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Full Length Research Paper

Semen characteristics, morphology and testicular parameters of Uda (*ovis aries*) in Ibadan, Nigeria

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The spermiogram and biometrical observations on the testes and epididymis of 20 adult Uda rams were investigated. The study was aimed at providing information on the reproductive potentials of Uda rams in Ibadan, and thus improved evaluation of breeding soundness in rams. The average age of the rams was 26.40±7.39 months, and their mean body weight was 39.80±3.00kg. The lengths of the right testes (14.34±1.24) and epididymis (20.50±2.94) as well as the circumference of the right testes (19.08±2.46) were significantly higher than their left counterparts (12.86±2.49, 18.50±3.36 and 16.40±4.35, respectively). The longer testes had a higher number of sperm cell abnormalities (23.43±4.97) compared to the shorter testes (15.03±6.91). Progressively, decreasing number of abnormalities was observed from the testes down the length of the epididymis from the head to the tail. The sperm cell abnormality that occurred most was the curved tail while the rudimentary tail was the least observed. The age was positively and significantly correlated with the body weight (r= 0.59, P<0.01) as well as most of the right testicular and epididymal parameters. The right testicular and epididymal volume was positively correlated with sperm motility of right caput epididymis (r= 0.82, P<0.05), and some other spermiogram parameters than the left which did not show any positive correlation. In conclusion, higher correlation observed between age and testicular measurements than with body weight implies that age is an important factor for selection of the Uda breed of ram.

Key words: Testes, epididymis, semen, Uda, ram, Ibadan.

INTRODUCTION

The Uda breed of ram (*Ovis aries*) is one of the four distinct indigenous breeds of ram in Nigeria, and is popularly known as the black and white or brown and white found in the Sudano-Sahelian vegetational region (Ibrahim et al., 2012). Uda is an excellent mutton producer and is highly esteemed during Moslem holidays, on local markets and for export (Devendra and McLeroy, 1982). The mammalian testes are reliable predictors of spermatozoa production (Ibrahim et al., 2012). The two major functions of the testes are production of spermatozoa through a process called spermatogenesis (Blood et al., 2007), and the production of the male

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sexual hormones called androgens. Investigations of testicular biometrics are important for various aspects of reproduction such as puberty characterization, sexual maturity determination, evaluation of spermatogenesis, monitoring the normality of the testis and gauging potential sperm production (da Silva Santos et al., 2013). Biometric parameters such as scrotal circumference, testicular weight and testicular length are vital measurements in the andrological evaluation of a breeding animal (Omar, 2016). Among these parameters, scrotal circumference is often used because it is easy to measure, and displays a high correlation with body weight and reproductive capacity, particularly sperm production but should not be used in isolation for the selection of breeders (da Silva Santos et al., 2013).

A complete andrological evaluation (a breeding soundness examination), including an evaluation of semen quality, should be performed to certify the reproductive capacity of a male (da Silva Santos et al., 2013). The epididymis is an extremely convoluted structure that is closely attached to the dorsal part of the lateral surface of the testes and functions in storage, maturation and absorption of sperm cells (Ibrahim et al., 2012).

Although, research has been done on Uda breed of ram, there is dearth of information on the andrological study as regards semen quality of Uda rams found in Ibadan. This study will provide information about the morphological changes of spermatozoa during epididymal transit, breeding soundness, fertility potential and correlation between the testicular and epididymal biometrics in Uda ram. This will therefore be of great importance to animal breeders and artificial insemination programmes as well as add knowledge to the existing information on some sheep breeds.

MATERIALS AND METHODS

The study was conducted on 20 apparently healthy Uda rams presented for slaughter at Bodija abattoir located in Ibadan North local Government Area of Oyo state, Nigeria on geographic grid reference of longitude 3°54′39″ E and latitude 7°25′35″ N. Prior to slaughter, the rams weighed 39.80±3.00 kg, and were aged 26.40±7.39 months. Immediately the animals were slaughtered, the intra-scrotal testes were maintained at a warm condition (37°C) and transferred to the laboratory within 10 min.

Each testicle with attached epididymis was weighed using a micro-sensitive electronic weighing balance (Lark) to the nearest 0.01 g, and the volume was based on Archimedes principle of water displacement evaluated from the volume of water they replaced. The testes collected were separated from the epididymis and weighed individually. The detached right and left epididymides were also weighed individually. The testicular circumference, testicular lengths and epididymal lengths were measured using a flexible metric tape and recorded.

Semen was obtained from all 40 testicles (20 pairs) from the parenchyma of the testis and from the caput, corpus and cauda epididymis through incisions about 1 cm long made with a scalpel blade. The semen samples were analysed to determine the percentage sperm motility, viability and morphological characteristics as described by Zemjānis (1962).

Percentage motility was evaluated by adding a drop of semen to a drop of 2.9% buffered sodium citrate (Analar, England) on a warm glass slide covered with a glass slip and viewed at a magnification of x40. Only sperm cells moving in a unidirectional motion were included in the motility rating, while sperm cells moving in circles or backward direction were discarded (Zemjānis, 1962).

Percentage viability was done by staining one drop of semen and one drop of warm Eosin-Nigrosin stain (Qualikems, New Delhi) on a warm slide. A thin smear was then made out of the mixture of semen and stain. The smear was air-dried and observed under the microscope. The ratio of the *in vitro* dead sperm cells was observed and it is based on the principle of Eosin penetrating and staining the dead autolysing sperm cells whereas viable sperm cells repel the stain (Zemjānis , 1962).

A drop of semen was placed with two drops of Wells and Awa stain (BDH, England). The semen and stain were thoroughly mixed together, and a smear was made on another slide. The smear was dried and viewed under light microscope (Olympus CX21, Tokyo Japan) starting from lower magnification to higher magnification. The presence of abnormal cells out of at least 400 sperm cells from several fields on the slide was noted (Zemjānis, 1962).

The mean and standard deviation of the values of the parameters for the right and left testes, and epididymides was calculated. The T-test was done to compare the parameters of the right and left testes and epididymides. The morphological characteristics of the spermatozoa from the right and left testes and epididymides were also compared using Analysis of variance (ANOVA). Simple correlation of the testicular and epididymal parameters was also calculated.

RESULTS

The mean age and body weight in the present study were recorded as 26.40±7.39 months and 39.80±3.00 kg, respectively. Table 1 showed the means of right and left epididymal and testicular weight, testicular weight, epididymal weight, testicular length, epididymal length, testicular circumference and testicular and epididymal volume. Significant difference was not observed between the mean values of the right and left weight of epididymis and testes, testicular weight, epididymal weight and testicular and epididymal volume of the right testes and epididymis. Significant difference was observed between the mean values of the right and left testicular length, epididymal length and testicular circumference.

Table 2 shows the spermiogram of the Uda rams. Significant difference was observed for the motility values between the right and left epididymal head and body while for other motility values, no significant difference was observed. Significant difference was not observed for the mean sperm livability values between the right and left testes, epididymal head, epididymal body and epididymal tail.

Table 3 shows that Uda rams age was positively and significantly correlated with the body weight (r = 0.59) as well as with most of the right testicular and epididymal biometrics such as the weight of right epididymis and testes (r = 0.61), weight of right testes (r = 0.55), weight

Table 1. Testicular and epididymal Biometrics of the Uda rams.

Variable	Mean ±SD (Right)	Mean ±SD (Left)
Weight of epididymis and testes (g)	236.50±55.56	187.85±81.99
Testicular weight (g)	200.99±55.07	156.33±79.41
Epididymal weight (g)	35.55±6.49	32.12±8.294
Testicular length (cm)	14.34±1.24*	12.86±2.49*
Epididymal length (cm)	20.50±2.94*	18.50±3.36*
Testicular Circumference (cm)	19.08±2.46*	16.40±4.35*
Testicular and epididymal volume (cm^3)	230.00±53.51	184.00±80.94

* indicates that P < 0.05.

Table 2. Spermiogram (motility and livability) of the Uda rams.

