and Gechi districts, respectively (Table 6).

Table 6: Post-exposure vaccination recorded data from health centers

Health center	Post-exposure vaccination													Suspected deaths		
	2015		2016		2017		2018		2019		Total					To tal
	M	F	M	F	M	F	M	F	M	F	M	F	To tal	M	F	
Bedele	38	23	26	14	46	23	57	43	61	48	228	151	379	7	4	11
Dabo	15	11	32	12	27	15	25	19	23	18	122	75	197	5	2	7
Gechi	34	18	41	21	41	24	49	24	38	26	203	113	316	6	3	9
Total	87	52	99	47	114	62	131	86	122	92	553	339	892	18	9	27

Discussion

Twelve interview meetings comprising 156 informants were conducted in 12 *Kebeles*. Dogs were the species most affected by rabies. Rabies was the most common dog disease with an average score of 64 and 50-80 range scores. The most common months of rabies occurrence were from June to October. Animal rabies cases were estimated at 1.75 bovines, 18 dogs, 2.37 equines, 2.28 cats and 0.37 shoats had been recorded per 10,000 animals annually. The annual average post-exposure rabies vaccinations records were 75, 39, and 63 in Bedele, Dabo Hana, and Gechi districts respectively. Also, the average annual rabies death in a human was 2.2, 1.4, and 1.8 in Bedele, Dabo Hana, and Gechi districts respectively.

The current study showed dogs were the highest rabies-affected animals followed by bovine, human, cat, equine, and shoat. This could be because dogs serve as crop guards from Monkey and Baboon where they could be exposed to infected foxes in the study areas. At night, dogs stay outdoor as a home guard and were exposed to the risk factors of rabies more than other domestic animals, which is in agreement with Jemberu *et al*. (2013. This study showed that rabies occurred commonly in the big rainy season from June to October and fewer scale outbreaks from December and March. This might be due to the high movement of stray dogs in search of food and breeding season which bring

together several dogs and exacerbate the spread of the infection. This finding is in agreement with the previous reports who found the seasonal occurrence of rabies outbreaks between July and September (Moges, 2015; Helao *et al*., 2019).

Slaughtering and sharing rabies suspected food animals for consumption was the most common practice. No rabies cases, nevertheless, were reported from individuals who consumed suspicious cattle. This might be due to the lack of public awareness about the potential risk of rabies transmission through the handling of infected animal carcasses in the study areas, which is in agreement with WHO (2012). When butchering and preparing the flesh of rabid cattle, people do not use personal protective equipment, and even they are regarded as being culturally protected from contracting rabies. There had not been any human case of death occurred due to consumption of rabid cattle meat which is in agreement with WHO (2018) that showed no human cases have been documented following the consumption of cooked meat from a rabid animal. However, butchering or eating rabid animal meat may potentially transmit rabies.

Most PDS teams ranked cultural medicine as better than post-exposure prophylactic vaccination and thought that cultural medicine was more accessible and easy to administer, which they believe can protect animals and humans after exposure. This agrees with the reports of Aga *et al*. (2015). Ali *et al*. (2010) also reported that 58.3% of the respondents had a strong belief in traditional medicine for rabies prevention and treatment.

Rabies exposure was more common in males (62%) than females (38%). This could be due to the socio-cultural difference among societies. According to Teklu *et al*. (2017), females typically carry indoor tasks such as cooking and childcare; while males typically carry out outdoor tasks like ploughing, crop harvesting, and livestock keeping. This is also true in the current study locations.

Conclusions

This study assessed the status and burden of rabies in three districts of the Buno Bedele zone using the PDS technique and triangulation of five years of recorded data. The study identified major dog diseases and rabies was the most common dog disease. Dogs were the species most affected by rabies followed by

bovine, human, cat, equine, and shoat. It indicated a high incidence of suspected rabies cases in humans and livestock, which might significantly affect the livelihood of the society. A big knowledge gap of rabies diseases in society regarding the transmission, quarantining of suspected cases, the decision taken to the suspected animal, post-exposure vaccine, and cultural medicine was observed. Five years of recorded data of suspected animals from veterinary clinics showed a high occurrence of rabies disease. In addition, five years of recorded data of post-exposure rabies vaccinations from health centers showed high human exposure to rabies disease. In general, this study showed that rabies was an important zoonotic disease in the area and needs special public health awareness. The prevailing traditional salvaging practice of infected cattle and the reduced preference for post-exposure vaccine over traditional medicine is detrimental and demands serious public health awareness intervention.

Acknowledgments

First, I thank my God, the lord of all creations who granted me his blessings throughout my life and gave me the endurance to do and complete this research paper. Importantly, my acknowledgment goes to DTRA project symbol OSRO/RAF/703/USA "Strengthening Epidemio-surveillance Capabilities and Underlying Regulatory Frameworks in Eastern Africa" for funding this specific study.

References

Aga, M., Hurisa, B. and Urga, K., 2015. Current situation of rabies prevention and control in developing countries: Ethiopian perspective. *J. Anc. Dis. Prev. Rem.,* 4(1), 1- 6.

Aluma, A., Saskia, H., Bryony, J., Jeffrey, M., Purvi, M., and Cyrille, P., 2009. Introduction to Participatory Epidemiology and its Application to Highly Pathogenic Avian Influenza Participatory Disease Surveillance A Manual for Participatory Disease Surveillance Practitioners. pp 1-56

Ali, A., Mengistu, F., Hussein, K., Getahun, G., Deressa, A., Yimer, E. and Tafesse, K., 2010. Overview of rabies in and around Addis Ababa, in animals examined in EHNRI zoonosis laboratory between, 2003 and 2009. *Ethiop. Vet. J.,* 14(2), 91-101.

Ali, A., Yimer, E. and Sifer, D., 2013. A Study on knowledge, attitude, and practice of rabies among residents in Addis Ababa, Ethiopia. *Ethiop. Vet. J*., 17(2), 19 – 35.

Catley, A., 2005. *Participatory epidemiology: A guide for trainers.* African Union - Inter-African Bureau for Animal Resources, Nairobi.

Deresa, A., Ali, A., Beyene, M., Newayeselassie, B., Hussein, K. and Yimer, E., 2010. The status of rabies in Ethiopia: A retrospective recorded review. *Ethiop. J. Hlth. Dev*., 24(2), 127-132

Esayas, F., Hiko, A., Ali, A., Kedir, A. and Kokorevica, L., 2012. Infectiousness of brain and salivary gland suspension from rabies suspected dogs. *Int. J. Pub. Hlth. Epidemiol.* 1(3), 031 – 034.

Fekadu, M., 1982. Rabies in Ethiopia. *Am. J. Epidemiol.*, 115 (2), 266-273.

Helao, M., Hikufe, E.H., Freuling, C.M., Athingo, R., Shilongo, A. and Ndevaetela, E.E., 2019. Ecology and epidemiology of rabies in humans, domestic animals, and wildlife in Namibia, 2011- 2017. *PLoS Negl Trop. Dis.* 13(4), e0007355.

Hemachuda, T., Laothamatas, J. and Ruprecht, C.E., 2002. Human rabies: a disease of complex neuropathogenic mechanisms and diagnostic challenges. *Lancet Neurol.* 1, 101109.

Jemberu, WT., Molla, W., Almaw, G. and Alemu, S., 2013. Incidence of rabies in humans and domestic animals and people's awareness in North Gondar Zone, Ethiopia. *PloS. Negl. Trop. Dis.* 7(5), e2216.

LRDO, 2019. Livestock Resource and Development districts (Bedele, Gechi, and Dabo Hana) Office, Buno Bedele Zone, 2019.

Moges, N., 2015. RABIES IN ETHIOPIA: Review Article. *Academic J. Anim. Sci.* 4(2), 74-81.

Ramos, J.M., Melendez, N., Reyes, F., Gudiso, G., Biru, D., Fano, G., *et al*., 2015. Epidemiology of animal bite and other potential rabies exposures and anti-rabies vaccine utilization in a rural area in southern Ethiopia. *Ann. Agric. Environ. Med.,* 22(1), 76 – 79.

Teklu, G. G., Hailu, T. G. and Eshetu, G. R., 2017. High incidence of human rabies exposure in northwestern Tigray, Ethiopia: A four-year retrospective study. *Plos. Negl. Trop. Dis.*, 11(1), e0005271.

Tschopp, R., Bekele, S. and Aseffa, A., 2015. Dog demography, animal bite management, and rabies knowledge, attitude, and practices in the Awash basin, eastern Ethiopia. PLOS Negl. Trop. Dis., 10 (2), e0004471. https://doi.org/10.1371/journal.pntd.0004471.

WHO, 2007. WHO recommendations on rabies post-exposure treatment and the correct technique of intradermal immunization against rabies: Geneva, Switzerland.

WHO, 2012. World Health Organization. Strategic Framework for Elimination of Human Rabies Transmitted by Dogs in the South-East Asia Region. WHO Library.

WHO, 2018. World Health Organization. WHO Expert Consultation on Rabies, third report: WHO Technical Series Report No.1012, Geneva, 2018.

Review Article

One Health – A holistic solution for sustainable management of globalization-driven public health challenges

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Summary

Globalization is an inevitable and extremely complex phenomenon that involves transnational integration of culture, economy, environment, politics, and other social interest. Globally, we are witnessing multitude changes such as a rapid population growth, urbanization, international trade and commerce, agricultural intensification, and encroachment into the natural ecosystem. Further mismatching of food demand and supply, growth disparities, increasing food prices, and over utilization of natural resources are among the challenges to the economic status of a nation and its health sector. The health impacts of globalization can be both positive and negative; of course, its impacts vary based on factors such as geographical location, gender, age, literacy, and socioeconomic status. Globalization has played pivotal role in health improvements via dissemination of new medical knowledge, low-cost health technologies, fast transactions of medical supply and improvement of human rights. Thus it has shown potential positive impacts by minimizing the gaps in health inequalities between rich and poor people in the global South and North and improved the idea of healthcare for all. On the other hand, there are also shortcomings of globalization to global health, such as the spread of infectious diseases due to rapid mobility, which is emerging as the greatest threat to all. The interconnectedness of globalization and One Health is complex. Whereas, globalization is one of the main challenges to ensure global health security. One Health is a remedy to manage the negative health consequences of globalization, especially in least developed world. It is undeniable that the connection between humans, animals, and the environment calls for the attention of multi-sectorial institutes to collaborate to closely monitor and reduce the risks and consequences on health and wellbeing. One Health approach is increasingly recognized and streamlined into national and international plans and strat-

egies for effective management of zoonotic diseases, food safety, antimicrobial resistance, and climate change. Human practices such as, changes in land use and how food is produced are driving ecological and evolutionary conditions that facilitate disease spillover events and contribute to antimicrobial resistance. These changes are occurring rapidly on a large scale, both locally and globally. The pursuit of understanding human, veterinary and environmental health issues separately leads to an incomplete understanding of disease dynamics and, therefore, missed synergy for a joint mitigation of the problems. One Health actions support the primary prevention of such problems, enabling more timely and effective containment and response to public health threats at the human-animal-environment interface. In short, systematic and sustained One Health approach becomes more important than ever in order to promote and ensure health security and avert the negative impacts of globalization. Therefore, there is a need to focus on the creation of socially and environmentally sustainable forms of globalization that provide the greatest benefits and least costs, shared more equitably than the status queue.

Keywords: *Globalization, One Health, Impacts, Opportunities.*

Introduction

Though there is no universally agreed definition of globalization; economists typically use the term to refer to international integration in commodity, capital, and labor markets (Bordo *et al.,* 2003). Globalization has revealed itself in the 19th century and the second half of the 20th century. It has often been marked by periods of accelerated integration and by periods of dramatic reversals (as in the inter-war period) sometimes with devastating outcomes. The two most recent episodes of globalization were characterized by increased integration in trade, capital flows, and movement of labor, although there are differences in the importance that each of these elements played in the two episodes (World Trade Organization, 2007a).

The increasing interconnectedness and interdependence of nations, peoples, and economies are described by globalization. It has played its role in the improvement of health via the dissemination of new health-related research findings, affordable health technologies, and human rights (Labonté, 2015). Globalization is believed to impact a wider range of public health both positive and negative. Globalization plays its role in the creation, exchange, and application of knowledge, ideas, norms, beliefs, values, cultural identities, and other

thought processes as its consequence. in recent decades, the rapid spread of communication and information technologies resulting in a more widespread and intense flow of information across national boundaries via the mass media, advertising agencies, think tanks, consultancy firms, public relations bodies, educational institutions, scientists, and religious groups (Lee, 2000). Globalization also influences lifestyles (for example, diet, and smoking) and health-seeking behavior. Less directly is the impact on knowledge creation and dissemination concerning health and health care through scientific research, policy ideas, training, and business and management practices (Evans and Newnham, 1998). To supplement these principles of state security, analysts have argued, "globalization has made individual human suffering an irrevocable universal concern. While governments continue to be important, global integration of world markets and instant communication have given a role and a profile to those in business, civil society, NGOs, and intergovernmental organizations" (Axworthy, 2001).

Since the patterns of global change are complex, the complicated interconnection of humans, pet animals, livestock, and wildlife and their social and ecological environment is obvious and integrated approaches to human and animal health and their respective social and environmental contexts are required. Zinsstag *et al.,* (2012) also noted that from early historical times, the history of integrative thinking of human and animal health is briefly reviewed and ended with the establishment of universities in Europe and followed by the beginning of comparative medicine at the end of the 19th century. It is well established that humans do not live in isolation, are a part of the large living ecosystem, and are regarded to be interconnected to the inhabiting ecosystem (Scott, 2008) and that is why it is said everything is designed to become interconnected.