Variable (%)	Mean ±SD (Right)	Mean ±SD (Left)
Testicular sperm motility	21.00±11.43	16.00±8.21
Epididymal head sperm motility	52.00±16.42*	64.00±16.67*
Epididymal body sperm motility	62.00±13.61*	70.00±14.51*
Epididymal tail sperm motility	85.00±9.18	89.20±10.08
Testicular sperm viability	72.00±19.63	72.00±17.35
Epididymal head sperm viability	93.20±6.91	91.00±3.84
Epididymal body sperm viability	92.60±3.41	94.60±2.64
Epididymal tail sperm viability	97.40±1.23	97.40±1.23

*indicates that P < 0.05.

of right epididymis (r = 0.60), length of right epididymis (r = 0.73) and circumference of right testes (r = 0.60). The weight of the left epididymis was negatively correlated with the weight of the right epididymis and testes (r = -0.80), weight of the right testes (r = -0.83), length of right testes (r = -0.80), length of right epididymis (r = -0.86) and circumference of right testes (r = -0.83) at P<0.05 (Table 3).

From Table 4, the right testicular and epididymal volume was positively correlated (P<0.05) with motility of right testes (r=0.45), motility of right caput epididymis (r=0.82), motility of right corpus epididymis (r=0.61), motility of right cauda epididymis (r=0.71), motility of left cauda epididymis (r=0.71) and sperm viability of right caput epididymis (r=0.80). The left testicular and epididymal volume was not correlated with other spermiogram parameters except a negative correlation with sperm viability of left corpus epididymis (r=-0.92, P<0.05).

The sperm viability of right testes was positively correlated with motility of right testes (r=0.51), motility of right caput epididymis (r=0.64), motility of right corpus epididymis (r=0.58), motility of right cauda epididymis (r=0.67), motility of left testes (r=0.84), sperm viability of right corpus epididymis (r=0.61), sperm viability of right cauda epididymis (r=0.84), sperm viability of left testes (r=0.95) and sperm viability of left cauda epididymis (r=0.84). The motility values of the right testes, right caput epididymis, right cauda epididymis, left testes and left caput epididymis were positively correlated (P<0.05) with their corresponding sperm viability values.

From Table 5, it was also observed that the longer testis (right) had a higher number of sperm cell abnormalities compared to the shorter one (left) with the rudimentary tail abnormality being the least observed, and the curved tail abnormalities being the most observed. The number of sperm cell abnormalities progressively decreased from testes down the length of the epididymis (from caput to cauda).

DISCUSSION

The mean body weight of 39.80±3.00 kg reported for Uda rams in this study is higher than those reported by Hassan et al. (2009) in Bangladesh native rams. All the testicular and epididymal biometrics were higher than those reported by Gemeda and Workalemahu (2017) in bucks. The testes weight, epididymal weight and length were lower while testicular length and epididymal and testicular volume were higher than those reported in Uda rams by Ibrahim et al. (2012).

Table 3. Correlation between the right and left epididymal and testicular biometrics of the Uda ram.

Variable	A	B	C	D	E	F	G	H	I	J	K	L	M	N
A	-	0.59*	0.61*	-	0.55*	-	0.60*	-	-	-	0.73*	-	0.60*	-
B	0.59*	-	-	-	-	-	-	-	-	-	0.60*	-		-
C	0.61*	-	-	-	0.99*	-	-	-0.80*	0.94*	-	0.75*	-	1.0*	-
D	-	-	-	-	-	1.0*	-	-	-	1.0*	-	0.84*		1.0*
E	0.55*	-	0.99*	-	-	-	-	-0.83*	0.96*	-	0.75*	-	1.0*	-
F	-	-	-	1.0*	-	-	-	-	-	0.99*	-	0.86*	-	0.99*
G	0.60*	-	-	-	-	-	-	-	-	-	-	-	-	-
H	-	-	-0.80*	-	-0.83*	-	-	-	-0.80*	-	-0.86*	-	-0.83*	-
I	-	-	0.94*	-	0.96*	-	-	-0.80*	-	-	0.59*	-	0.94*	-
J	-	-	-	1.0*	-	0.99*	-	-	-	-	-	0.83*	-	1.0*
K	0.73*	0.60*	0.75*	-	0.75*	-	-	-0.86*	0.59*	-	-	-	0.79*	-
L	-	-	-	0.84*	-	0.86*	-	-	-	0.83*	-	-	-	0.86*
M	0.60*	-	0.99*	-	1.0*	-	-	-0.83*	0.94*	-	0.79*	-	-	-
N	-	-	-	1.0*	-	0.99*	-	-	-	1.0*	-	0.86*	-	-

*Correlation is significant at the 0.01 level (P<0.01). A; Age (months); B, body weight (kg); C, weight of right epididymis and testes (g); D, weight of left epididymis and testes (g); E, weight of right testes (g); F, weight of left testes (g); G, weight of right epididymis (g); H, weight of left epididymis (g); I, length of right testes (cm); J, length of left testes (cm); K, length of right epididymis (cm); L, length of left epididymis (cm); M, circumference of right testes (cm); N, circumference of left testes (cm).

The testes weight recorded were higher than those reported in Balami and Yankassa rams (Ibrahim et al., 2012), West African Dwarf rams (Ahemen and Bitto, 2007) but lower than values reported for Dorper rams by Besta (2006). The testicular lengths reported in this study were higher than those reported by Hassan et al. (2009) in rams. The testicular length and epididymal, and testicular volume recorded were higher than those reported in Balami and Yankassa rams (Ibrahim et al., 2012).

The epididymal weight recorded was similar to values reported for Dorper rams (Besta, 2006), higher than values reported for West African Dwarf rams (Ahemen and Bitto, 2007) and Yankassa rams but lower than values reported for Balami rams (Ibrahim et al., 2012). The epididymal length recorded was lower than those reported in Balami and Yankassa rams (Ibrahim et al., 2012).

Sperm motility in the right testes was higher than the left though not statistically significant which is similar to the finding of Ajani et al. (2015). It is often expected that the longer epididymis should have higher motility as more space will encourage maturity of the spermatozoa in the epididymis, but this was not so in the Uda rams used for this study as there was significant increase in the motility of spermatozoa in the head (caput) and body (corpus) of the left epididymis (the shorter) compared to the right (the longer).

The significant correlation between the age and body weight as well as most of the right testicular and epididymal biometrics is similar to the findings of Koyuncu et al. (2005) in Kivircik lambs. However, in contrast to the findings of Koyuncu et al. (2005) in which body weight was more closely correlated with testicular measurements than age, this study showed higher correlation between age and testicular measurements than with body weight. Significant correlations were found between the paired testicular weight and all testicular dimensions and size. This was similar to the findings of Abdou et al. (1978) and Koyuncu et al. (2005).

Correlation between the testicular circumference and testicular length is similar to the findings of da Silva Santos et al. (2013) in buffalo.

Table 4. Correlation between the right and left epididymal and testicular spermiogram of the Uda rams.

Variable	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
A	-	-	0.45*	0.82*	0.61*	0.71*	-	-	-	0.71*	-	0.80*	-	-	-	-	-	-
B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.92*	-
C	0.45*	-	-	0.83*	0.93*	-	-	0.53*	0.64*	-	0.51*	-	0.41*	-	-	0.82*	-0.68*	-
D	0.82*	-	0.83*	-	0.83*	0.56*	-	-	-	-	0.64*	0.54*	-	-	-	0.47*	-0.56*	-
E	0.61*	-	0.93*	0.83*	-	-	-	0.52*	0.64*	-	0.58*	-	-	-	-	0.77*	-	-
F	0.71*	-	-	0.56*	-	-	0.84*	-0.69*	-0.47*	-	0.67*	-	-	0.84*	0.76*	-0.49*	-	0.84*
G	-	-	-	-	-	0.84*	-	-0.80*	-0.71*	-	0.84*	-	-	-	0.95*	-0.54*	-	-
H	-	-	0.53*	-	0.52*	-0.69*	-0.80*	-	0.96*	-	-	-	-	-0.80*	-0.61*	0.92*	-	-0.80*
I	-	-	0.64*	-	0.64*	-0.47*	-0.71*	0.96*	-	0.53*	-	-	-	-0.71*	-0.54*	0.95*	-	-0.71*
J	0.71*	-	-	-	-	-	-	-	0.53*	-	-	0.98*	-0.76*	-	-	-	-	-
K	-	-	0.51*	0.64*	0.58*	0.67*	0.84*	-	-	-	-	-	0.61*	0.84*	0.95*	-	-	0.84*
L	0.80*	-	-	0.54*	-	-	-	-	-	0.98*	-	-	-0.73*	-	-	-	-	-
M	-	-	0.47*	-	-	-	-	-	-	-0.76*	0.61*	-0.73*	-	-	0.51*	-	-0.46*	-
N	-	-	-	-	-	0.84*	-	-0.80*	-0.71*	-	0.84*	-	-	-	0.95*	-0.54*	-	-
O	-	-	-	-	-	0.76*	0.95*	-0.61*	-0.54*	-	0.95*	-	0.51*	0.95*	-	-	-	0.95*
P	-	-	0.82*	0.47*	0.77*	-0.49*	-0.54*	0.92*	0.95*	-	-	-	-	-0.54*	-	-	-0.48*	-0.54*
Q	-	-0.92*	-0.68*	-0.56*	-	-	-	-	-	-	-	-	-0.46*	-	-	-0.48*	-	-
R	-	-	-	-	-	0.84*	-	-0.80*	-0.71*	-	0.84*	-	-	-	0.95*	-0.54*	-	-

*Correlation is significant at the 0.05 level (P<0.05). A, Volume of right testes and epididymis; B, volume of left testes and epididymis; C, sperm motility of right testes (%); D, sperm motility of right caput epididymis (%); E, sperm motility of right corpus epididymis (%); F, sperm motility of right cauda epididymis (%); G, sperm motility of left testes (%); H, sperm motility of left caput epididymis(%); I, sperm motility of left corpus epididymis (%); J, sperm motility of left cauda epididymis (%); K, sperm viability of right testes (%); L, sperm viability of right caput epididymis (%); M, sperm viability of right corpus epididymis (%); N, sperm viability of right cauda epididymis (%); O, sperm viability of left testes (%); P, sperm viability of left caput epididymis (%); Q, sperm viability of left corpus epididymis (%); R, sperm viability of left cauda epididymis (%).

Testicular length could provide a useful estimate of testicular growth because of its high correlations with the other testicular measurements. This is in agreement with the findings of Land and Carr (1975), Kumi-Diaka et al. (1985), Koyuncu et al. (2005), Hassan et al. (2009), Ibrahim et al. (2012) and Omar (2016).

The number of sperm cell abnormalities progressively decreased from testes down the length of the epididymis (from caput to cauda). This observation may be linked to the effectiveness of epididymal function across the length of the epididymis. The curved tail and curved mid-piece abnormalities occurred most. This is similar to the finding of Ajani et al. (2015) with observation of curved tail as the mostly occurring abnormality.

In conclusion, higher correlation observed between age and testicular biometrics than with body weight implies that age is an important factor for selection of the Uda breed of ram.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

Table 5. Percentage morphological abnormalities of spermatozoa from the right and left testes, caput, corpus and cauda epididymides.

Abnormality	Testes		Caput epididymis		Corpus epididymis		Cauda epididymis	
	Mean±SD (Right)	Mean±SD (Left)	Mean±SD (Right)	Mean±SD (Left)	Mean±SD (Right)	Mean±SD (Left)	Mean±SD (Right)	Mean±SD (Left)
Tailless head	2.17±2.52^{abcd}	1.20±0.71	1.37±1.64	0.43±0.56^{a}	0.90±0.53^{b}	1.46±1.06^{e}	0.27±0.28^{ce}	0.38±0.52^{d}
Headless tail	1.69±1.33^{abcde}	1.04±0.96^{f}	1.12±1.62^{g}	0.47±0.36^{a}	0.42±0.37^{b}	0.67±0.63^{c}	0.13±0.19^{dfg}	0.37±0.46^{e}
Rudimentary tail	0.07±0.14^{a}	0.20±0.17^{bcd}	0.35±0.51^{aefghi}	0.00±0.00^{be}	0.00±0.00^{cf}	0.11±0.14^{g}	0.00±0.00^{dh}	0.05±0.10^{i}
Bent tail	3.11±1.06	2.19±1.27^{ab}	3.77±1.39^{ac}	2.86±1.70	3.76±0.89^{bd}	3.21±1.77	2.77±0.69	2.37±1.27^{cd}
Curved tail	3.94±1.39	2.78±1.70^{ab}	4.52±1.08^{ac}	3.50±1.20	4.24±0.57^{d}	3.48±1.91	3.28±0.57	2.88±1.74^{cd}
Bent mid-piece	2.22±0.51	2.02±1.13	2.99±0.78^{ab}	2.09±1.64	2.07±0.68	1.79±1.01^{a}	1.82±0.41^{b}	2.18±1.27
Curved mid-piece	3.75±1.13	3.02±2.13	4.01±0.90^{abc}	2.39±1.74^{ad}	3.94±0.71^{de}	2.86±1.74	2.59±0.89^{b}	2.43±1.47^{ce}
Looped tail	0.27±0.35^{a}	0.20±0.28^{b}	0.22±0.20^{c}	0.98±1.43^{abcdef}	0.21±0.44^{d}	0.22±0.33^{e}	0.05±0.10^{fg}	0.84±1.03^{g}
Total cells	23.43±4.97	15.03±6.91	26.26±4.02	15.03±7.78	20.22±0.84	17.22±7.29	11.28±1.57	12.61±6.96

Means with same superscripts in the same line are significantly different at P<0.05.

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Full Length Research Paper

Effect of topical application of mixture of cod liver oil and honey on old (chronic) wounds and granulation tissue in donkeys

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A comparative study was conducted from November 2015 to May 2016 with the objective of investigating the beneficial effects of the mixture of cod liver oil and honey (group 1) comparing with routine treatment (group 2), combination of chlorhexidine (0.3%) and cetrimide (3%) in healing of contaminated old wounds in donkeys at Bahir Dar, North western Ethiopia. Out of 18 donkeys, 12 (6 male and 6 female) were treated with mixture of cod liver oil and honey and 6 donkeys (3 male and 3 female) were routinely treated. At the 35th day of treatment in group 1, the areas of the wounds were markedly decreased from 4.2% to 66.7% and in group 2 from 66.7% to 85.7% out of 100% of the initial area. The treatment outcome between group 1 and group 2 were significantly different ($p<0.05$).In group 1, no swelling and hyperemia of perilesional skin appearance, no inflammatory exudate, reduced wound area and short time to clinical healing of wound were recorded after treatment. This study also demonstrated that difference in wound healing process between sex groups, in which wound healing in male was significantly ($p<0.05$) faster than female. Mixture of cod liver oil and honey is beneficial in treatment of old traumatized wounds in the donkeys. This effect was primarily mediated by formation of healthy mature scars, clinical healing in short period of time. The owners, institutions or organization working with donkeys and governments may use this mixture for treating old traumatized wounds.

Key words: Cod liver oil, donkey, healing, honey, wound.

INTRODUCTION

More than 72% of the world's equine population resides in developing countries kept for draft purpose (Swann, 2006). Ethiopia has more than 6 million donkeys, the second largest donkey population in the world next to China, 1.9 million horses and over 350,000 mules (FAOSTAT, 2012). Equines are important animals to the resource-poor communities in rural and urban areas of Ethiopia, providing traction power and transport services at low cost (Dinka et al., 2006).

In Ethiopia, the rugged terrain characteristics, absence of well-developed modern transport networks and the prevailing low economic status of the community necessitate the use of equines for transportation (Alemayehu, 2004). In rural and per-urban area, people

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used equines to transport crops, fuel wood, water, building materials and people can be transported by carts or on their back from farms and market to the home (Mohammed, 1991).