Like globalization, One Health is not a new concept and of course, the outcomes of the interaction that exist between ecosystems, animals, and people have modified, and continue to modify in the course of human events and history. A rational and evidence-based argument of the history of One Health should first be established on consensuses of the accepted definition of the term. However, given the many disciplines and sciences involved, coming up with a clear definition is not an easy task. Furthermore, some visionary scholars have, over a long period, tried to create awareness and advance the concept to improve the mitigation of risks and outcomes that arise at the interface between animal, human, and ecosystem health. The One Health concepts and approach of the current century encompass a re-conceptualization of disease mitigation

efforts in response to the increasing ecosystem changes over the past 100 and more years. Changes that is associated with the parallel exponential growth and concentration of the global human population. Therefore, the idea of One Health should have to be recognized as the continuously growing association between animals, humans, and the ecosystem they share (Evans *et al.,* 2014). In conditions in which vaccines and other pharmaceutical resources are unobtainable, some governments are fighting against the pandemic disease tempted to equate biosecurity with border security. Such an approach was taken to the extreme when Ebola struck West Africa in 2014, and it may have seemed at first to be better than allowing freedom of international movement to continue in the face of contagion. Therefore, the objective of this review paper is to assess the interconnectedness and impact of Globalization on One health.

Globalization

The definition of globalization is described in many ways; some define globalization in the way that it has intensified the integration of national economies in the global market through trade, investment, and financial impact. This means there is a strong and complex global exchange of goods, services, finance, productivity, and people. Others define it as the "process of denationalization of markets, laws, and politics in the sense of connecting peoples and individuals for the sake of the common good (Delbrück, 1999). Furthermore, some researchers define globalization as a free, comprehensive, and fast movement, exchange, and transfer of information, knowledge, finances, goods, services, and people between national economies globally. However, globalization is not just a simple phenomenon, and not just an economic process, it contains new trends in the economy, leading to significant changes in the allocations of workforces, reorganization, and relocation of corporations. There is a growing agreement that globalization is a form of accelerated international economic activities that require rapid movement of information, capital, goods, and services, leading to the establishment of transnational structures and the global integration of cultural, economic, environmental, political, and social processes, and global interdependency. This is a more dynamic process than a phenomenon that in itself involves and transfers many aspects of financial, technological, economic, social, cultural, and geopolitical activities. This is process is institutionalized in openness and the strengthening of international understanding in trade, technology, and financial flow that affect the price and allocation of resources, including manpower in a way that reduces the impact of national policies (Frankel and Romer, 1999).

Globalization and human health

For almost 200 years, globalization has been perceived as a positive development, albeit with costs and benefits, and as progress and modernization, a broadening of humanity's scope from the local and parochial to the cosmopolitan and international levels. This attitude has changed dramatically with the Great Recession of 2008, the waves of migration of the last decade, and the emergence of global pandemics (e.g. COVID-19, Swine /Bird flu, SARS, MERS-CoV) For many, globalization is now being viewed as economic dislocation, increasing inequality, unwanted immigration, and being a vehicle for the transmission of disease (Kobrin, 2020). Globalization is a key challenge to public health, especially in developing countries, but the linkages between globalization and health are complex. Although a growing amount of literature has appeared on the subject, it is piecemeal and suffers from a lack of an agreed framework for assessing the direct and indirect health effects of different aspects of globalization (Woodward *et al*., 2001). As borders disappear, people and goods are increasingly free to move, creating new challenges to global health. These cannot be met by national governments alone but must be dealt with instead by international organizations and agreements (Pang and and Guindon, 2004). Woodward *et al,* (2015) state that the links between globalization and health are complex and globalization is a multifaceted phenomenon that can affect health in many ways. Some of the effects of globalization are, high mobility and emerging infectious diseases, illegal trading of drugs and biomedical with potential health risks, and 'brain-drain' in the health sector as a result of labor migration from developing to developed regions. The novel coronavirus (COVID-19) is challenging the world. When there was no vaccine and limited medical capacity to treat the disease, non-pharmaceutical interventions (NPI) is the main strategy to contain the pandemic. Unprecedented global travel restrictions and stay-at-home orders are causing the most severe disruption of the global economy since World War II. With international travel bans affecting over 90% of the world population and widespread restrictions on public gatherings and community mobility, tourism largely ceased in March 2020. Early evidence on impacts on air travel, cruises, and accommodations have been devastating. While highly uncertain, early projections from UNWTO for 2020 suggested international arrivals could decline by 20 to 30% relative to 2019. Tourism is especially susceptible to measures to counteract pandemics because of restricted mobility and social distancing. The WHO has estimated that by the year 2020, non-communicable diseases, such as cancers, diabetes, obesity, and cardiovascular diseases, will cause about two-thirds of the global

disease burden. This rapid increase again illustrates the globalized risks for conditions that are mainly caused by diet, even in less developed countries that have coexistent under-nutrition. There were 151 million cases of diabetes worldwide in 2001, and this was estimated to increase by 46% to 221 million cases in 2010, with the steepest growth in the developing world (Zimmet *et al.,* 2001). The same holds for obesity (Shell, 2001). The global spread of antibiotic-resistant Pneumococcus was first identified in Spain in the early 1980s and rapidly jumped to South Africa and USA before spreading to all other parts of the world. Each year, approximately two million children die in developing countries as a result of lower respiratory infections, mostly pneumonia. The exact origin of this Pneumococcus strain is still unknown but is likely to come from the Far East (McGee *et al.,* 2001). Perhaps the most profound changes that have taken place during the past 10 years have come through the globalization of ideas and information, facilitated through the revolution in information technology. The internet, satellite television broadcasting, and high-speed data links across the globe have dramatically increased access to all kinds of information, even in the most remote corners of the world. Despite early concerns about a 'digital divide' that would further disadvantage the poor, these fears have been overestimated. The reality is that this divide is narrowing between rich and poor countries that have increased access to cell phone communication and the internet (The Economist, 2004).

Globalization is likely to affect many aspects of public health, one of which is vaccine-preventable communicable diseases. Important forces include increased funding initiatives supporting immunization at the global by WHO & FAO level; regulatory harmonization; widespread intellectual property rights provisions through the World Trade Organization agreements; the emergence of developing-country manufacturers as major players in vaccine supply; and the appearance of new communicable disease threats, including those potentially linked to bioterrorism. All of these forces can affect, either positively or negatively, the development and availability of vaccines. Harnessing these will be a challenge for policymakers and immunization stakeholders (Scheffler and Pathania, 2005). There are many studies on the consequences of globalization on the availability of pharmaceutical products (Huynen *et al.,* 2005).

Globalization is causing profound and complex changes in the very nature of our society, bringing new opportunities as well as risks. In addition, the effects of globalization are causing a growing concern for our health, and the intergenerational equity implied by 'sustainable development' forces us to think

about the right of future generations to a healthy environment and a healthy life (Martens *et al.,* 200). As the world around us is becoming progressively interconnected and complex, human health is increasingly perceived as the integrated outcome of its ecological, social-cultural, economic, and institutional determinants. Therefore, it can be seen as an important high-level integrating index that reflects the state and, in the long term, the sustainability of our natural and socio-economic environments (World Bank, 2020a).

It is important to note that global tourism has been exposed to a wide range of crises in the past years (Figure 1). Between 2000 and 2015, major disruptive events including the September 11 terrorist attacks (2001), the severe acute respiratory syndrome (SARS) outbreak (2003), the global economic crisis unfolded in 2008/2009, and the 2015 Middle East Respiratory Syndrome (MERS) outbreak (Gössling *et al.,* 2020, World Bank, 2020a, 2020b). None of them led to a longer-term decline in the global development of tourism, and some of them are not even notable in Figure 1, with only SARS (-0.4%) and the global economic crisis (-4.0%) leading to declines in international arrivals (Frederick, 2005). This would suggest that tourism, as a system has been resilient to external shocks. However, there is much evidence that the impact and recovery from the COVID-19 pandemic is unprecedented. The relationships between pandemics and travel are central to understanding health security and global change (Brown *et al.,* 2016). Although tourism research has developed at least a cursory realization of the potential systemic effects of global climate change, there has not been the same appreciation of the systemic effects of pandemics, with studies tending to focus on individual country impacts, rather than the system-level challenges and vulnerability. Several studies have demonstrated the important role of air travel in accelerating and amplifying propagating influenza and coronaviruses (Gössling *et al.,* 2016).

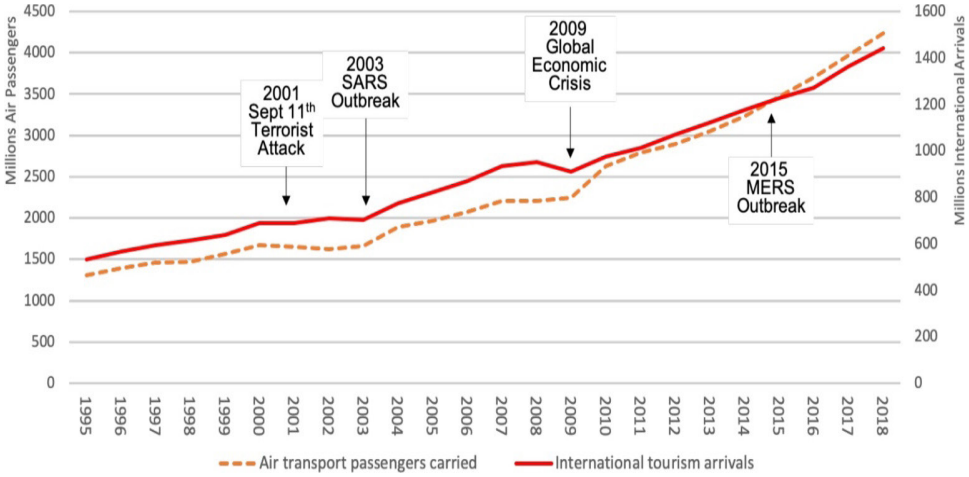


Figure 1. Impact of major crisis events on global tourism (World Bank, 2020a, 2020b).

Most importantly, there have been several warnings that pandemics posed a major threat to society and tourism from both tourism (Scott *et al.,* 2015, Bloom, and Cadarette, 20190) and health researchers (Fauci and Morens, 2012, NASEM, 2018), as well as government agencies (Jonas, 2013) and institutions (Labonte *et al.,* 2014). The main reasons for the increasing pandemic threat in the 21st century are: a rapidly growing and mobile world population; urbanization trends and the concentration of people; industrialized food production in global value chains; increased consumption of higher-order foods including meat; and, the development of global transport networks acting as vectors in the spread of pathogens (Hon, 2013). One of the central realizations of research on pandemics is that travel is central to epidemiology and disease surveillance (Nicolaides *et al.,* 2019). Recognizing that travel and tourism is both a contributor to disease spread and its economic consequences and is dramatically affected by it because of Non-pharmaceutical Interventions (NPIs) (Bai *et al.,* 2020).

The world has experienced several major epidemics/pandemics in the last 40 years, yet none had similar implications for the global economy as the COVID-19 pandemic. COVID-19 is not as contagious as measles and not as likely to kill an infected person as Ebola, but people can start shedding the virus several days in advance of symptoms so that spread rapidly (Rothe *et al.,* 2020, Li *et al.,* 2020). As a result, asymptomatic people transmit COVID-19 before they

know to self-isolate or take another measure like physical distancing in public or wearing mouth/nose coverings to prevent the spread of the virus through speaking, coughing, or sneezing. With very limited testing in many countries, also due to the unavailability of tests, unknowingly asymptomatic transmission is thought to be substantive (ECDC, 2020). There is also a rapid increase in and spread of confirmed COVID-19 cases from its epicenter (Shrestha *et al.,* 2018).

Globalization and animal health

The containment of pandemic threats such as avian influenza and severe acute respiratory syndrome within months of the outbreak is a few examples of successful applications of the One Health paradigm (McMichael, 2004). Understanding this versatile human-animal interface is crucial for characterizing the permanent yet continuously evolving risks of cross-species transmission of pathogens between animals and humans. Humans and microbes are engaged in a moral, self-interested, co-evolutionary struggle (Reperant *et al.,* 2013). The inextricable connectivity between animals, humans, and the environment has been recognized for centuries. Diamond and Ford, (2000) have put forth 'Questions of the animal origins of human disease lie behind the broadest pattern of human history and some of the most important issues of human health today.' The complex relationships between humans and animals have resulted in a human-animal interface since pre-historical times (Mackenzie *et al.,* 2013). There are various emerging and reemerging infections, which are mainly originated from wild, and domestic animals. The incidence or a spread of a pathogen into new areas or a new host species is called "disease emergence" (Hubálek, 2003). Yet, majorities of Zoonoses are not prioritized by health systems and are labeled as "neglected" or "endemic" (Gebreyes *et al.,* 2014).

In recent decades, many infectious diseases have emerged with often devastating consequences for global health and impacts on trade and economies. These include SARS, Nipah virus, avian influenza, pandemic H1N1, and MRSA. Systematic reviews have determined that ~75% of emerging infections in the past two decades are zoonotic in origin (Woolhouse and Gowtage-Sequeria, 2005). Therefore, human-animal linkages play a key role in infectious-disease emergence. Changing agricultural practices and the spillover of pathogens from wildlife reservoirs are both driving the emergence of infectious diseases from animals (Burroughs *et al.,* 2002). Many pathogens exist in wildlife reservoirs often without causing significant morbidity or mortality in the reservoir spe-

cies. As human development encroaches on wildlife habitats, and humans continue to make contact with wildlife through hunting or trading in wild animals, there is an increased risk of pathogen spillover into human populations (Daszak *et al.,* 2000)]. One public health response to changing environmental health risks is to be vigilant for the occurrence of sentinel events that can signal the appearance of a new hazard or a breakdown in ongoing control efforts for a recognized health threat. Animals have served in numerous cases as "sentinels" for environmental threats near where humans are living or working (Van der *et al.,* 1999). A classic example of this is the historical use of canaries by coal miners to detect the presence of toxic gas in coalmines (Burrell and Seibert, 1916). An example of animals serving as sentinels for infectious pathogen hazards in the environment occurred with the 1979 outbreak of anthrax in Sverdlovsk, Russia (Meselson *et al.,* 1994). Climate change is also expected to affect the distribution of vector species and other animals. One predicted effect is an increase in malaria-prone habitats in Africa (Ermert *et al.,* 2011). The same changes may also make formerly habitable areas less hospitable and alter the overall range distribution of a particular arthropod vector (Ahlenius, 2005).

Infectious diseases share common pathophysiology between domestic and wildlife animals. These diseases are not only one of the largest sources of non-tariff barriers to international trade, but also, affect the dynamism and fluidity of the agronomic input markets, either by creating higher costs of production in industries directly dependent on these inputs or generating commercial levies to the export country on certain animal products as a direct consequence of a disease epidemic. As a result, an increase in the complexity of the patterns of consumption and access to competitive global markets is generated. Additionally, affect the monumental annual costs that several countries worldwide spend on control and eradication programs of TADs, as well as, the resilient and compensatory payments by governments to the livestock sector after a disease outbreak (Vandeputte *et al.,* 2011). According to the Organization of the United Nations Food and Agriculture Organization (FAO), pests and animal diseases cause the loss of more than 40% of the global food supply. This is a clear threat to the residual economies of developing countries and the food security of its inhabitants. In general livestock products have increased their international trade by 4% only in the last two decades. However, as a result of the emergence and re-emergence of various animal diseases, such as bovine spongiform encephalopathy (BSE), the annual growth of meat products decreased by 2% in the late 1990s (Morgan, and Prakash, 2006). There-

fore, the cost of trans-boundary animal diseases (TADs) related to agricultural products, the country's economy, and international markets are monumental. Thus, it is very important to create public policies focused to assure countries' food security (especially in developing nations) to avoid negative economic impacts caused by TADs, especially on the more susceptible social stratus.

The globalization era starting in full in the 1990s brought entirely new conditions for epizootic disease spread and control at the global level. Significantly, intensified global trade in animals and their products without any effective sanitary filter has facilitated the mass worldwide spreading of pathogens. The huge daily flow of exported nonpathogenfree animal commodities has led to the rapid deterioration of the global epidemiological situation. The international animal health information system covers only a very small part of animal infections and provides insufficient data on their occurrence. The importing countries have minimal or zero information for objective risk assessment to avoid pathogen introduction (Václav, 2017).

Swanepoel *et al.* (1999) described that Rabies and Marburg are zoonotic diseases with serious health implications for humans and both have a common reservoir, the bat. These two diseases are very good examples for the application of the One Health approach. Globalization also favors the international trade of domestic and wild animals, some of which include illegal trade and importation of wildlife. The magnitude of the global movement of animals is staggering. In terms of sheer numbers, 37,858,179 individually counted live amphibians, birds, mammals including rodents, and reptiles were legally imported to the United States from 163 countries from 2000–to 2004 (Jenkins *et al.,* 2007). There are several unfortunate examples of the failure of partners across these areas to work together. They include governmental decisions in the People's Republic of China to kill dogs as a control measure for rabies and advocating the extermination of storks as a control measure for avian influenza in Thailand (Wildlife Conservation Society, 2007). In the case of the dogs, an integrated team of animal and public health professionals might have implemented alternate control measures, such as leash laws and rabies vaccination of dogs. The storks were luckier in which wildlife conservationists and other partners in the animal health sector eventually intervened to convince governmental authorities that the killings of storks was not an appropriate control measure for avian influenza. Prevention efforts should include reducing both the supply and the demand for exotic animals. However, navigating the myriad responsibilities of the different sectors for human, livestock, companion

animal and wildlife health continues to be a challenge. Guidelines addressing the infectious disease risks associated with exotic animals that may help raise awareness of the risks and decrease the demand for exotic animals have been published (NAPHV, 2006). However, no single agency can solve this problem alone; it is only through partnership with other federal agencies, wildlife associations, veterinary medical associations, and private industry that we will be able to better control the global movement of animals and reduce the risk of introducing emerging infectious diseases into new locations. The "One Medicine Initiative" announced by Roger Mahr, the 2006 President of the American Veterinary Medical Association, has led to the 2007 formation in the United States of a "One Health Task Force" to bring wildlife, environmental, human, and domestic animal sectors together for a coordinated approach to improving and protecting human and animal health (Enserink, 2007). This coordinated approach, actively supported by multiple stakeholders, takes into account the larger ecologic context of infectious diseases and improves our ability to prevent disease rather than simply reacting to new outbreaks as they emerge. The close collaboration of cross-disciplinary initiatives, as well as the efforts of the informed readership of Emerging Infectious Diseases (EID) to make important contributions in stemming the magnitude of live animal trade that poses risks to human, animal, and ecosystem health, is very important (Marano *et al.,* 2007).

Globalization and environmental health

Globalization affects the socio-economic and political aspects of human life and connects world economies via trade, capital flows, innovative opportunities, and cultural ties. It heightens global changes in population size, distribution, mobility, scales, and types of economic activity, all of which have consequences for the environment. Intensified economic and agricultural activities have already resulted in a rapid increase in greenhouse-gas emissions, degradation of land, and depletion of natural resources in addition to environmental pollutions. The emissions of pollutants have further adverse implications for global climate change and ecological imbalance. Moreover, the effects of these emissions may result in lower sustainable economic growth and development through welfare retarding channels (Ganguly, 2003).

Globalization brings new environmental health risks such as the rise of non-communicable diseases (coronary heart disease, cancers) and forms of substance abuse such as alcoholism, and tobacco-related diseases (Feachem,

2001). It is also time that globalization is changing the nature of the infectious disease. Infectious diseases such as tuberculosis, malaria, plague, and cholera are interpreted as re-emerging. For example, the re-emergence of malaria is seen in areas (developed and developing countries) where it had been thought it was eradicated (Baum, 2001). In addition, there are other newly emerging diseases like HIV, Ebola, SARS, and Avian Flu (Lee, 2000). However, with the increase in global travel, tourism, population migration, and displacement, other diseases like malaria and cholera, HIV is proving to be a public and environmental health threat (World Health Organization, 1997). The increase and intensification of worldwide mobility in both people and trade also have key public health risks and implications for the transportation of food (expired/contaminated) and the increased incidence of trans-border food-borne diseases (Reperant and Osterhaus, 2017). The global environmental changes have brought about the intensity of modern consumer driver economics and holding risks for public health. These changes to the earth's basic life-supporting processes pose long-term risks to the health of populations (McMichael, 2004).

Gold mining operations are often associated with spectacular fish kills and consequences to aquatic resources. Most aquatic organisms were killed along the main stem of the Tisza River in Hungary, and most water supplies were closed when a dam failure at a tailing pond in Romania triggered the release of about 100,000 m3 of cyanide-containing waste in January 2000 (Roggla, 2000). More sustainable mining practices require mitigation measures for existing tailings and improved processes and safety procedures for ongoing activities (Dudka and Adriano, 1997). Highly toxic chemicals, such as cyanide or mercury, should be replaced by less harmful extraction agents, such as halogens, or a zero-emission policy should be enforced (Akcil, 2003). Such technical measures should be supplemented by clear international regulations (Hamor, 2004) and corporate social responsibility in the mining industry, which is based on open information policies (Jenkins and Yakovleva, 2006).

Contamination of groundwater from municipal solid waste landfills, hazardous waste sites, accidental spills, and abandoned production facilities is a prominent cause of water pollution. Several hundred thousand sites can be found throughout the world, where 100 million tons of waste have been and still are discarded. Many of them contain large amounts of hazardous or radioactive material (Giusti, 2009). Even though many of the official contaminated sites are under control, the large majority of them are expected to release chemicals into the environment. In addition, thousands of oil, gasoline, and other chemi-

cal spills occur each year on the land and in water from a variety of types of incidents, including transportation and facility releases. Estimating the number and fluxes of toxic chemicals from such contaminated sites to the groundwater is difficult (Baun and Christensen, 2004). In many cases of spills, waste disposal sites, and abandoned facilities, their primary contaminants are chlorinated ethane, PCBs and polychlorinated dibenzo-p-dioxins (PCDDs) from wastes of pesticide manufacturing (Engelhaupt, 2008), methyl mercury from contaminated soils and wastewater (Selin, 2009), radionuclides from former nuclear weapons test sites (Kersting *et al.,* 1999) and radioactive waste repositories (Ewing, 2006), and nitro aromatic explosives from ammunition plants (Eighmy *et al.,* 1995), to name just a few. Discarded materials are, however, often not well characterized and heterogeneous (Baun and Christensen, 2004]. Apart from some predominant contaminant species, the leachate composition from the landfill materials cannot be predicted in detail (Eighmy *et al.,* 1995).

One Health approach as a remedy to globalization driven health challenges

Earlier healers, religious persons, and pioneer physicians have been devoted to the health care of both people and animals until the 18th century when Health Science evolved into veterinary and human medicines (Schwabe, 1984; Driesch and Peters, 2003). A Chinese text by Xu Dachun ('on the origin and development of medicine') from the 18th century states that 'The foundations of veterinary medicine are as comprehensive and subtle as those of human medicine and it is not possible to place one above the other' (Driesch and Peters, 2003). Medical science reached a climax point towards the end of the first millennium with specific hippiatric texts like the Kitab al Baytara in the sphere of Arab influence. Here, veterinary medicine has remained largely in the hands of equerries until the 18th century, whereas, human medicine was integrated into the medieval Universities (Rüegg, 2004). Claude Bourgelat, the founder of the first veterinary school in Lyon in 1762, was heavily criticized when he recommended human clinical training for the veterinary curriculum (Driesch and Peters, 2003). In the 19th century, German Pathologist Dr. Rudolf Virchow developed a strong interest in linking human and veterinary medicine as a form of comparative medicine based on the discovery of similar disease processes in humans and animals with the advent of cellular pathology (Saunders, 2000). The German scientist, Rudolf Virchow proposed the term zoonosis in 1855 (Cardiff *et al.,* 2008), and he was also the first to suggest the term One Health, stressing the need and interconnectedness between human and

veterinary medicine in all aspects (Osburn *et al.,* 2009). Calvin Schwabe (Herbold, 2005) and William Osler (Kahn *et al.,* 2007) have subsequently promoted the one health concept internationally. Globally, the term one health has re-emerged as an innovative solution to combat zoonoses, AMR, food safety issues under the premise that international animal health is a public good. More importantly, some animal diseases, for instance, Rinderpest, rather than human epidemics, were the stimulus for medical research in South Africa, and tsetse fly control was motivated primarily by cattle trypanosomiasis (Dukes, 2000).

The term one health has emerged as a front-runner as an innovative solution to combat trans-boundary animal diseases (TADs) under the premise that international animal health is a public good. The definition of the One Health approach adopted by the European Union as ´´The improvement of health and well-being via (a) the prevention of risks and the mitigation of effects of crises that originate at the interface between humans, animals, and their shared environments, and (b) promoting a cross-sectoral, collaborative, "whole of society" approach to health hazards, as a systemic change of perspective in the management of risks´´(Okello *et al.*, 2017). Furthermore, the Executive Summary of the American Veterinary Medical Association (AVMA) One Health Initiative Task Force (OHITF) defines One Health as ´´The collaborative effort of multiple institutes working at the local, national, and international level to provide the best health service for their people, animals, and the environment´´ (King *et al.,* 2008). Globalization has intensified the links and connectivity among human, animal and environmental health within an ecological setting, aggressively working to assess the effects of leading to the emergence of contagious diseases of animals origins that have impacts on human health and animal health, (Tabor, 2002).

According to WHO (2021), The Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (WOAH), the United Nations Environment Programme (UNEP) and the World Health Organization (WHO) welcome the newly formed operational definition of One Health from their advisory panel, the One Health High Level Expert Panel (OHHLEP), whose members represent a broad range of disciplines in science and policy-related sectors relevant to One Health from around the world. The four organizations are working together to mainstream One Health so that they are better prepared to prevent, predict, detect, and respond to global health threats and promote sustainable development. The One Health definition developed by the OHHLEP states: ***One Health*** *is an integrated, uni-*

fying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems. It recognizes the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and inter-dependent. The approach mobilizes multiple sectors, disciplines and communities at varying levels of society to work together to foster well-being and tackle threats to health and ecosystems, while addressing the collective need for clean water, energy and air, safe and nutritious food, taking action on climate change, and contributing to sustainable development (Figure 2).

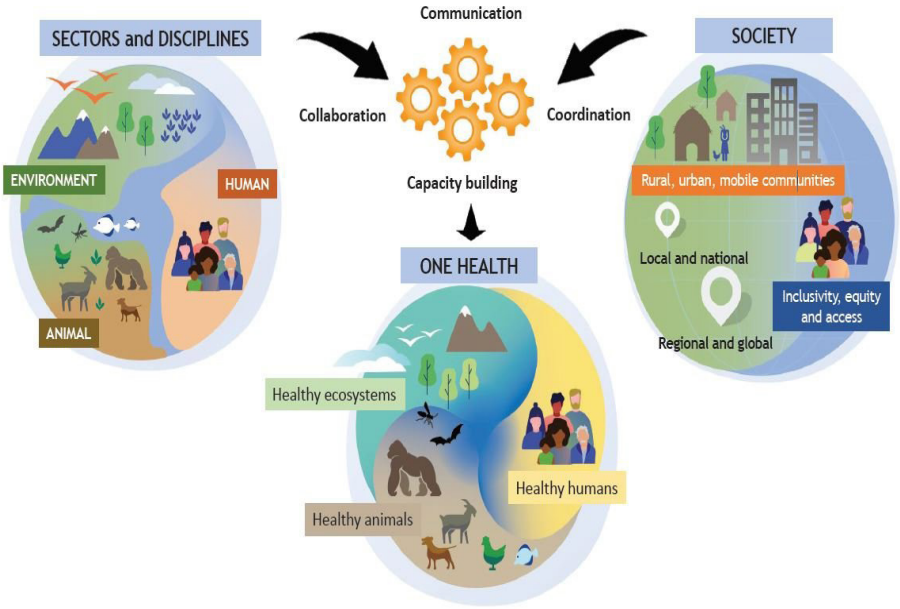


Figure 2. Tripartite and UNEP support OHHLEP's definition of "One Health" (WHO, 2021).

In the increasing globalized world the exponential growth in both human and animal populations, rapidly changing farming systems and urbanization. These have encouraged increased forest encroachment, marked changes in ecosystem. Moreover, this closer interface among humans, animals, and wildlife resulted in the increase of globalization of trade animals and animal products. These characteristics of the present globalized world provide sustained exposure of humanity to myriads of challenges that will require sustainable global

governance and solutions. Among these challenges is the spread of emerging and/or re-emerging infectious diseases in the animal-human-environment interface. These diseases are medically, socially, economically, and environmentally expensive, with their wide consequences requiring interdisciplinary solutions. The "One Health" approach can proffer such solutions, which can be harnessed as a global strategy by health organizations and policy makers worldwide in response to global needs (Bolajoko *et al.,* 2022).