Despite their uses, the husbandry practices of working equines are poor. Some methods of hobbling to restrain equines cause discomfort and inflict (Alujia and Lopez, 1991; Mohammed, 1991). As per Alujia and Lopez (1991) report, loading of donkeys without padding and over loading in long distance causes external injury on donkeys and mules. Poorly designed harnesses or yokes that may be healthy and ragged have an effect on the animal health and safety (Alujia and Lopez, 1991).

Wound is an open mechanical injury of the epidermis, underlying the tissues and organs. It is characterized by pain, and gaping bleeding functional disturbance. The most common cause of wounds in working equine are over loading, accidents, improper position of load predisposing to falling, hyena bites, donkey bites, injuries inflicted by horned Zebu (DACA, 2006). Some hobbling methods, inappropriate harnesses or yokes that may be heavy and ragged, long working hours may cause discomfort and inflict wounds (Mekuria et al., 2013).

Wounds are one of the primary welfare concerns of working equines (Sells et al., 2010). The type of wound in working donkeys includes tissue damage with or without blood or exudates or pus, abscess formation, or any secondary bacterial complication. Bites (lacerated wounds) will be identified by irregular edges with underlying tissues removed as well as hemorrhage (Sevendsen, 2008).

Wounds can be either traumatic or surgical in origin; both types can fail to heal and become chronic although traumatic wounds are more commonly affected by healing difficulties. The incidence and prevalence of traumatic wounds in equine is considered to be high (Singer et al., 2003) and a high percentage become chronic, adding more complexity to wound healing management strategies.

Skin lacerations and other traumatic injuries of the integument are frequently seen in equine practices and range from relatively minor cuts to severe, potentially debilitating injuries. The challenges facing the practitioner managing these injuries are numerous, and treatment is dictated by the nature and size of the wound, the area of the body on which the wound occurs, and several aspects of wound healing unique to horses. The age of the wound, integrity of the local blood supply, degree of contamination, location of the injury, skin loss, and local tissue damage must all be considered when deciding on the most appropriate method for managing a particular wound. In addition to biologic factors, the physical size of equine patients and the environment in which they are kept present unique management challenges not encountered in the treatment of soft tissue injuries in other species (Jeremy, 2006).Wounds are of great concern in donkeys as they affect animal productivity and their treatment represents an economic burden to the owners particularly in developing countries (Magda and Khaled, 2011). Granulation tissue is the pebbly or granular appearing tissue which develops in healing wounds anywhere on the horse's body. It is composed of small blood vessels and fibroblasts, but has no nerve supply (Christina, 2002).

Treatment methods that are employed in the management of wounds focus on rapid and efficient evaluation, scrupulous, aseptic surgical techniques, and conscientious and prolonged aftercare (Griffiths et al., 2003).

Many therapeutic agents are used for topical treatment of wounds including yeast cell derivatives (Crowe et al., 1999), cod liver oil (Kietzmann and Braun, 2006), honey (Iftikar et al., 2009), sugar (Cavazana et al., 2009), corticosteroids (Jorissen and Bachert, 2009) and phenytoin (Qunaibi et al., 2009). Honey and cod liver oil are increasingly used as natural products and biological therapies in clinical practice. To accelerate wound healing, modern honey wound dressings have become more widely available and used in wound management (Zumla and Lulat, 1989). This is largely due to the growing clinical problems of antibiotic-resistant bacteria and the combined difficulties for the practitioners in managing chronic wounds such as burns and leg ulcers (Lay-flurrie, 2008). Besides antimicrobial effects of honey (Cooper and Molan, 1999), it has anti-inflammatory and antioxidant properties (Gheldof and Engeseth, 2002), promotes moist wound healing and facilitates debridement (Majtán, 2009; pieper, 2009). Cod liver oil is a nutritional supplement derived from liver of cod fish. It has high levels of omega 3 fatty acid, vitamin A and vitamin D. Terkelsen et al. (2000) reported that cod liver oil was beneficial in wound healing as it enhances epithelization and revascularization.

Management of wound in the study area was practice locally which leads to delay in clinical healing, development of old wounds with or without granulation tissue. Due to this poor practice, the owners lost their money, time, working efficiency, and the donkey itself.

Hence, considering the importance of donkey wound management, the topic was built up to investigate the beneficial effects of the mixture of cod liver oil and honey in the contaminated old wounds without any other topical disinfectants or antimicrobial devices. The general objectives of the study include, investigation of the clinical wound healing process with a mixture of cod liver oil and honey in treatment of old wounds and granulation tissue in donkeys and the specific objectives include evaluation of the time taken for clinical healing of old chronic wound.

MATERIALS AND METHODS

Study area

The study was conducted from November 2015 to May 2016 in and

Figure 1. Natural bee honey (A) and cod liver oil (B).

around Bahir Dar (Amhara Region), North Western part of Ethiopia. It is located 564 km from Addis Ababa, capital of Ethiopia. The study area is covers a total of 197,199 hectares of land which has a summer rainy season with the highest rain fall between June and September (1200-1600 mm) and winter dry season (December to March) with mean annual temperature of 23°C. Located 11'29''N latitude, 37'29''E longitude and with altitude range of 1500-2300 meters above sea level (ANRSAB, 1999).

Topography of the area is characterized with slight slopping covering about 70% of a total land of area, and marked with Lake Tana and Abay River (ANRSAB, 1999). The land is covered by various, low woods, and mainly evergreen plants of various types of, with vegetation cover of land. The main agricultural product is teff, barley, sorghum, wheat, maize and all pulse crops (ANRSAB, 1999). The region has 1.4 million cattle, 1.3 million sheep and goats and 2.8 million equines of which the figure in Bahir Dar and its surroundings are estimated to have about 58 horses, 550 mule and 19517 donkeys (CSA, 2010).

Study population

The study was conducted on both sexes of the local breeds of donkeys affected with chronic and old wounds. Donkeys in the study area were mainly used for water, grain, stone, and fire wood transportation. Wounds are mainly due to car accident, heavy loading, loading without padding, improper tying of legs with rope and loading of hot flour. Those donkeys exposed to wound and/or granulation tissue were treated with cod liver oil and honey mixture (n=12) and with routine treatment (n=6) within a given time.

Selection of animals

A total of 18 adult donkeys of both sexes having wounds admitted in the Donkey Sanctuary Veterinary Service Centers for treatment of wounds were used.

Materials used during the study

Cod liver oil, tap water, natural honey, savlon (trade name), cotton gauze, ordinary ruler, shaving blades, 50 ml syringe, cotton and curved and straight scissors (Figure 1).

Treatment of old wounds in donkeys with mixture of cod liver oil and honey

12 donkeys (6 male and 6 female) were presented in the Donkey Sanctuary Veterinary Service Center. After clinical examination, all donkeys were suffering with old and heavily traumatized wound along with the infection on the wound surface. Most wounds were located on the caudal back region of the donkeys. All of these wounds were caused due to heavy loading, loading without padding, improper loading of hot flour, and frequent loading with water for cultivation of chat in the area.

Treatment procedure

Before treatment history was recorded related to age, sex, breed and consent for treatment from the owner of the donkey was obtained. Treatment of wounds was carried out in the following steps: (1) the whole area around the wound up to about 5 cm length from the wound edges was clipped with curved scissor and shaved with shaving blade. (2) The Presence of Inflammatory Exudates (PIE) and Perilesional Skin Appearance (PSA) were assessed. (3) Its area was calculated by multiplying the two largest dimensions to assess the initial size and evaluate the progress in the healing process using ordinary ruler. (4) Grossly contaminated wounds were washed with slight warm water with body temperature water using syringe. (5) Then wound surface was covered with piece of gauze soaked in a mixture of an equal volume of cod liver oil and honey. The amount of the mixture varied according to the wound size. Generally, about 20 ml of the mixture will be used for 100 cm^2 dressing area on the body of the animal. (6) The frequency of changing dressing was decided with how rapidly the mixture is diluted with exudates. The bandage was changed daily up to seven days, every third day for two weeks and then once a week till clinical union of the wound took place. (7) At the end of treatment, the time required for clinical union of the wound, the remaining area, the perilesional skin appearance and the presence of inflammatory exudate were recorded.

Treatment of old wounds in donkeys with routine treatment savlon (chlorhexidine 0.3 + cetrimide 3%)

To investigate the whole effect of the mixture of cod liver oil and honey on the healing of wounds, its effects were compared with the routine treatment. Six donkeys (3 male and 3 female) with old wounds were prepared for this treatment. The wound area was aseptically prepared about a 5 cm from the edge of the wound. Hemorrhage was controlled by pressing of the wound surface for 10 minduring preparation. The wound surface was covered with piece of gauze soaked in savlon. The bandage was changed periodically daily for seven days, and every third day for two weeks and then once a week till the 35th day of treatment. In addition a combination of penicillin (8 mg) and dihydrostreptomycinsulphate (10 mg) per kg

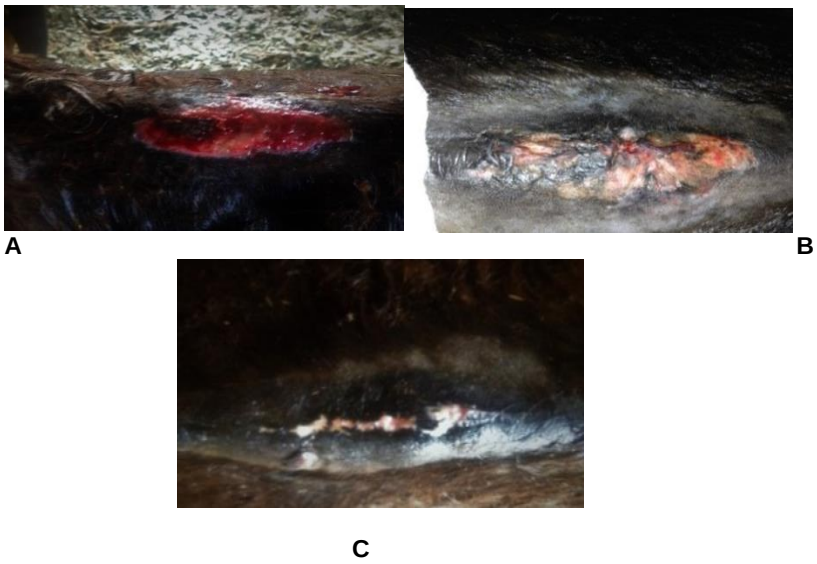

Figure 2. A. After cleaning with tap water. **B.** After one week of treatment with mixture of cod liver oil and honey. **C.** After three weeks of treatment with a mixture.

body weight or 1 ml penicillin and streptomycin per 25 kg bodyweight was given for three days to control the bacterial infection of the wound. The area of the wound, the time required for clinical union, PSA and PIE of the wound were recorded.

Statistical analysis

The data was entered into Mc-soft excel spread sheet and analyzed by STATA version 12.0. Independent t-test for the two groups of treatment and dependent t-test for sex difference within one group of treatment were utilized.

RESULTS

Treatment of old wounds in donkeys with mixture of cod liver oil and honey

The clinical healing of wounds in treated cases with the mixture of cod liver oil and honey took place in a period of time ranged from two to five weeks. It was found that surgical debridement at the beginning of treatment was important in most cases due to presence of granulation tissue. Washing of the wound with tap water was seen to be very successful in removal of debris, necrotic tissue and pus as well as helpful in refreshment of the wounds surface (Figures 2 and 3).

After one week of treatment, the wounds surface appeared bright red in color, moist and not elevated above wound edges. After two to three weeks of treatment, wounds in all cases showed cleanness and healthy surface and observable decrease in the wound surface. Wounds areas were markedly decreased after five weeks of treatment. After four weeks of treatment in eight cases, the remaining wound areas out of 100% of the initial area were from 4.2 to 37.5% and there was no swelling and hyperemia at the perilesional skin appearance (PSA) and no inflammatory exudate (PIE) over the surface of the wound. The rest four cases showed that for 50 to 66.6% area from the initial area, there was mild swelling and hypereamia and thick crust over the wound surface (Table 1).

Wound healing difference between Sex group

Area, perilesional skin appearance (PSA), presence of inflammatory exudate (PIE) and time required to clinical union of wound were significantly different ($p< 0.05$) between both sex groups. In two cases, male donkeys showed remaining wound areas of 30 and 37.5% and in five cases, it was between 4.2 to 17% out of 100% of the initial area. The perilesional skin appearances and presence of inflammatory exudate in male were none except one which showed mild swelling and hyperemia and thick crust over the wound surface while in the female donkey, there was mild swelling and hypereamia and thick crust over the surface of the wound except one case which showed no exudate. The time required for clinical union in male cases took place between a time range of two to five weeks whereas in females four to five weeks.

Treatment of old wounds in donkeys with routine treatment savlon (Chlorhexidine0.3%+cetrimide 3%)

All treated cases with routine treatment, not showing

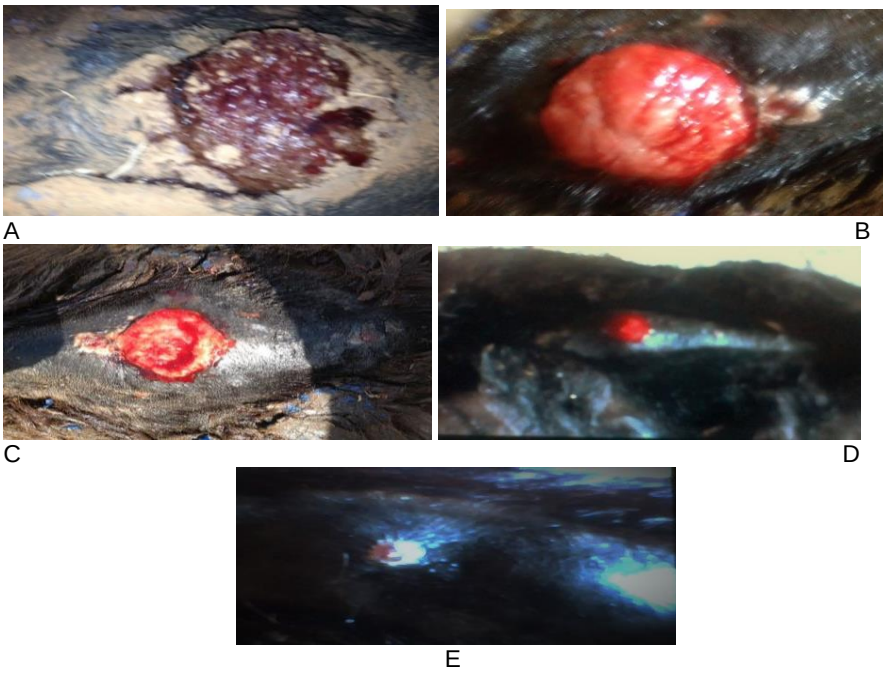

Figure 3. A. Before cleaning. **B.** After cleaning. **C.** After surgical removal of granulation tissue. **D.** After two weeks of treatment with mixture of cod liver oil and honey. **E.** After four weeks of treatment with mixture.

clinical union of wounds within a given period of time (35 days) and the area of wounds were not decreased(Figure 4). After six weeks of treatment only thick exudate was observed (Table 2).

Wound healing difference between two treatment groups

The times taken to clinical union of wound, area, perilesional skin appearance (PSA), and presence of inflammatory exudate (PIE) were significantly different ($p< 0.05$) between the two treatment different groups (Tables 3, 4, 5, 6, 7, 8, 9 and 10).

DISCUSSION

When treating equine wounds, the primary goal is to obtain rapid wound healing with a functional and aesthetically satisfactory outcome. Dressing are used to enhance and support the healing process by decreasing contamination, oedema or exudate, protesting against movement and further trauma and optimizing moisture, temperature, pH and gaseous exchanges at the wound site (Knottenbelt, 2003). Equines are known for their tendency to wound probably due to their inquisitive nature, large size and confinement in areas with potential obstacles such as metal or wire leads in their known difficulties with healing. This study shows the beneficial effect of mixture of cod liver oil and honey in treatment of old traumatized chronic wounds in donkeys. In clinically treated wounds, it was found that surgical debridement washing with slight warm water were valuable steps in removing granulation tissue and debris and in minimizing infection. Formation of granulation tissue in wounds usually occurs as the result of weakness of the initial inflammatory response of wound which leads to chronic inflammation which further inhibits wound contraction and promotes exuberant granulation tissue formation (Wilmink and Weeren, 2005).