Regarding One Health in Ethiopia, it is at its infant stage to implement the approach to mitigate the existing, emerging and, reemerging infectious diseases. So far, encouraging achievements have been made to advocate for and implement the One Health approach. Some of the developments achieved so far include the establishment of the National One Health Steering Committee and Technical Working Groups, prioritization of infectious diseases of human and animal health importance, the development of prevention and control strategies for prioritized zoonotic diseases, joint disease surveillance and outbreak investigation, capacity building and other One Health promotions. Despite these key achievements made so far, there are still challenges that require additional investments and efforts. Some of the challenges include poor collaboration and cooperation among institutes in data sharing and communication, institutionalization of the One Health, inadequate and sustained advocacy for One Health among health practitioners and stakeholders, shortage of funds to support One Health joint actions including research, etc. Hence, it is critical to continue raising awareness on One Health approach and foster collaboration across disciplines and sectors (Erkyihun *et al*., 2022).

Conclusions

Globalization is rather a complex phenomenon, which goes beyond economic, socio-cultural, and political integration. It is the interactive co-evolution of multiple technological, cultural, economic, institutional, social, and environmental trends. Globalization is associated with several flows that have direct and indirect effects on health, environment, food safety, biosecurity, and tourism. Some argue that globalization has positive effects on development as it gives rise to exposure to new technologies and skills as well as new industries and more jobs in developing countries. Conversely, globalization is also believed to have a downside in that it will force poorer countries to be dependent on developed countries and follow their directions. The risk of spreading drug-resistant in-

fectious agents or pandemic diseases has been heightened with globalization. As the movement of people continues to increase, the circulations of pathogens are also likely to rise. Hence, the trend of globalization has expanded from the impact on the individual to the impact on the community, from the technical health care problems to social problems.

Therefore, provided the fact that globalization has a huge impact (positive impacts of economic social developments, and/ or adverse consequences) on the health of humans, animals and the environment, a systematic and interdisciplinary collaboration through the ONE HEALTH approach is a remedy for reducing the global health challenges due to globalization.

Ethics approval and consent to participate

Not applicable

Availability of supporting data

The data used for this manuscript are available from the authors on request.

Conflict of interests

The authors declare that there is no conflict of interest.

Authors' contributions

FA conceived the concept of the preparation of the manuscript, collected all necessary data, and drafted the paper; while GM and BM are involved in the critical revision of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We would like to thank the College of Veterinary Medicine and Agriculture of Addis Ababa University for the provision of materials.

References

Ahlenius, H., 2005. Climate Change and Malaria, Scenario for 2050 UNEP/GRID-Arendal. Arendal, Norway: GRID-Arendal).

Akcil, A., 2003. Destruction of cyanide in gold mill effluents: Biological versus chemical treatments. *Biotechnol. Adv.,* 21, 501–11.

Axworthy, L., 2001. Human security and global governance: putting people first. *Glob. Gov.,* 7, 19–23.

Bai, Y., Yao, L., Wei, T., Tian, F., Jin, D.Y., Chen, L., *et al*., 2020. Presumed asymptomatic carrier transmission of COVID-19. *JAMA*, 323 (14), 1406.

Baum, F., 2001. Health, equity, justice and globalization: Some lessons from the people's health assembly. *J. Epidemiol. Community Health*, 53, 195 – 196.

Baun, D.L., Christensen, T.H., 2004. Speciation of heavy metals in landfill leachate: a review. W*aste Manag Res*., 22, 3–23

Bloom, D.E. and Cadarette, D., 2019. Infectious disease threats in the 21st Century: Strengthening the global response. *Front. immunol.,* 10, 549.

Bolajoko, M. and Balogun, A.H., 2022. Challenges and future perspectives for the application of One Health, Editor(s): Joana C. Prata, Ana Isabel Ribeiro, Teresa Rocha-Santos, One Health, Academic Press, 329-343.

Bordo, M.D., Taylor, A.M. and Williamson, J.G., 2003. 'Introduction', in Bordo, M.D, Taylor, A.M and Williamson, J. G. (eds): Globalization in Historical Perspective, Chicago and London: University of Chicago Press, 1-10.

Brown, A., Ahmad, S., Beck, C. and Nguyen-Van-Tam, J., 2016. The roles of transportation and transportation hubs in the propagation of influenza and coronaviruses: A systematic review. *J. Travel Med.,* 23 (1), tav002.

Burrell, G.A. and Seibert, F.M., 1916. Gases found in coalmines. Miners' Circ. 14. Bur. Mines, Dep. Inter., Washington, DC.

Burroughs, T., Knobler, S. and Lederberg, J., eds., 2002. The Emergence of Zoonotic Diseases: Understanding the Impact on Animal and Human Health. Workshop Summary. Washington, DC: Inst. Med., Natl. Acad. Press).

Cardiff, R., Ward, J., Barthold, S., 2008. One Medicine—One Pathology': are veterinary and human pathology prepared? *Lab. Invest*., 88, 18–26

Daszak, P., Cunningham, A.A. and Hyatt, A.D., 2000. Emerging infectious diseases of wildlife—threats to biodiversity and human health. *Science*, 287, 443–449.

Delbrück, J., 1999. Globalization of law, politics, and markets implications for domestic law a European perspective. *Indiana J. Glob. Leg. Stud.,* 1(1), 9–36.

Diamond, J. and Ford, L.E., 2000. Guns, germs, and steel: the fates of human societies. *Perspect. Biol. Med.*, 43, 609.

Driesch, A. and Peters, J., 2003. History of Veterinary Medicine. In: Geschichte der Tiermedizin, Stuttgart, *Schattauer*, 247– 250.

Dudka, S. and Adriano, D.C., 1997. Environmental impacts of metal ore mining and processing a review. *J. Environ. Qual.,* 26, 590–602

Dukes, T.W., 2000. That other branch of medicine: an historiography of veterinary medicine from a Canadian perspective. *Can. Bull. Med. Hist.*, 17, 229–243.

Eighmy, T.T., Eusden, J.D., Krzanowski, J.E., Domingo, D.S., Stämpfli, D., Martin, *et al.,* 1995. Comprehensive approach toward understanding element speciation and leaching behavior in municipal solid waste incineration electrostatic precipitator ash. *Environ. Sci. Technol.,* 29, 629–646.

Engelhaupt, E., 2008. Happy birthday, love canal. *Environ. Sci. Technol.*, 42, 8179–8186.

Enserink, M., 2007. Initiative aims to merge animal and human health science to benefit both. *Science*, 316, 1553.

Erkyihun, G. A., Fikru, R. G., Bedaso, M. E. and Gezahegne, M. K., 2022. A review on One Health approach in Ethiopia. *One Health Outlook*, 4, 8.

Ermert, V., Fink, A.H., Morse, A.P. and Paeth, H., 2011. The impact of regional climate change on malaria risk due to greenhouse forcing and land-use changes in tropical Africa. *Environ. Health Perspect.*, 120, 77–84.

European Centre for Disease Prevention and Control (ECDC), 2020. COVID-19 Situation update worldwide.

Evans, B.R. and Leighton, F.A., 2014. A history of One Health, *Rev. Sci. Tech.*, 33 (2), 413-420.

Evans, G. and Newnham, J., 1998. Dictionary of international relations. London: *Penguin Books*, 77, 261–552.

Ewing, R. C., 2006. The nuclear fuel cycle: a role for mineralogy and geochemistry. Elements 2,331.

Spain, J.C, Hughes, J.B., Knackmuss H-J., 2000. Biodegradation of Nitro aromatic Compounds and Explosives. Boca Raton: Lewis, Pp. 434.

Fauci, A.S., and Morens, D.M., 2012. The perpetual challenge of infectious diseases. *N. Engl. J. Med.*, 366 (5), 454–461.

Feachem, R.G.A., 2001. Globalization is good for your health mostly. *Brit. Med. J.,* 323, 504 – 506.

Frankel, A.J. and Romer, H.D., 1999. Does trade cause growth? *Am. Econ. Rev.*, 89(3), 379-399.

Frederick M.*B., 2005.* Globalization and disasters: Issues of public health, state capacity and political action. *J. Int. Aff.,* 59 (2), 231–265.

Ganguly, S, R., 2003. Paradoxes of globalization, liberalization and gender equality: The world of the lower middle classes in West Bengal, India. *Gen. Soc.*, 17(4), 544 -566.

Gebreyes, W.A., Dupouy-Camet, J., Newport, M.J, Oliveira, C.J, Schlesinger, L.S. and Saif, Y.M., 2014. The global one health paradigm: Challenges and opportunities for tackling infectious diseases at the human, animal, and environment interface in low-resource settings. *PLoS Negl Trop Dis*; 8, e3257.

Giusti, L., 2009. A review of waste management practices and their impact on human health. *Waste Manag*., 29, 2227–2239.

Gössling, S., Daniel, S. and Michael Hall, C., 2020. Pandemics, tourism and global change: a rapid assessment of COVID-19. *J. Sustain. Tour,* 29(1), 1-20.

Hamor, T., 2004. Sustainable mining in the European Union: the legislative aspect. Environ *Manag*, 33(2), 252–261.

Herbold, J., 2005. Emerging Zoonotic Diseases: An Opportunity to Apply the Concepts of Nidality and One-Medicine. *Environ. Health Prev. Med*., 10, 260-262.

Hon, K.L., 2013. Severe respiratory syndromes: Travel history matters. *Travel Med Infect Dis*, 11 (5), 285–287.

Hubálek, Z., 2003. Emerging human infectious diseases: Anthroponoses, zoonoses, and sapronoses. *Emerg Infect Dis,* 9, 403.

Huynen, M.M., Martens, P. and Hilderink, H.B., 2005. The health impacts of globalization: a conceptual framework. *Global Health*, 1, 14.

Jenkins, H. and Yakovleva, N., 2006. Corporate social responsibility in the mining industry: exploring trends in social and environmental disclosure. *J. Clean. Prod.,* 14, 271–84.

Jenkins, P.T, Genovese, K. and Ruffler, H., 2007. Broken screens: the regulation of live animal importation in the United States. Washington DC: Defenders of Wildlife.

Jonas, O.B., (2013): Pandemic Risk. World Bank, Washington, DC. © World Bank.

Kahn, L, Kaplan, B. and Steele, J., 2007. Confronting zoonosis through collaboration between medicine and veterinary medicine. *Vet Ital*, 43, 5-19.

Kersting, A.B., Efurd, D.W., Finnegan, D.L., Rokop, D.J., Smith, D.K. and Thompson, J.L., 1999. Migration of plutonium in ground water at the Nevada Test Site. *Nature,* 397, 56–59.

King, L, Anderson, L, Blackmore, C, Blackwell, M, Lautner, E, Marcus, L, *et al.,* 2008. Executive summary of the AVMA One Health Initiative Task Force report. *J. Am. Vet.Med. Assoc*, 233, 259-260.

Kobrin, S.J., 2020. How globalization became a thing that goes bump in the night. *J. Int. Bus. Policy,* 3, 280–286.

Labonté, R., 2015. Globalization and Health. *IESBS*, 215, 198–205.

Labonte, R., Mohindra, K. and Schrecker, T., 2011. The growing impact of globalization for health and public health practice. *Annu. Rev. Public Health*, 32 (1), 263–283.

Lee, K., 2000. Global sneezes spread diseases. *Health Matter*s, 41, 14 – 15.

Lee, K., 2000. The impact of globalization on environmental health: Implications for the faculty of public health medicine. *J. Public Health Med,* 22 (3), 253 – 262.

Lee, K., 2000. Globalization and health policy: a conceptual framework and research and policy agenda. In: Bambas A, Casas JA, Drayton H *et al*, eds. Health and human development in the new global economy: the contributions and perspectives of civil society in the Americas. Washington, DC: Pan American Health Organization, Pp.15–41.

Li, R., Pei, S., Chen, B., Song, Y., Zhang, T., Yang, W., *et al.,* 2020. Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV2). *Science,* 368(6490), 489-493.

Mackenzie, J.S., Jeggo, M., Daszak, P. and Richt, J.A., 2013. One Health: The Human-Animal-Environment Interfaces in Emerging Infectious Diseases. Heidelberg: Springer.

Marano, N., Arguin, P.M. and Pappaioanou, M., 2007. Impact of globalization and animal trade on infectious disease ecology. *Emerg Infect Dis*., *13*(12), 1807–1809.

Martens, P, McMichael, A.J. and Patz, J, 2000. Globalization, Environmental Change and Health. *Glob. Chang.Human Health,* 1, 4-8.

McGee , L., McDougal, L., Zhou, J., Spratt, B.G., Tenover, F.C., George, R., *et al.,* 2001. Nomenclature of major antimicrobial-resistant clones of *Streptococcus pneumoniae* defined by the pneumococcal molecular epidemiology network. *J. Clin. Microbiol.*, 39, 2565–2571.

McMichael, A. J., 2004. Environmental and social influences on emerging infectious diseases: Past, present and future. *Philos Trans R Soc B Biol Sci*; 359, 1049-1058.

Meselson, M., Guillemin, J., Hugh-Jones, M., Langmuir, A., Popova, I., Sholokhov, A., *et al*., 1994. The Sverdlovsk anthrax outbreak of 1979. *Science*, 266, 1202–1208.

Morgan, N. and Prakash, A., 2006. International livestock markets and the impact of animal disease. *Rev. Sci. Tech.*, 25, 517-528.

National Academies of Sciences, Engineering, and Medicine; Health and Medicine Division; Board on Global Health; Forum on Microbial Threats, 2018. Understanding the Economics of Microbial Threats: Proceedings of a Workshop. Washington (DC): National Academies Press (US).

National Association of State Public Health Veterinarians, 2006. Compendium of measures to prevent disease associated with animals in public settings.

Nicolaides,C.,Avraam,D.,Cueto Felgueroso,L., González,M.C.and Juanes, R., 2019. Hand hygiene mitigation strategies against global disease spreading through the air transportation network. *Risk Anal.*, 40 (4), 723–740.