Application of mixture of cod liver oil and honey, after debridement, on and around the wounds, brought significance in the requirement of the infection, and observable decrease of wound surface after two weeks to five weeks of treatment. These effects of the mixture appeared to be mediated by the effects of honey, and vitamin A and omega-3 fatty acids in cod liver oil. Gethin et al. (2008) reported that honey dressings were associated with significant reduction in non-healed chronic superficial ulcers. Terkelsen et al. (2000) reported that vitamin A had an important role in accelerating wound healing process. McDaniel et al. (2008) reported that omega-3 fatty acids in cod liver oil can increase in the wound healing through increasing pro-inflammatory cytokines production at wound sites. After application of the mixture, all wounds were covered with protective bandages which were advantageous in controlling

Table 1. Area, PSA, PIE, time taken to clinical union of the wound in group 1.

Number	Sex	Area(size)of wound before treated with oil-honey mixture (cm²)	PSA before treatment	PIE before treatment	Area of after treatment (cm²)	PSA after treatment	PIE after treatment	Time taken to clinical union of wound (in weeks)
W1	M	4*3=12	Swollen and hypermia	Thin	2*1=2(17%)	No swelling and hypereamia	None	2
W2	F	7*4=28	Swollen and hypermia	Thin	5*3=15(53.6%)	Mild swelling and hypermia	Thick crust over the wound surface	4
W3	M	6*3=18	Swollen and hypermia	Thin	1*1=1(5.6%)	No swelling and hypereamia	None	3
W4	M	8*5=40	Swollen and hypermia	Thin	4*3=12(30%)	No swelling and hypereamia	None	3
W5	F	6*4=24	Swollen and hypermia	Thin	4*3=12(50%)	Mild swelling and hypermia	Thick crust over wound surface	5
W6	M	5*3=15	Swollen and hypermia	Thin	1*1=1(6.6%)	No swelling and hypereamia	None	3
W7	M	7*5=35	Swollen and hypermia	Thin	2*2=4(11.4%)	No swelling and hypereamia	None	3
W8	F	6*5=30	Swollen and hypermia	Thin	5*4=20(66.7%)	Mild swelling and hypermia	Thick crust over wound surface	5
W9	F	5*4=20	Swollen and hypermia	Thin	3*3=9(45%)	No swelling and hypereamia	Thick crust over wound surface	4
W10	M	4*3=12	Swollen and hypermia	Thin	1*0.5=0.5(4.2%)	No swelling and hypereamia	None	5
W11	F	6*3=18	Swollen and hypermia	Thin	3*2=6(33.3%)	No swelling and hypereamia	None	4
W12	F	8*4=32	Swollen and hypermia	Thin	6*2=12(37.5%)	Mild swelling and hypermia	Thick crust over wound surface	5

bleeding, reducing the tendency for granulation tissue formation, absorbing exudates, keeping the wounds moist which helps epithelization, protecting the wound from contamination, dust and flies and keeping the topical mixture used better in contact with the wound surface. In wounds treated with routine treatment (group 2) there was very slow clinical healing process and not significant decrease in wound size when compared to those treated with the mixture (group 1). Similar observations reported by Iftikhar et al. (2010) found that honey increased epithelization in wound models in rats. Majtán et al. (2010) observed that honey increased metalloproteinase-9 in cultured human keratinocytes. Metalloproteinase-9 was observed to degrade type IV collagen in the basement membrane and further facilitate migration of keratinocytes (kyriakides et al., 2009). Regarding cod liver oil, it was reported that topical application of cod liver oil ointment to surgically-induced full thickness

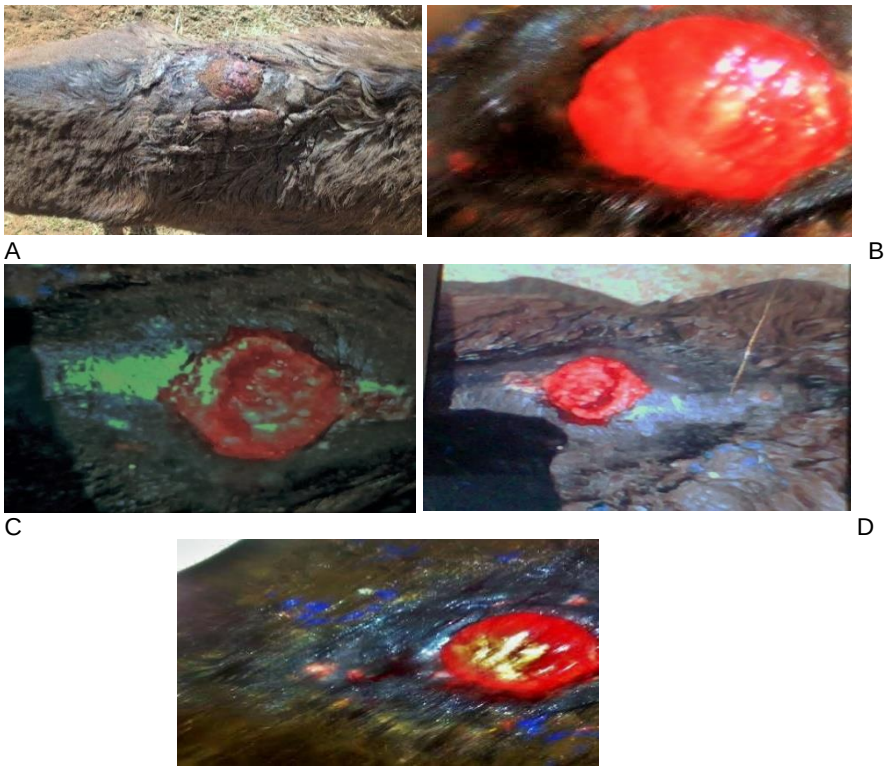

Figure 4. **A.** Before cleaning the wound. **B.** After cleaning. **C.** After surgically removing the granulation tissue. **D.** After three weeks of treatment. **E.** After five weeks of treatment.

Table 2. Area, PSA, PIE and time taken to clinical union of the wound.

Number	Sex	Area (size) of wound before treatment (cm²)	PSA before treatment	PIE before treatment	Area (size) of wound after treatment(cm²)	PSA after treatment	PIE after treatment	Time to clinical union of the wound (in weeks)
W1	F	7*2=14	Swollen and hyperemia	Thin	6*2=12(85.7%)	Swollen and mild hypereamia	Thick	No clinical union took pace
W2	F	6*2=12	Swollen and hyperemia	Thin	4*2=8(66.7%)	Mild swelling and hypereamia	Thick	No clinical union took pace
W3	M	5*3=15	Swollen and hyperemia	Thin	5*2-10(66.7%)	Mild swelling and hypereamia	Thick	No clinical union took pace
W4	M	6*4=24	Swollen and hyperemia	Thin	5*4=20(83.3%)	Swollen and hypereamia	Thin	No clinical union took pace
W5	M	4*3=12	Swollen and hyperemia	Thin	3*2=6(50%)	Mild swlling and hypereamia	Thick	No clinical union took pace
W6	F	3*2=6	Swollen and hyperemia	Thin	2*2=4(66.7%)	Swollen and hypereamia	Thin	No clinical union took pace

dermal wounds on the ears of mice resulted in faster epithelization than those coated with vaseline vehicle (Terkelsen et al., 2000). Vitamin A and D in cod liver oil are responsible for such effects. The mixture produced good results in clinically admitted wounds at the end of fourth week. This appeared due to formation of healthy scar that showed higher degree of maturity with an increasing number of fibrocytes and parallel collagen fibers.

This study also demonstrated some sex differences in

Table 3. Area of wound between group 1and 2 after treatment.

S/N	Group 1	Group 2
W1	2*1=2(17%)	6*2=12(85.7%)
W2	5*3=15(53.6%)	4*2=8(66.7%)
W3	1*1=1(5.6%)	5*2-10(66.7%)
W4	4*3=12(30%)	5*4=20(83.3%)
W5	4*3=12(50%)	3*2=6(50%)
W6	1*1=1(6.6%)	2*2=4(66.7%)
W7	2*2=4(11.4%)	
W8	5*4=20(66.7%)	
W9	3*3=9(45%)	
W10	1*0.5=0.5(4.2%)	
W11	3*2=6(33.3%)	
W12	6*2=12(37.5%)	

Table 4. Statistical analysis of area between group 1 and 2, ($p< 0.05$).

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
var1	6	1.5	0.34	0.84	0.62	2.38
var2	6	2.83	0.17	0.41	2.40	3.26
Diff	6	-1.33	0.49	1.21	-2.60	-0.06

Table 5. PIE between group 1 and 2 after treatment.

S/N	Group 1	Group 2
W1	None	Thick
W2	thick crust over the wound surface	Thick
W3	None	Thick
W4	None	Thin
W5	thick crust over the wound surface	Thick
W6	None	Thin
W7	None	
W8	thick crust over the wound surface	
W9	thick crust over the wound surface	
W10	None	
W11	None	
W12	thick crust over the wound surface	

Table 6. Statistical analysis of PIE between group 1 and 2, ($p< 0.05$).

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
var1	6	0.33	0.21	0.52	-0.21	0.88
var2	6	2.33	0.21	0.52	1.79	2.88
Diff	6	-2	0.37	0.89	-2.94	-1.06

clinical healing of wound. Clinical requirement of wound in male donkeys were significantly ($p<0.05$) faster than female donkeys. This may be due to the fact that in male there is larger dermal thickness compared to female, a parameter that has been reported to be under the influence of male hormones (Azzi et al., 2005). A direct

Table 7. PSA between group 1 and 2 of treatment.

S/N	Group 1	Group 2
W1	No swelling and hypereamia	Swollen and mild hypereamia
W2	Mild swelling and hyperemia	Mild swelling and hypereamia
W3	No swelling and hypereamia	Mild swelling and hypereamia
W4	No swelling and hypereamia	Swollen and hypereamia
W5	Mild swelling and hyperemia	Mild swelling and hypereamia
W6	No swelling and hypereamia	Swollen and hypereamia
W7	No swelling and hypereamia	
W8	Mild swelling and hyperemia	
W9	No swelling and hypereamia	
W10	No swelling and hypereamia	
W11	No swelling and hypereamia	
W12	Mild swelling and hyperemia	

Table 8. Statistical analysis PSA between group 1 and 2, ($p< 0.05$).

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
var1	6	0.33	0.21	0.52	-0.21	0.88
var2	6	1.5	0.22	0.55	0.93	2.10
Diff	6	-1.17	0.40	0.98	-2.20	-0.13

Table 9. Time taken to clinical union of the wound between group 1 and 2 of treatment.

S/N	Group 1	Group 2
W1	2 weeks	no clinical union took pace
W2	4 weeks	no clinical union took pace
W3	3 weeks	no clinical union took pace
W4	3 weeks	no clinical union took pace
W5	5 weeks	no clinical union took pace
W6	3 weeks	no clinical union took pace
W7	3 weeks	
W8	5 weeks	
W9	4 weeks	
W10	5 weeks	
W11	4 weeks	
W12	5 weeks	

Table 10. Statistical analysis of time taken to clinical union of the wound between group 1 and 2, ($p< 0.05$).

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
var1	6	1.33	0.21	.52	0.79	1.88
var2	6	3	0	0	3	3
Diff	6	-1.67	0.21	0.52	-2.21	-1.12

relationship between dermal thickness and collagen content, and thereby skin strength or mechanical resistance, has been suggested (Shuster et al., 1975). In relation to skin injury, the expression of collagen is markedly increased in fibroblasts in the dermis. This is followed by an extensive remodeling phase, in which the

collagen content is degraded by the coordinated action of several collagenolytic proteases, whose expression and activation have been reported to depend on plasmin (Pins et al., 2000).

CONCLUSION AND RECOMMENDATIONS

This study demonstrated that cod liver oil and honey mixture was beneficial in healing of old traumatized wounds in donkeys before and after treatment, the time required to clinical healing of the wound, perilesional skin appearance and presence of inflammatory exudate. In conclusion, usage of mixture of cod liver oil and honey for old wound help in donkeys early clinical healing, reduce extra expenditure of money and time. Study also demonstrated that there was a significant difference in wound healing between sex groups.

Based on the important major findings and conclusion drawn, the following recommendations are forwarded: The owners, institutions or organization working with donkeys can use this mixture for treating old traumatized wounds as it enhances early wound healing, formation of healthy scar and can reduce risk of antibiotic resistance and also the knowledge of importance and usage of the mixture of cod liver oil and honey in wound healing may be transferred to the community for adoption.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Abbreviations: FAOSTAT, Food and Agricultural Organization Statistical; **DACA,** Drug Administration and Control Authority; **PSA,** perilesional skin appearance; **PIE,** presence of inflammatory exudate; **SIS,** small intestine submucosa; **LYCD,** live yeast cell derivatives; **BCE,** before christian era; **PI,** povidone iodine; **VEGF,** vascular endothelial growth factor; **ATP,** adenosine triphosphate; **DNA,** deoxyribonuclic acid; **MRSA,** methicillin resistant *Staphylococcus aureus*; **HP,** hydrogen peroxide; **ANRSAB,** Amhara National Regional State Agriculture Bureau; **CSA,** Central Statistical Agency; **CM,** centimeter; **ML,** milliliter; **MG,** milligram; **KG,** kilogram.

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Cross-sectional study on the prevalence and risk factors for major skin diseases of cattle in Hawassa city, Southern Ethiopia

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A cross-sectional study was conducted on the major skin diseases of cattle in Hawassa city with the objectives of estimating the prevalence and assessing the effect of different risk factors in the occurrence of these skin diseases in the study area. The study was conducted with thorough physical examination of cattle and laboratory examination of samples collected from all encountered cases. Out of 662 cattle examined for the presence of skin diseases, 146(22.05%) cattle had different skin diseases, namely ring worm (6.2%), lice infestation (6%), tick infestation (5.3%), wart (4.4%), lumpy skin disease (0.6%), mange mites (0.3%) and dermatophilosis (0.2%).There was a significantly higher ($p<0.001$) prevalence of skin diseases in young (38.5%) than the old (10.3%) animals. However, the other considered risk factors namely; sex, origin and management were not statistically associated with the occurrence of skin diseases. In general, unless feasible control measures are timely implemented the encountered skin diseases can have varied and adverse effect on cattle production, tanning industry and health of the public and concomitantly pose huge economic loss.

Key words: Skin diseases, ring worm, ectoparasites, wart, risk factors, cattle, Hawassa.

INTRODUCTION

The livestock subsector of Ethiopia is the second major sources of foreign currency, through export of live animals and hides and skins (Ayele et al., 2003; Teshome and Derso, 2015). Although the country produces about 2.7 million of hide, 8.1 million of sheepskin and 7.5 million of goatskin per annum for tannery industry, yet as many as one quarter to one – third of all the skins processed at tanneries are unsuitable for export due to various defects (Kassa et al., 1998; Abebayehu et al., 2011). Up to 65% of these defects are believed to occur in the pre-slaughter stage of production while the animal is still alive (Kassa et al., 1998; Wondwossen, 2000). The most common cattle skin diseases reported in Ethiopia are dermatophilosis, lumpy

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skin disease, dermatophytosis, pediculosis, acariasis, photosensitization and warts (Chalachew, 2001; Teshome and Derso, 2015).