Okello, A., Paul, E., Gibbs, J., Vandersmissen, A. and Welburn, S., 2011. One Health and the neglected zoonoses: Turning rhetoric into reality. *Vet Rec.*, 169, 281-285.

Osburn, B., Scott, C. and Gibbs, P., 2009. One World – One Medicine – One Health: Emerging veterinary challenges and opportunities. *Rev. Sci. Tech.*, 28, 481-486.

Pang, T. and Guindon, G.E., 2004. Globalization and risks to health. EMBO reports, 5 Spec No (Suppl 1), S11–S16.

Reperant, L.A. and Osterhaus, A., 2017. AIDS, Avian flu, SARS, MERS, Ebola, Zika… what next? *Vaccine*, 35(35), 4470–4474.

Reperant, L.A., Cornaglia, G., and Osterhaus, A.D., 2013. The importance of understanding the human-animal interface: from early hominins to global citizens. *Curr. Top. Microbiol. Immunol.*, 365, 49–81.

Roggla, G., 2000. UN report on cyanide spill warns of risk. *Lancet*, 355, 1530.

Rothe, C., Schunk, M., Sothmann, P., Bretzel, G., Froeschl, G., Wallrauch, C., et al., 2020. Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. *N.Engl.J.Med*, 382 (10), 970–971.

Rüegg, W., 2004. A History of the University in Europe: Volume III: Universities in the Nineteenth and Early Twentieth Centuries (1800–1945), Cambridge University Press; Cambridge.

Saunders, L.Z., 2000. Virchow's contributions to veterinary medicine: celebrated then, forgotten now. *Vet. Pathol.,* 37, 199–207

Scheffler, R.M. and Pathania, V., 2005. Medicines and vaccines for the world's poorest: Is there any prospect for public-private cooperation? *Glob. Health,* 1, 10.

Schwabe, C., 1984. Veterinary Medicine and Human Health. Baltimore: Williams and Wilkins, 3rd edition, Pp. 713.

Scott, C., 2008. Calvin Schwabe one health project. The intersection of human, animal and environmental health. http//www.vetmed.ucdavis.edu/onehealth. Accessed 5/6/2022.

Scott, D. and Gössling, S., 2015. What could the next 40 years hold for global tourism? *Tour. Recreat. Res*., 40 (3), 269–285.

Selin, N.E., 2009. Global biogeochemical cycling of mercury: a review. *Annu. Rev. Environ. Resour*., 34, 43– 63.

Shell, E.R., 200. New world syndrome. *Atlantic Monthly*, 287, 50–53.

Rothe, C., Schunk, M., Sothmann, P., Bretzel, G., Froeschl, G., Wallrauch, C., *et al*., 2020. Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. *N.Engl. J.Med*, 382 (10), 970–971.

Rüegg, W., 2004. A History of the University in Europe: Volume III: Universities in the Nineteenth and Early Twentieth Centuries (1800–1945), Cambridge University Press; Cambridge.

Saunders, L.Z., 2000. Virchow's contributions to veterinary medicine: celebrated then, forgotten now. *Vet. Pathol.,* 37, 199–207

Scheffler, R.M. and Pathania, V., 2005. Medicines and vaccines for the world's poorest: Is there any prospect for public-private cooperation? *Glob. Health*, 1, 10.

Schwabe, C., 1984. Veterinary Medicine and Human Health. Baltimore: Williams and Wilkins, 3rd edition, Pp. 713.

Scott, C., 2008. Calvin Schwabe one health project. The intersection of human, animal and environmental health. http//www.vetmed.ucdavis.edu/onehealth. Accessed 5/6/2022.

Scott, D. and Gössling, S., 2015. What could the next 40 years hold for global tourism? *Tour. Recreat. Res*., 40 (3), 269–285.

Selin, N.E., 2009. Global biogeochemical cycling of mercury: a review. *Annu. Rev. Environ. Resour*., 34, 43– 63.

Shell, E.R., 200. New world syndrome. *Atlantic Monthly*, 287, 50–53.

Shrestha, K., Acharya, K.P. and, Shrestha, S., 2018. One health: The interface between veterinary and human health. *Int. J. One Health*, 4, 8-14.

Swanepoel, R., Smit, S.B., Rollin, P.E., Formenty, P., Leman, P.A., Kemp, A., *et al*., 1999. Migration of plutonium in ground water at the Nevada Test Site. *Nature*, 397, 56–59.

Tabor, G., 2002. Defining Conservation Medicine. In: Aguirre A, Ostfeld R, Tabor G, Haouse C, Pearl M, editors. Conservation Medicine. New York: Oxford University Press. Pp 407.

The Economist, 2004. Canyon or mirage—a new paper questions the notion of a worsening digital divide between rich and poor. *The Economist,* 370, 70.

Václav, K., 2017. Foundation of Global Epizootiology – a New Branch of Life Sciences, *Agricultura tropica et subtropica* (*ATS*), 50(3), 147–152.

Van der Schalie, W.H., Gardner, H.S. Jr., Bantle, J.A., De Rosa, C.T., Finch, R.A., Reif, J.S., *et al.,* 1999. Animals as sentinels of human health hazards of environmental chemicals. *Environ. Health Perspect*., 107(4), 309-315.

Vandeputte, S., Humblet, M., Fecher-Bourgeois, F., Gosset, C., Albert, A., Vernaillen, F., *et al*., 2011. Instruments de Gestion Économique des Crises Sanitaires Touchant les Animaux de Production en Europe. *Rev. Sci. tech*., 30, 683-701.

W.H.O., 2021. Tripartite and UNEP support OHHLEP's definition of "One Health", Joint Tripartite (FAO, OIE, WHO) and UNEP Statement.

Wildlife Conservation Society, 2007. The conservation implications of avian influenza (H5N1).

Woodward, D., Draer, N., Beaglehole, R. and Lipson, D., 2001. Globalization and health: a framework for analysis and action. *Bull. WHO*, 79, 875–881.

Woolhouse, M.E. and Gowtage-Sequeria, S., 2005. Host range, emerging, and reemerging pathogens. *Emerg. Infect. Dis*. 11, 1842–1847.

World Bank, 2020a. Air transport, passengers carried and World Bank, (2020b): International tourism, number of arrivals.

World Health Organization, 1997. Health and environment in sustainable development: Five years after the earth submit. Geneva.

World Trade Organization (WTO), 2007a. International Trade Statistics 2007, Geneva, WTO.

Zimmet, P, Alberti, K.G. and Shaw, J., 2001. Global and societal implications of the diabetes epidemic. *Nature,* 414, 782–787.

Zinsstag, J., Meisser, A., Schelling, E., Bonfoh, B. and Tanner, M., 2012. 'From "two medicines" to "One Health" and beyond'. *Onderstepoort J. Vet. Res*., 79(2), 5.

Short communication

Distribution of *E. coli* biotypes shed by dairy calves in selected dairy farms in Bishoftu, Ethiopia

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Abstract

A longitudinal study was undertaken to investigate *E. coli* using standard biochemical and sugar fermentation tests. Faecal samples were taken from calves purposively from three selected dairy farms in Bishoftu Ethiopia. Four different sampling times were used to observe the detection rate of *E. coli*. The overall detection of *E. coli* was 84/104 (80.70%). The detection of *E. coli* isolates in different sampling points ranged from 16.34% to 25.00% in which the occurrence of *E.coli* has a significant association. All *E. coli* isolated showed different sugar fermentation patterns. *E. coli* was biotyped into 14 biotypes and variation occurred for samples taken during the first to fourth sampling points, the pattern ranging from 20.20% to 31.00%. Among 14 biotypes, biotypes VI and III dominate with 55.95% and 16.67% respectively. *E. coli* biotype (predominantly group VI) distribution concerning sampling time points have a significant association (p=0.039).Diverse natures and variations of *E. coli* were observed in calves with different sampling points as the main determinant. Farm management practice could reduce the occurrence of the pathogen in farm animals, particularly neonatal calves.

Keywords: Biotype; Calf diarrhea; *Escherichia coli*; Ethiopia, Risk factors.

Introduction

Calf mortality due to diarrheagenic *Escherichia coli* (*E. coli*) in neonatal calves is a major problem on dairy farms. Different determinants including seasons, age, hygiene, husbandry practices, and production systems could be potential

risk factors for the occurrence of disease caused by *E. coli* (Radostits *et al.,* 2007; Berber *et al.,* 2021).

Escherichia coli, an enteric Gram-negative bacillus from the family *Enterobacteriaceae*, is a noninvasive commensal that grows in mass culture in human and animal gut lumen. They can be also out-competent and inhibitory activities for other pathogenic bacteria (Zihler *et al*., 2009). However, pathogenic *E. coli* strains are related to gastroenteric diseases such as diarrhea, and hemorrhagic colitis, and extra intestinal infections in humans and animals. Diarrhea caused by this pathogen affects newborn calves, lambs, and children resulting in significant losses (Boschi-Pinto *et al*., 2008; Malik *et al*., 2011).

Biochemical reactions and sugar fermentation studies have conventionally been used for the identification of bacterial distributions either in human or animal subjects (Krishnan *et al*., 1987). Having information on biochemical characterization and sugar fermentation tests of *E. coli* is important to suggest the distribution of the pathogen. In Ethiopia, no sufficient biotype identification studies have been conducted on *E. coli*. Identification of the diversity of *E. coli* biotypes and its potential deriving factors in different ecological settings is very important to characterize the distribution of the bacterium in a specific area. Therefore, the objective of this study was the isolation and biotype characterization of *E. coli* from both diarrheic and healthy calves' feces in association with determinants for the occurrence of calf diarrhea in dairy farms.

Materials and methods

Study area

The study was conducted on dairy farms in Bishoftu and its surroundings in Ethiopia. The area is located 47 km Southeast of Addis Ababa at 9°N and 39°E at an altitude of about 1900m above sea level. The area receives a mean annual rainfall of 865 mm with mean temperatures in the range of 15°C to 28°C. The mean relative humidity of the area is 61.3% (CSA, 2009). There are many small-scale and large-scale exotic and cross-breeding dairy farms in these areas.

Study population

Calves aged from 1 week to 4 months old that did not get treated with antibiotics were the study populations.

Study design and sampling methodology

A longitudinal study on three dairy farms in four different sampling times in the winter and spring seasons was conducted. Lists of farms were purposively selected and a total of 104 calves, 45 with diarrhea and 59 apparently healthy, were included. Fecal materials were collected and were transported in the icebox in sterile tubes to Veterinary Microbiology Laboratory, Addis Ababa University. Samples were stored at 4°C, for a maximum of overnight, until the time of processing.

Isolation and identification procedures

Isolation of *E. coli*

The isolation and identification of *E. coli* were undertaken according to the techniques recommended by Quinn *et al.* (2002) and the International Organizations for Standardization (ISO 16654:2001). Fecal samples were inoculated on MacConkey agar and incubated at 37° C overnight. From each plate, two isolated lactose fermenting colonies that were inoculated on an Eosin-methylene blue (EMB) agar medium having a characteristic metallic sheen were considered presumptive *E. coli.* All the isolates were stained by Gram's staining method to determine the Gram negativity of the isolates.

Primary and secondary identification

Oxidase test with dark purple colors along the streak line and catalase test having an appearance of oxygen gas (bubble) indicated the positive reaction was conducted as primary identification for *E. coli. Escherichia coli* isolates were preliminarily identified by IMViC biochemical tests (Sagar, 2017).

Biotyping of isolated *E. coli*

Sugar fermentation reactions were used for biochemical typing/ identification of *E. coli.* The isolates were characterized by dulcitol, raffinose, rhamnose, salicin, sucrose, inositol, lactose, maltose, and xylose. The isolates were inoculated into 1% of each sugar medium after being cultured in phenol red broth. The tubes were incubated at 37 °C for 7 days and measured every 24 hrs. The production of yellow color was seen as a favorable reaction, and appropriate controls were maintained (Quinn *et al.*, 2002; Aklilu *et al.,* 2013).

Data management and analysis

Data were classified, filtered, and coded using Microsoft Excel 2007 and exported to SPSS version 20 for appropriate statistical analysis. Descriptive statistics and Chi-square ($\chi 2$) test were used to measure the association between determinants. A p-value of ≤ 0.05 was considered statistically significant.

Results

Isolation and identification of *E. coli* isolates

From 104 diarrheic and apparently healthy calves, 84 (80.70%) samples were found to be positive for *E. coli*. The isolation rate in the diarrheic and healthy calves categories was recorded at 37/45 (82.20%) and 47/59 (79.60%) respectively. Some of the sick calves were diarrheic at the beginning of the study while others became diarrheic during the second, third, and fourth sampling point in the study period.

Association of *E. coli* positivity with different factors

The association of *E. coli* positivity was assessed concerning several factors relevant to diarrhea and the study design as presented in (Table 1). *E. coli* positivity was found to be variable among the four points of sampling. *E. coli* was isolated at the highest rate at the first time point (25%), followed by the second time point (23.07%) and the third and fourth time points with a similar isolation rate (16.34%). This variation was found to be statistically significant (p=0.031).

Table1: Association of *E. coli* positivity in diarrheic calves with different factors

Variable		No. tested *E. coli*			*P*- value
		Positive (%)	Negative (%)		
Age at occurrence *E.coli*	7 days	1	1 (100)	0	0.560
	8-15 days	11	8 (72.70)	3 (27.30)	
	16-30 days	21	21 (100)	0	
	31-60 days	39	31 (79.50)	8 (20.50)	
	61-90 days	21	13 (61.90)	8 (38.10)	
	90-120 days	11	10 (90.90)	1 (9.10)	
Sex	Male	39	35 (89.70)	4 (10.30)	0.582
	Female	65	49 (75.40)	16 (24.60)	
Health status	Diarrheic	45	37 (82.20)	8 (17.70)	0.428
	Healthy	59	47 (79.70)	12 (20.30)	
Farm	1	76	63 (82.90)	13 (17.10)	0.916
	2	12	8 (66.60)	4 (33.40)	
	3	16	13 (81.25)	3 (18.75)	
Sampling time points	1st	26	25 (96.20)	1 (3.80)	0.031
	2nd	26	23 (88.50)	3 (11.50)	
	3rd	26	16 (61.54)	10 (38.50)	
	4th	26	16 (61.54)	10 (38.50)	

M=Male; F=Female; P=Probability

Dynamics of *E. coli* biotypes in calves

A total of 14 different biotypes were identified throughout the study time points. Overall, biotype VI (47 isolates, 55.95%) was the most dominant, followed by biotypes III (14 isolates, 16.67%), V and VII (each with 5 isolates, 5.95%), I (3 isolates, 3.57%), IX (2 isolates, 2.38%), and II, IV, XII, VIII, X,XI, XIII and XIV (each accounting for 1 isolate, 1.19%) (Table 2).

Table2: Designation of biotypes of *E. coli* isolates based on fermentation reactions

Biotype No.	Isolates	The proportion of isolates No (%)	Fermented sugars
I	295D, 4515D, 4514C	3 (3.60)	Dulcitol, raffinose, rhaminose, salcine, sucrose. lactose, maltose and xylose
II	3345B	1 (1.19)	Dulcitol, raffinose, salcine, sucrose. lactose, maltose, inositol and xylose
III	4503A, 295B, 21B, 4511B, 4515A, 20B. 4505B, 4503C, 369B, 360A, 363C, 20A, 364C, 3346B	14 (16.67)	Dulcitol, raffinose, rhaminose, sucrose. lactose, maltose, inositol and xylose
IV	295A	1 (1.19)	Dulcitol, raffinose, rhaminose, salcine, sucrose. lactose, maltose and inositol
V	4518B, 4506B, 4512A, 3346A, 4511A	5 (5.59)	Raffinose, rhaminose, sucrose. lactose, maltose, xylose and inositol
VI	4509C, 4513A, 4509A, 364A. 369A, 364B, 4515B, 4509B. 4510C,4503B, 368D, 4507D, 295C, 4518A, 4505D, 22D, 4512D, 361D, 4503D, 4510D, 4511D, 364D, 21D, 22B, 3345C, 4513D, 363D, 362D, 360D, 4504A, 21A, 368B, 362A, 4507A, 4510A, 4504C, 366C, 4506A, 22C, 4507B, 363B, 4514B, 4504B, 368C, 360B, 4512C, 366B,	47 (55.95)	Dulcitol, raffinose, rhaminose, salcine, sucrose. lactose, maltose, xylose and inositol
VII	4510B, 4513B, 366A, 4514A, 361A	5 (5.59)	Raffinose, rhaminose, salcine, sucrose. lactose, maltose, inositol and xylose
VIII	361C	1 (1.19)	Dulcitol, rhaminose, salcine, lactose, maltose, xylose and inositol
IX	4506C, 4518C	2 (2.38)	Raffinose, rhaminose, salcine, sucrose. lactose, maltose and inositol
X	4512B	1 (1.19)	Dulcitol, rhaminose, sucrose. lactose, maltose, xylose and inositol
XI	4505A	1 (1.19)	Dulcitol, raffinose, rhaminose, sucrose. lactose, maltose and xylose

Biotype No.	Isolates	The proportion of isolates No (%)	Fermented sugars
XII	22A	1 (1.19)	Dulcitol, raffinose, rhaminose, sucrose. lactose, maltose and inositol
XIII	21C	1 (1.19)	Rhaminose, salcine, xylose and inositol
XIV	362C	1 (1.19)	Rhaminose, salcine, lactose, maltose, xylose and inositol
Total		84 (100)	

Biotype diversity was found to be variable among the four points of sampling, and this variation was found to be statistically significant (p=0.039) (Table 3).

Table 3: Association of biotypes in diarrheic calves with different factors

Variable	Categories	Number of Biotypes (%)	*P- value*
Age at occurrence *E.coli*	7 days	1 (1.20)	
	8-15 days	8 (9.60)	0.225
	16-30 days	21 (25.10)	
	31-60 days	31 (37.00)	
	61-90 days	13 (15.50)	
	90-120 days	10 (11.90)	
Sex	Male	35 (41.70)	0.499
	Female	49 (58.30)	
Health status	Diarrheic	37 (44.00)	0.135
	Healthy	47 (56.00)	

Variable	Categories	Number of Biotypes (%)	*P- value*
Farm	1	63 (75.00)	0.358
	2	8 (9.50)	
	3	13 (15.50)	
Sampling time points	1st	26 (31.00)	
	2nd	24 (28.60)	0.039
	3rd	17 (20.20)	
	4th	17 (20.20)	

The distribution of the biotypes along the sampling time points was observed as indicated in Table 4 below.

Table4: Distribution of *E. coli* biotypes and variables

Variable		The abundance *of E. coli* biotypes in fecal samples, Number (%)													
		I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**XIII**	**XIV**
Sampling time points	1st	0 (0)	0 (0)	5(6)	1(1.2)	3(3.6)	11(13.1)	4(4.8)	0(0)	0(0)	0(0)	0(0)	0(0)	1(1.2)	1(1.2)
	2nd	0(0)	1(1.2)	7(8.3)	0 (0)	2(2.4)	11 (13.1)	1(1.2)	1(1.2)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	3rd	1 (1.2)	0 (0)	2(2.4)	0 (0)	0 (0)	9 (10.7)	0(0)	0(0)	1(1.2)	2(2.4)	1(1.2)	1(1.2)	0(0)	0(0)
	4th	2 (2.4)	0 (0)	0 (0)	0 (0)	0 (0)	14 (16.7)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Age at the occurrence of diarrhea	7 d	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(1.2)
	8-15d	0(0)	0(0)	2(2.4)	0(0)	2(2.4)	3(3.6)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(1.2)	0(0)
	16-30d	0(0)	1(1.2)	6(7.2)	0(0)	2(2.4)	9(10.8)	2(2.4)	1(1.2)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	31-60d	2(2.4)	0(0)	4(4.8)	1(1.2)	1(1.2)	17(20.2)	3(3.6)	0(0)	0(0)	2(2.4)	1(1.2)	0(0)	0(0)	0(0)
	61-90d	1(1.2)	0(0)	2(2.4)	0(0)	0(0)	8(9.6)	0(0)	0(0)	1(1.2)	0(0)	0(0)	1(1.2)	0(0)	0(0)
	91-120d	0(0)	0(0)	0(0)	0(0)	0(0)	10(11.9)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Health status	Diarrheic	3(3.6)	1(1.2)	10(11.9)	0(0)	3(3.6)	17(20.2)	2(2.4)	0(0)	0(0)	0(0)	0(0)	0(0)	1(1.2)	0(0)
	Healthy	0(0)	0(0)	4(4.8)	1(1.2)	2(2.4)	30(35.7)	3(3.6)	1(1.2)	2(2.4)	1(1.2)	1(1.2)	1(1.2)	0(0)	1(1.2)
Farms	1	1(1.2)	1(1.2)	10(11.9)	1(1.2)	4(4.8)	37(44)	3(3.6)	0(0)	1(1.2)	2(2.4)	1(1.2)	1(1.2)	1(1.2)	0(0)
	2	1(1.2)	0(0)	2(2.4)	0(0)	0(0)	2(2.4)	1(1.2)	1(1.2)	0(0)	0(0)	0(0)	0(0)	0(0)	1(1.2)
	3	1(1.2)	0(0)	2(2.4)	0(0)	1(1.2)	8(9.5)	1(1.2)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

d=day(s)

Discussion

Different pathogenic strains of *E. coli* cause diarrhea in animals and humans. Diarrhea is the most significant cause of morbidity and mortality in neonatal dairy calves throughout the world (Uhde *et al*., 2008). In the present study, 84(80.70%) *E. coli* isolates were obtained from 104 calves' fecal samples based on the biochemical and sugar fermentation tests. The isolation rate of *E. coli* from calves in this study was higher than the previous isolation studies in different countries, which ranged from 8.10% (Mebrhatu and Kebede, 2017) to 73.90% (Adesiyum *et al*., 2001). In another case, the present study could show a lower isolation rate than that of 84.30% of *E. coli* from diarrheic calves (Aklilu *et al*., 2013). The sampling point is a significantly different factor that indicates *E. coli* positivity decrease at the sampling point increase within increased age of the calves. That implies the pathogen is potentially risky at the early age of neonates. In addition to this, the result of the current study varies from the reports in different areas, which might be due to the variations in the management conditions of the farms including hygienic conditions and housing system of the farms (Bekele *et al*., 2009; Charles and Lani, 2010).

Biochemical and sugar fermentation reactions have conventionally been used for the identification of bacteria. In the present study, the fermentation reactions made by all *E. coli* isolates were variable. The identified 14 biotypes were similar to previous findings of Aklilu *et al.* (2013) in diarrheic lamb in Debre Birhan, Ethiopia. Whereas, the number of biotypes is different from a study that found 12 *E. coli* biotypes (Gargan *et al*., 2013). The distribution of the isolates into different biotypes indicates the diversity of the bacterium due to the presence of enzymes that ferments a given sugar. Sampling points are the only significantly different variable for the association of *E. coli* distribution in neonatal calves in the study area that suggests that seasonal variation could be an important determinant for the occurrence of the disease in calves.

Conclusions

The isolation rate and biotype diversity of *E. coli* in calves were found to be high in the study area. The distribution of the pathogen based on sugar fermentation is also grouped into 14 different biotypes. Both *E. coli* positivity and biotype distribution were significantly associated with sampling points and sampling times during the study. The high isolation rate and diverse nature of the study organism that caused diarrhea in calves could suggest that calve

diarrhea due to *E. coli* could be an economically important health problem in dairy farms. Determinants need to be identified and farm management practices should be improved to improve the health status of farm animals in general and calves in particular.

Acknowledgments

The authors are grateful to Addis Ababa University for the facilities used, and to private dairy farms for their allowance and cooperation.

References

Adesiyum, A., Kaminjolo, J., Ngeleka, M., Mutani, A., Borde, G., Hardwood, W., and Harper, W., 2001. A longitudinal study on enteropathogenic infections of livestock in Trinidad. *Rev. Soc. Bras. Med. Trop.*, 34, 29-35.

Aklilu, M., Sisay, T., Tefera, G. and Tekalign, B., 2013. Identification and biotyping of *Escherichia coli* from diarrheic lambs in and around Debre Birhan town, Ethiopia. *J. Environ. Anal. Toxicol.,*3, 188.

Bekele, M., Alemayehu, R., Fufa, A. and Kasahun, A., 2009. Prevalence and incidence rate of calf morbidity and mortality and associated risk factors in smallholder dairy farms in Hawassa, Southern Ethiopia. *Ethiop. Vet. J.*, 13(2), 59-68.

Berber, E., Çanako ˘glu, N., Sözdutmaz, ˙I., Simsek, E., Sursal, N., Ekinci, G., *et.al*, 2021. Seasonal and age-associated pathogen distribution in newborn calves with diarrhea admitted to ICU. *Vet. Sci.*, 8, 128.

Boschi-Pinto, C., Velebit, L. and Shibuya, K., 2008. Estimating child mortality due to diarrhoea in developing countries. *Bull. WHO,* 86, 710–717.

Charles, S. and Lani, V., 2014. Calf scours- cause, prevention, and treatment. NDSU Repository. North Dakota State University. URL:www.ag.adsu.edu./pubs/ansci/beet/as776.pdf.

CSA, 2009. Central Statistical Agency, Federal Democratic Republic of Ethiopia. Agricultural sample survey 2009/10. Report on livestock and livestock characteristics, in Statistical Bulletin No. 468, CSA, Addis Ababa, Ethiopia.

ISO, 2001. International Organization for Standardization (ISO 16654:2001). Microbiology of Food and Animal Feeding Stuffs- Horizontal Method for the Detection of *Escherichia coli* O157. 1st ed. Geneva, Switzerland.

Gargan, R., Brumfitt, J., and Hamilton-Miller., 2013. A concise biotyping system for differentiating strains of *Escherichia coli. J. Clin. Pathol.,* 35, 1366-1369.

Malik, S., Amit, K., Amit, Verma.,Manoj, Gupta., Som, S., Arvind, S. *et al*., 2011. Haematological profile and blood chemistry in diarrhoeic calves affected with colibacillosis. *J. Anim. Health Prod*., 1, 10–14.

Mebrhatu, T. and Kebede, D., 2017. Bacteriological study of calf colisepticemia in Alage dairy farm, Southern Ethiopia. *BMC Res. Note*, 10, 710.

Quinn, P., Markey, B., Carter, M., and Carter, R., 2002. Veterinary Microbiology and Bacterial Disease. Black Well Science Ltd., London, UK. Pp.1–648.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W. and Constable, P.D., 2007. Veterinary Medicine. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats, and Horses. 10th ed., Elsevier, UK. Pp. 673–762.

Sagar, A., 2017. Bacterial Identification: Biochemical Test and Identification of *E. coli*. Online Microbiology Notes 2015. URL: https://microbiologyinfo.com/biochemical-test-and-identification-of-e-coli/. Accessed on 15 Jan 2017.

Uhde, F., Kaufmann, T., Sager, H., Albini, S., Zanoni, R., Schelling, E. and Meylan, M., 2008. Prevalence of four enteropathogens in the faeces of young diarrhoeic dairy calves in Switzerland. *Vet. Rec.,* 163, 362–366.

Zihler, A., Le Blay, T., deWouters, C., Lacroix, C., Braegger, A., Lehner, P., Tischler, T., Rattei, H. and Stephan, R., 2009. In-vitro inhibition activity of different bacteriocin producing *Escherichia coli* against *Salmonella* strains isolated from clinical cases. *Lett. Appl. Microbiol*., 49, 31-38.

Short communication

A One Health approach to investigate bats as a potential source of zoonotic mycoses in selected areas of Mpumalanga province, the Republic of South Africa

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Abstract

A One Health approach pilot study was carried out in selected villages within the Mnisi Traditional Authority's area, Manyeleti Game Reserve, and Hans Hoheisen Wildlife Research Station in Mpumalanga Province, the Republic of South Africa from July to December 2018. The study's main objectives were to identify positive and negative human-bat-environment interactions and microbiological screening of bats' faecal samples for zoonotic fungi. Thirty-three purposively selected participants were asked to complete a structured questionnaire with multiple-choice and open-ended questions, and a total of 55 faecal samples were collected, 25 from identified bat roosting sites and 30 from captured bats. Ninety seven percent of respondents were aware of the presence or absence of bats in their immediate surroundings. However, the majority of them (87.9%) were uneasy about the presence of bats in their buildings, and nearly half (48.5%) were unsure whether bats play a positive or negative role in the environment. Some respondents (15.2%) stated that bats play beneficial roles in the environment, such as pollinating plants, spreading seeds of indigenous plants, catching harmful insects, and so on. More than half of the respondents (66.7%) stated that bats can be a nuisance; 18.2% of those polled

reported contracting fungal diseases as a result of cleaning bat droppings without adequate protection. The analysis of faecal samples revealed that bats can harbour pathogenic fungi such as *Aspergillus fumigatus*, and *A.flavus*. We concluded that bats can harbour fungal pathogens that cause human diseases. Further research should be conducted to compile a complete list of fungi pathogens in bats in the study area.

Keywords. Bats; Faecal sample; One Health Approach; Questionnaire survey; Republic of South Africa; zoonotic fungi.

Introduction

Bats are mammals that are distributed everywhere in the world except in the hottest desert and Polar Regions. There are about 1200 species of bats worldwide that makeup one-fourth of all mammalian species (Gauteng & Northern Regions Bat Interest Group, 2020a). It is recorded that 59 species of bats occur in South Africa. Thirty-nine of them are found in Gauteng, Limpopo, Mpumalanga, and part of Northwest Province (Ecosolutions, 2018). Bats are involved in many ecosystem services that are important for the survival of a human being. At night, bats come out of their roosting sites and forage on various food items such as insects, nectars, fruits, seeds, frogs, fish, small mammals, and blood (Kunz *et al*., 2011).

Despite the above-mentioned positive roles in the ecosystem, bats are potentially a source of zoonotic diseases (Hayman *et al.,* 2013). They serve as a source of fungal diseases such as aspergillosis (Seelan *et al*., 2008), histoplasmosis, cryptococcosis, and blastomycoses (Taylor *et al*., 2000).

Although bats were identified as sources of highly pathogenic and zoonotic diseases (Kohl and Kurth, 2014), some fungal diseases with zoonotic potential have lacked adequate attention in international public health efforts leading to the lack of appropriate attention in the preventive strategies of the diseases (Seyedmousavi *et al.,* 2015). Therefore, this coupled with the lack of adequate knowledge on bats as a source of zoonotic diseases can pose a risk to the public that had direct or indirect contact with bats. Immunocompromised individuals are at a higher risk of developing a fungal infection than immunocompetent individuals. This is because immunocompromised individuals lack the basic mechanisms of cellular defence. The immunity of individuals can be compro-

mised due to prolonged treatment against malignant diseases, autoimmune diseases, immune-suppressive therapy, and immunosuppressive diseases such as leukaemia, lymphoma, or AIDS. Those individuals with deficient immunity are characterized by increased susceptibility to opportunistic infections (Silva, 2010). A review by Armstrong-James *et al.* (2014) showed that opportunistic fungal infections have been a primary driver for mortality from HIV infection since the first cases of AIDS were identified in San Francisco and New York in the early 1980s.In the Bushbuckridge community, HIV has a prevalence of over 25% in pregnant women, and human tuberculosis (TB) is a significant cause of death in the population (Bushbuckridge Local Municipality, 2016). Secondary infections with fungal diseases such as aspergillosis, histoplasmosis, cryptococcosis, or blastomycosis could thus pose a serious risk in the immunocompromised population of Mnisi Traditional Authority, Manyeleti Game Reserve, and Hans Hoheisen Wildlife Research Station in the Republic of South Africa.

Therefore, the objectives of this study were to determine positive and negative human-bats-environment interactions and microbiological screening of bat faecal samples for zoonotic fungi.

Materials and methods

Study area

The study was conducted in selected villages within Mnisi Traditional Authority's area (i.e., Clare A, Share, and Utah), Manyeleti Game Reserve, and Hans Hoheisen Wildlife Research Station, Mpumalanga Province in the Republic of South Africa from July to December 2018. Mpumalanga Province is located at latitude: 25° 33' 55.2096" S and longitude: 30° 31' 40.476" E. The province has an elevation of 1401.096 meters above sea level (Country Coordinate, 2018)

The Mnisi Traditional Authority's area is located in the Mpumalanga Province in the north-eastern corner of the Bushbuck-ridge Local Municipality and it is the core research and engagement area of the Mnisi Community Programme, Faculty of Veterinary Science, University of Pretoria. The Mnisi Community Programme is a multidisciplinary platform for research, teaching, learning, and community engagement within the 'One Health' philosophy (Berrian *et al*., 2016; UP, 2022).

Manyeleti Game Reserve is situated adjacent to the Mnisi community and has open access to Kruger National Park. Hans Hoheisen Wildlife Research Station is situated at Orpen gate of Kruger National Park. Manyeleti Game Reserve and Hans Hoheisen Wildlife Research Station are thus part of the Great Limpopo Trans Fontier Conservation Area (Berrian *et al.*, 2016).

Study design

A cross-sectional study was conducted on three focus groups: households and public buildings in selected villages within the Mnisi Traditional Authority's area, lodges and tourist facilities in the Manyeleti Game Reserve, and Hans Hoheisen Wildlife Research Station buildings. These areas were chosen because there was a high risk of bats and humans coming into contact, either directly or indirectly. The sample size for environmental samples (bat faeces) and the questionnaire survey was determined on purpose based on the sample, and respondents' availability, respectively. Similarly, the number of bats sampled in this study was determined purposively in line with approval from the Animal Ethics Committee of the University of Pretoria under project number V062-18, Section 20 of the animal diseases act 1984 (act no. 35 of 1984), and a bat catch permit obtained from Mpumalanga Tourism and Park Agency (permit number MPB. 5622), the Republic of South Africa.

Study animals

Bats from Mnisi Traditional Authority, Manyeleti Game Reserve, and Hans Hoheisen Wildlife Research Station were included in the study.

Study methodology

Questionnaire

To identify suitable sites for sample collection, a structured questionnaire comprised of multiple-choice and open-ended questions was used with a total of thirty-three purposively selected participants in all three focus groups. Participants were also asked about their perceptions of bats and their role in disease risks, whether positive (e.g., mosquito control) or negative (e.g., pathogen source), as well as their current control measures for bat populations in their immediate environment, if any. The questionnaire also looked into potential

risk factors related to human activity at the study sites that could promote pathogen transmission.

Faecal sample collection

Faecal samples were directly collected from the 30 captured bats across four different sites: Manyeleti game reserve, Share village (household), Share village (church), and Hans Hoheisen Wildlife Research Station. Furthermore, about 25 faecal samples (2 – 5 faecal pellets) were collected from the environment at identified bat roosting sites. The environmental faecal samples were placed in solid plastic sample bottles with leak-proof lids and zip-locked plastic bags separately and transported to the Hans Hoheisen Wildlife Research Station Laboratory in a cold-chain.

Laboratory analysis of faecal samples

In a BSL2+ cabinet (Healforce, IMP), the samples were plated onto potato dextrose agar (Selecta media, #510159), and plate with antibiotics (chloramphenicol) (Selecta media, # 510184), then were incubated at 37^{0}C. The plates were sealed with Parafilm (Parafilm® M All-Purpose Laboratory Film, 100MM x 75M, Lasec). Plates were checked regularly for growth. Once growth was present, a smear was made and stained with Lactophenol cotton blue (Merck, 1137410100) and examined under a microscope (Primo star, Zeiss) with the 40x objective. The plates were then returned to the incubator (IB 25G, Labotec) for a further six weeks until final examination of the plates was done and discarded. Fungal species was confirmed based on the morphology of the fungal growth on culture plates, the temperature at which the fungi grew as well as the results of the lactophenol cotton blue stain (Quinn *et al.,* 2011).

Data management and analysis

All questionnaire and laboratory results data were recorded on a specially designed sheet and then entered into Excel spreadsheet. R statistical software was used for the analysis (R Development Core Team, 2008). For analysis, a precision of 5% and a confidence interval of 95% were used. The proportion analysis result was described using descriptive analysis.

Results

Questionnaire results

Demographic information of participants

During this pilot study, 33 people were interviewed from Hans Hoheisen Wildlife Research Station, Manyeleti Game Reserve, Share, Utah, and Clare A villages (Table 1).

Table 1. Frequency distribution of study participants

Locations	Number of participants (n)	Percent (%)
Hans Hohenstein Wildlife Research Station	6	18.2
Manyeleti Game Reserve	6	18.2
Share	8	24.2
Utah	3	9.1
Clare A	10	30.3
Total	**33**	100

Perception of participants on the presence and positive roles of bats

Almost all (97.0%) of those polled were aware of the presence or absence of bats in their immediate surroundings. However, the majority of them (87.9%) were not comfortable with the presence of these animals in their buildings, and nearly half of the respondents (48.5%) were unsure whether bats play a positive or negative role in the environment. However, some respondents (15.2%) stated that bats play beneficial roles in the environment, such as pollinating plants, spreading seeds of indigenous plants, catching harmful insects (such as mosquitoes), and aiding in research (Table 2).

Table 2. Perception of participants on the presence of bats

Variables	Category	Frequency (n)	Percent (%)
Do bats live in or around your building?	Yes	25	75.8
	No	7	21.2
	I don't know	1	3.0
Are you comfortable with the presence of bats in your building?	Yes	4	12.1
	No	29	87.9
Do bats have a positive role in your area?	Yes	5	15.2
	No	12	36.4
	I don't know	16	48.5
Do you know any problems caused by bats?	Yes	22	66.7
	No	4	12.1
	I don't know	7	21.2

Knowledge of participants in the management of bats and their droppings

In terms of bat management in buildings, the majority of respondents (84%) did nothing to remove them. However, some respondents (16%) stated that they tried to remove bats with brooms or by closing the opening where they came in. In terms of bat droppings management, 60% of participants stated that they did not use any type of protective clothing when cleaning droppings. When cleaning bat droppings, staff at Hans Hoheisen Wildlife Research Station and Manyeleti Game Reserve wear protective clothing such as rubber gloves and/or masks (Table 3).

Table 3. Knowledge of participants on the management of bats and their droppings (n=25)

Variables	Category	Frequency (n)	Percent (%)
How do you manage the bats population (if any) that dwells in your building?	I do nothing	21	84.0
	I hire an exterminator	0	0.0
	I attempt to remove them	4	16.0
Do you regularly clean bats dropping?	Yes	19	76.0
	No	6	24.0
Do you use any protective wearing when you clean bats dropping?	Always	9	36.0
	Sometimes	1	4.0
	Never	15	60.0

Knowledge of participants on bats as a source of diseases

Approximately 78.8% of those polled were unfamiliar with diseases that affect both bats and humans. Only 21.2% of respondents were aware of diseases shared by bats and humans (such as rabies). Furthermore, the majority of respondents (75.8%) were found to not know the source of diseases common to bats and humans.

Some respondents (18.2%) claimed to have contracted fungal diseases as a result of cleaning bat droppings without adequate protection. They also stated that the proximity of bats and humans, a lack of care when cleaning bat droppings, and the nature of the pathogens shared by bats and humans were major factors in disease transmission (Table 4).

Table 4. Knowledge of participants on bats as a source of zoonotic disease transmission (n=33)

Variables	Category	Frequency (n)	Percent (%)
Do you know of any disease that affects both bats and humans?	Yes	7	21.2
	No	26	78.8
Who do you think is the source of the disease common to bats and humans?	Bats	4	12.1
	Humans	1	3.0
	Other animals	1	3.0
	I don't know	27	81.8
Have you contracted any fungal disease due to cleaning droppings?	Yes	6	18.2
	No	27	81.8
Do you know what the treatment is?	Yes	7	21.2
	No	26	78.8
What do you think of the risk factors for the transmission of diseases between bats and humans?	The proximity between bats and humans	4	12.1
	Lack of care when cleaning bat dropping	3	9.1
	Nature of pathogen shared	2	6.1
	All of the above	5	15.2
	I don't know	19	57.6

Laboratory result for faecal sample analysis

Culture analysis of faecal samples revealed that bats can harbour pathogenic fungi. In this study, we isolated two species of *Aspergillus* from faeces collected in Share village and Manyeleti Game Reserve from households, churches, and directly from bats (Table 5).

Table 5. Fungal pathogens isolated from faecal samples of bats at share village and Manyeleti game reserve

Type of sample	Villages	Site of collection	Positive samples (n)	Percent (%)	Isolated pathogen
Faeces collected from captured bats (n=30)	Manyeleti game reserve	Dormitory	1	3.33	*A. flavus*
	Share village	Church	2	6.67	*A. flavus*
	Share village	Church	1	3.33	*A. flavus* and *A. niger*
Faeces collected from the environment (n=25)	Share village	Household	1	4.0	*A. niger*
		Church	2	8.0	*A. flavus*

Discussion

The current result was in line with the findings of Kunz *et al*. (2011), Banskar *et al*. (2016), and Mahandran *et al.* (2018) who reported the roles played by bats in arthropod control, seed dispersal, and pollination. In addition, the perception of participants on the presence of bats in their environment was found to be similar to the perception of western cultures described by Lewis and Oliver (1996). In contrast, Nabhan *et al*. (2010) indicated that bats were considered symbols of fortune in China. The high proportion of public discomfort due to the presence of bats in their immediate environment in our current pilot study could be due to a lack of awareness of the positive role of bats, bad smelling associated with their urine and faeces, and damages they caused to infrastructures such as roof and wall of buildings.

There are various reasons to remove bats that dwell in buildings. For example, bat colonies that live in the building may sometimes be noisy or create unpleasant odours, or some people just don't want them in their houses (due to bat phobia) (Gauteng & Northern Regions Bat Interest Group, 2020b). There is little reason to remove bats from buildings where they are not causing a nuisance. South African bats do not feed on blood, and bats are not aggressive and will not attack people. However, bats should be prevented from entering human living quarters (Gauteng & Northern Regions Bat Interest Group, 2020c) because they can serve as a potential source of zoonotic diseases (Hayman *et al.,* 2013). In addition, all necessary protective clothing should be worn when

cleaning bat droppings, as faecal materials from these animals may contain pathogenic microorganisms (De Mandal *et al.*, 2015; Yinda *et al.*, 2016).

A review done by Kohl and Kurth (2014) indicated that European bats harbour highly pathogenic viruses that have been implicated in human deaths. Similarly, we isolated 12.77% pathogenic fungi (*A. favus* and *A. niger*) from captured bats and their dropping. These fungi are known for their potent mycotoxins and cause infections in immunocompromised individuals (Ráduly *et al.,* 2020).

Regarding fungal pathogens isolated from faecal samples of bats, our current result (12.77%, n=7/55) was in agreement with a preliminary survey conducted by Seelan (2006) who isolated fungi of the genus *Aspergillus* from anal swabs of bats (56.5%) at Kubah National Park, Matang, Sarawak in Malaysia. In our study, we confirmed that bats harbour pathogenic fungi such as *A. flavus* and *A. niger*. This showed that bats can contaminate the environment with their infected faecal material and can serve as a potential source of infection to humans. Moreover, bats dropping can serve as an ideal substrate for the growth of fungi from other animals such as rodents as it is rich in organic matters. This can lead to the maintenance of fungi at the site where the bat droppings build up. These conditions together with a lack of adequate ventilation in public spaces will increase the potential risk of inhalation of a sufficient load of fungi in the air and can result in the establishment of infection in humans.

Moreover, close contact with bats and/or their droppings can lead to infection with the pathogenic organisms they harbour. Hayman *et al*. (2013) mentioned that human activities can increase exposure to bats and, therefore, protective clothing should be worn whenever cleaning bats dropping or handling them. In addition, close contact with these animals should be avoided as much as possible to minimize the risk of environmental contamination with their droppings and transmission of infections to humans. Isolation of pathogenic fungi such as *Aspergillus* species from bats mist-netted at public spaces (church and tourist facilities) at Share village, and Manyeleti Game Reserve could probably increase the risk of transmission of fungal pathogens to humans. The risk of infection with fungal diseases is of particular concern in resource-limited areas, such as rural villages where the human population is immunocompromised due to the high prevalence of HIV and human TB, and health care services are limited.

As this was a pilot study, it might have some shortcomings and limitations. We only captured 30 bats for the faecal sample collection. For this reason, we didn't collect a large number of samples and isolate all zoonotic fungal pathogens of bats that may exist in this area. Since there is a possibility that the environmental samples could be contaminated by faeces of other species, such as rodents, the fungal pathogens isolated from the environmental samples were not necessarily from bats. Moreover, we didn't characterize, at a molecular level, the *Aspergillus* isolates we have isolated in this study.

Conclusions

In the current pilot study, the majority of the participants were aware of either the presence or absence of bats in their immediate environment. However, most of them didn't have the know-how on fungal pathogens common to bats and humans. Even though bats play a beneficial role in the environment, some of the bats sampled in the study were known to harbour fungal pathogens. The isolation of pathogenic fungi like *Aspergillus* from both environmental and bat samples indicates that these animals could serve as an important source of zoonotic fungal pathogens. The presence of zoonotic pathogens, combined with a lack of appropriate care when handling bats and/or their dropping, could expose the public to bat zoonotic fungal pathogens, resulting in serious health problems. Therefore, comprehensive research should be conducted to get a complete list of fungi pathogens in the study area, a poster should be prepared as part of an awareness creation program on the ecological roles of bats for the people of the study area, and a workshop should be organized on the risk of contact and infection with zoonotic fungal diseases from bats such as aspergillosis.

Acknowledgments

The authors would like to thank Mr. Mark Bourn for facilitating bat mist-netting and Ms. Violet Ndlovu for her fieldwork assistance. We'd also like to thank the Mnisi research platform and the Hans Hoheisen wildlife research station laboratories.

Conflict of Interest

The author has declared that no conflict of interests exists.

Funding

This study was part of Tilaye Shibbiru Mengistu's thesis for a collaborative Master of Science in Tropical Medicine between the Institute of Tropical Medicine, Antwerp, Belgium, and the University of Pretoria, South Africa. It was fully funded by the "DGD (Directorate General of Development Cooperation) Scholarship" under the Institute of Tropical Medicine, Antwerp, Belgium. The funder has no role or authority in the conduct of the study. The views expressed in this publication do not necessarily reflect those held by the funder.

References

Armstrong-James, D., Meintjes, G., and Brown, G.D.,2014. A neglected epidemic: fungal infections in HIV/AIDS. *Trends Microbiol.,* 22(3), 120 – 127.

Banskar, S., Mourya, D.T., and Shouche, Y.S., 2016. Bacterial diversity indicates dietary overlap among bats of different feeding habits. *Microbiol.* Res.,182, 99 – 108.

Berrian, A.M., van Rooyen, J., Martínez-López, B., Knobel, D., Simpson, G.J.G., Wilkese, M.S., and Conrada, P.A., 2016. One Health profile of a community at the wildlife-domestic animal interface, Mpumalanga, South Africa. *Prev. Vet. Med.,* 130, 119 – 128.

Bushbuckridge Local Municipality. 2016. Final Integrated Development Plan. Republic of South Africa.2015/2016.

Country Coordinate. 2018. GPS Coordinate of Mpumalanga South Africa. Accessed from https://www.countrycoordinate.com/city-mpumalanga-south-africa/#:~:text=The%20latitude%20of%20Mpumalanga%20is,time%20zone%20is%20Africa%2FJohannesburg. Accessed on 12 April 2018.

De Mandal, S., Zothansanga, Panda, A.K., Bisht, S.S., and Kumar, N.S., 2015. First report of bacterial community from a Bat Guano using Illumina next-generation sequencing. *Genom. Data,* 4, 99 – 101.

Ecosolutions. 2018. Bat species. Accessed from http://ecosolutions.co.za/bat-boxes/bat-species on 29 March 2018.

Gauteng and Northern Regions Bat Interest Group. 2020a. Conflict with bats in buildings. Accessed from http://batsgauteng.org.za/wp/about/interesting-bat-facts/ on 27 February 2020.

Gauteng and Northern Regions Bat Interest Group. 2020b. Conflict with bats in buildings. Accessed from http://batsgauteng.org.za/wp/unwanted-bats/bat-conflict/ on 27 February 2020.

Gauteng and Northern Regions Bat Interest Group. 2020c.Guide to Bat Exclusion: Should you Bat-proof your House? Accessed from http://batsgauteng.org.za/wp/unwanted-bats/excluding-bats-from-buildings/ on 27 February 2020.

Hayman, D.T.S., Bowen, R.A., Cryan, P.M., McCracken, G.F., O'Shea, T.J., Peel, A.J., *et al*., 2013. Ecology of zoonotic infectious diseases in bats: current knowledge and future directions. *Zoonoses Public Health,* 60, 2 – 21.

Kohl, C. and Kurth, A., 2014. European bats as carriers of viruses with zoonotic potential. *Viruses*, 6, 3110 – 3128.

Kunz, T.H., Braun de Torrez, E., Bauer, D., Lobova, T., and Fleming, T.H., 2011. Ecosystem services provided by bats. *Ann. N. Y. Acad. Sci.,* 1223, 1–38.

Lewis, J.R., and Oliver, E.D., 1996. Angels A to Z. Visible Ink. Detroit, Michigan.

Mahandran, V., Murugan, C.M., Marimuthu, G., and Nathan, P.T., 2018. Seed dispersal of a tropical deciduous Mahua tree, *Madhucalatifolia* (Sapotaceae) exhibiting bat-fruit syndrome by pteropodid bats. *Glob. Ecol. Conserv.*, 14, 1 – 11.

Nabhan, M.L., Aliperti, J.R., Feng, J., and Kunz, T.H., 2010. Bats and wind energy development in China: An emerging threat to cultural and ecological harbingers of good fortune. Unpublished manuscript. Boston University, Boston, MA.

Quinn, P.J., Markey, B.K., Leonard, F.C., Hartigan, P., Fanning, S., and Fitzpatrick, E.S., 2011. Veterinary Microbiology and Microbial Disease, 2nd Ed. Blackwell Publishing Ltd.p413 – 483.

Ráduly, Z., Szabó1, L., Madar, A., Pócsi, I., and Csernoch, L., 2020. Toxicological and medical aspects of aspergillus-derived mycotoxins entering the feed and food chain. *Front. Microbiol*., 10, 1 – 23.

R Development Core Team. 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org.

Seelan, J.S.S., Khan, F.A.A., Muid, S., and Abdullah, M.T.,2008. Bats (chiropteran) reported with *Aspergillus* species from Kubah National Park, Sarawak, Malaysia. *J. Trop. Biol. Conserv.,* 4, 81 – 97.

Seyedmousavi, S., Guillot, J., Tolooe, A., Verweij, P.E., and de Hoog, G.S., 2015. Neglected fungal zoonoses: hidden threats to man and animals. *Clin. Microbiol. Infect,* 21, 416–425.

Silva, R.F., 2010. Fungal infections in immunocompromised patients. *J. Bras. Pneumol.,* 36, 142 – 147.

Taylor, M.L, Chavez-Tapia, C.B., and Reyes-Montes, M.R., 2000. Molecular typing of *Histoplasma capsulatum* isolated from infected bats, captured in Mexico. *Fungal Genet. Biol.,* 30, 207–212.

UP (University of Pretoria). 2022. Mnisi Community Programme. Accessed from https://www.up.ac.za/mnisi-community-programme on 11 July 2022.

Yinda, C.K., Rector, A., Zeller, M., Conceição-Neto, N., Heylen, E., Maes, P., *et al.,* 2016. A single bat species in Cameroon harbours multiple highly divergent papillomaviruses in stool identified by metagenomics analysis. *Virol. Rep.,* 6, 74–80.

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2022

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ISSN: 1683-6324

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Ethiop. Vet. J., is the Official Scientific Organ of the Ethiopian Veterinary Association

ETHIOPIAN VETERINARY JOURNAL

(*Ethiop. Vet. J.*)

Objectives

- To stand as the official scientific organ of the Ethiopian Veterinary Association and serve as medium of communication with professionals in the fields of animal health and production and other related disciplines.
- To effectively disseminate research outputs in all spheres of veterinary science, veterinary public health, animal production and other related fields of study.
- To help promote agricultural development in Ethiopia and the subregion as a whole through generation via research of technologies, methods etc and dissemination of scientific knowledge to stakeholders and ultimately to end users.

Type of papers

- Original research papers
- Review articles
- Short communications

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This includes original articles presenting outputs from basic, applied and adaptive research activities related to animal and public health and diverse aspects of animal production in Ethiopia, and from regional and international sources. The material must not have been previously submitted or published elsewhere. The research article should not contain more than 6000 words.

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These include papers covering baseline data/information as well as advances in the field of veterinary medicine, public health and related fields of research and development. Often review articles will be prepared by specialist or researchers actively engaged in particular area of study for a considerable period of time with proven success. This must be supported by a strong track record

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This is devoted to entertain results of original research, selected clinical case reports, brief scientific notes including preliminary results, scientific observation, experimental techniques, recent technological advances and news of interest in the field. Short communication must be written in a synthesis form where all necessary information are presented in a condensed manner. The material must not have been previously published elsewhere. Short communication should not contain more than 3000 words

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Electronic copy in Word file should be addressed to the Editorial-Office. All manuscripts should be accompanied with a letter (or a filled Manuscript Submission and Copyright Transfer Form) signed by all authors, who clearly state that the paper, other than oral presentation or abstract, has not been submitted for publication elsewhere in any form. The Editorial Board reserves the right to accept or reject any paper submitted.

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The title page should consist of:

a) Title of the article, which should be concise and descriptive enough; should be in sentence case, and justified left; avoid abbreviations;
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Abstract

The second page should provide an abstract of not more than 300 words summarizing the background, objective, materials and methods, major findings and their significance, and conclusions. Avoid the use of undefined abbreviations.

Keywords

Below the abstract, in a separate line, keywords, up to six in number should be given in alphabetical order separated by ";".

Introduction

Give sufficient information on the associated background zooming in to the subject in question; articulate the specific research question, and the contribution of your current research in bridging the gap, followed by statement of objective(s) is in order.

Materials and methods

Under this section, briefly state the materials used, the selection of study animals, subjects, herds or materials, and the design for observational or experimental study and the nature of controls. Briefly describe the methods and procedures followed in sufficient detail and give reference; if new or modified methods are used, give justification for using them. Precisely specify apparatus, instrument or chemicals used in terms of manufacturer's name and ad-

dress in parentheses. Similarly, drugs, biologicals, medicinal plants or others used should be sufficiently described in terms of generic name(s) or scientifically accepted identifications, dose(s), and route(s) of administration. Describe sufficiently the statistical methods used and how the magnitude or precision of analysis results reached.

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Quantify qualitative findings and present them with appropriate indicators of measurement of error or uncertainty, such as by using confidence intervals. Likewise, when reporting quantitative data, authors must indicate the extent of variability by either using standard deviation or standard error. Present the results in logical sequence in the text, tables, or illustrations. The results have to be presented in the same order as the questions raised in the objective(s) and methods sections. Results should be concise and no need of interpretation. In clinical or therapeutic trials, report complications or losses or even dropouts of such observations giving numbers. Support all findings by using appropriate statistical analysis.

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Under this heading you should explain the findings and the associated interpretations. Relate the results with the objective of the study and with previ-

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Conclusion

This section should be separately presented with supporting evidences based on the major findings of the study. Appropriate recommendations can be made if necessary

Acknowledgements

Acknowledgments should be briefly stated after the Conclusions. Under this, technical, financial and material support can be mentioned.

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Authors should declare that they have no conflict of interest.

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Jobre, Y., Malone, J. B., McCarroll, J. C., Erko, B., Mukaratirwa, S. and Xinyu, Z., 2001. Satellite climatology and the environmental risk of Schistosoma mansoni in Ethiopia and east Africa. *Acta Trop*., (*In press*).

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D) Books or chapters in books:

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Malone, J. B and Jobre, Y., 1999. Predicting outbreaks of fasciolosis from Ollernshaw to satellites. In: Dalton, J.P. (Ed.), Fasciolosis. CBA International Publications, Cambridge, Pp. 151-183.

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