Skin diseases are accountable for significant and varied socio-economic impacts. Apart from quality degradation of hides and skin, skin diseases induce associated economic losses due to reduction of wool quality, meat and milk yield, losses due to culling and occasional mortalities related with cost of treatment and prevention of the diseases (Yacob et al., 2008; Salih et al., 2015). In addition, some skin diseases such as ringworm and sarcoptic mites are potential zoonosis (Quinn et al., 2002; McDaniel et al., 2014).

The huge impact of socioeconomic perspective still demands the nationwide detailed investigation of the distribution of important skin diseases and their determinants. Though some research works on bovine skin diseases have been done in some part of the country (Chalachew, 2001; Yacob et al., 2008; Teshome and Derso, 2015), most of them were based on tannery information, some others were focused only on dermatophilosis (Berhanu and Woldemeskel, 1999; Woldemeskel,2000; Woldemeskel and Taye, 2002; Kassaye et al., 2003) and most of them were done in areas other than the current study site and hence there is still information gap in some geographic location and on the remaining skin diseases.

Therefore, this study was conducted to assess the prevalence of major skin diseases of cattle in Hawassa city and to assess the effect of different risk factors associated with these skin diseases.

MATERIALS AND METHODS

Study area

The study was carried out from October 2014 to May 2015 in Hawassa city, which is the capital of south nation nationalities and people regional state (SNNPRS). Hawassa is located at 38°29'E and 7°05'N, 275 Km south of Addis Ababa. The elevation is about 1790 m above sea level and the respective minimum and maximum average temperature is 12 and 28°C. The mean annual rain fall is 960 mm. The short rainy season/'belg' start from February to May and the long rainy season /'meher' start from mid-June to October.

The total livestock population of the zone was 2,172,015 cattle, 858,206 sheep and goat, 155,356 equines and 2,123,579 poultry (CSA, 2017). The two rural villages, Chefe and Dato, are characterized by water lodged and marshy land and are located north east main part of the city following the water course tributary to Lake Hawassa called 'Tikur weha'. The area is known for huge indigenous cattle population and cash crops like Catha edulis (Chat) and 'Enset'.

Study animal and sampling method

The sampling was made first by randomly selecting small-scale rural and urban dairy farms from Hawassa, Chefe and Dato and then the study animals, comprising both sexes, both local and crossbred and different age group were selected using simple random sampling.

Accordingly, a total of 662 cattle, composed of 213 male and 449 female, were selected for physical and laboratory based examinations. Majority (528) of the study animals were cross bred and the rest (134) were local (Zebu) breeds. The age of the animals was estimated based on teeth eruption as suggested by Muylle (2016). The average age of the examined cattle was 2.6 years. For simplicity sake, the study animals were categorized into young (less than 2 years) and adult (above 2 years). The management was classified as poor and better based on the availability of additional feed, shelter and medicament when needed.

Study methodology

History taking and physical examination

History regarding, age, presence of itching, course and progression of the disease, measures taken, if any; the number of animal having the same problem, type of lesion and\or clinical signs observed and their chronology were recorded. The entire skin of the animals was thoroughly inspected and palpated for the presence of papules, vesicles, pustules, erythema, scabs, pigmentation abnormalities, alopecia, tumorous growth and ectoparasites. Distribution of the lesion on the body of the animal and symmetry were also considered.

Samples collection and processing

Depending on the case, appropriate samples were collected for microbiological and parasitological examinations. Lumpy skin disease was diagnosed tentatively based on the characteristic clinical findings and previous serological confirmation made in the area.

Skin scraping: *Skin scraping was collected from representative sites of cases presented with alopecia, Itching and erythematous lesions. Skin scrapings collected from suspected cases were placed in dry clean sterile test tubes. In addition to superficial skin scraping taken from the periphery of the circular lesion, hair specimens were also collected from lesions suspected of dermatophytosis using a pair of forceps. Similarly, for dermatophilosis suspected cases, exudative crusts were also collected by pairs of forceps along with a deep scraping with clean surgical blade. Moreover, the nodular lesions of demodicosis suspected cases were pressed in between two digits until the pus is expressed, the pus was then placed on a slide and crushed in between two glass slides or after placing cover slip (Greiner, 2012; Abu-Samra et al., 2014). The collected samples were properly labeled, packed and transported to the Veterinary Parasitology and Pathology* Laboratory*, Hawassa University for examination.*

Tick and lice collection and examination: Ticks and lice were manually collected by searching on different regions of the animals' body. Various predilections/feeding sites namely; base of the tail, ear, perianal area, sternum, scrotal area and the belly were the targeted sites. The collected ticks and lice were placed in universal bottles, preserved in 70% ethyl alcohol, labeled with the necessary information and transported to the laboratory for further identification.

Laboratory investigation: The squash smears prepared from demodicosis suspected cases were examined for the presence of demodectic mites at 10x magnification of light microscope (Greiner, 2012). Each scraping was transferred to clean and dry petri-dish and examined directly under stereomicroscope for the presence of

moving sarcoptic mites. Positive sample was then treated with few drops of 10% KOH and examined based on the standard procedures, mites were picked with dissecting needle along with some content, placed on clean slide, covered with cover slip and examined under 10x magnification to characterize and identify the mites according to their morphology (Urquhart et al., 1996; Greiner, 2012). Exudative crusts and/or deep skin scrapings taken from dermatophilosis suspected cases were moistened with warm saline solution on clean slide, smeared, dried with heat and subjected to Giemsa staining for demonstration of Dermatophilus congolensis (Quinn et al., 2011).The collected ticks and lice were examined under stereomicroscope and identified following the procedures given by Hoogstral (1956) and Walker et al. (2003). Scrapings and hairs collected from ringworm suspected cases were mounted for direct examination of dermatophytes in 25% KOH or NaOH mixed with 5% glycerol, heated for 1 h at 51 to 54°C to emulsify lipids, and examined under 40X magnification for fungal structures (Weitzman and Summerbell, 1995).

Data analysis

Data collected from the field and laboratory examination were recorded, entered in Microsoft Excel spread sheet and analyzed using STATA version 11 (STATA corp., College Station, TX). In the analyses the confidence level was held at 95% and $p<0.05$ was set for establishing significance.

Association with the considered risk factors (age, sex, management system, and origin) was assessed with chi square analysis.

RESULTS

Out of 662 cattle examined for the presence of skin diseases, 146 (22.05%) cattle were positive for different skin problems, namely ring worm (6.2%), lice infestation (6%), tick infestation (5.3%), wart (4.4%), lumpy skin disease (0.6%), mange mites (0.3%) and dermatophilosis (0.2%) (Table 1, Plates 1-3).

The common lice species/genera identified were *Bovicola bovis*, *Linognatus* and *Haematopinus* spps. Moreover, *Rhipicephalus* (*Boophilus*) *decolaratus*, *Rhipicephalus evertsi-evertsi*, *Amblyomma variegatum*, *A. coherence* and *A. lepidium* were the identified tick species.

The variation in the prevalence of skin diseases were statistically significant ($p<0.001$) between age categories, young cattle being more frequently affected (38.5%) than their adult counterparts (10.3%). Hence, young cattle were 5.44 times more likely to have skin diseases than adults (Odds Ratio (OR) =5.44, 95% CI=3.62, 8.18).

However, the other considered risk factors namely; sex, origin and management were not showing statistically significant effect in the occurrence of the problem (Table 2). Age was having statistically significant ($p<0.001$) effect on the occurrence of ringworm, lice infestation and wart. Moreover, the occurrences of wart and lice infestation were significantly influenced by both management and origin of the examined cattle.

DISCUSSION

This study revealed that, skin diseases caused by parasites, bacteria, viruses and fungus were common in Hawassa city in cattle with an overall prevalence of 22.05%. This finding is relatively higher than the report made by Yakob et al. (2008) and lower from that of Teshome and Derso (2015), who reported 15.41 and 27.68% from veterinary clinics of Adama and University of Gondar, respectively. The observed difference among the studies could be partly explained by the difference in the geographic location (that is, ecology and climate), cattle management practice and the study sites preferred for sampling. Although sampling at the veterinary clinics potentially incurs bias, the prevalence current reported and the previous work are still high because of several reasons. The prevailing poor veterinary services, poor animal husbandry, lack of awareness on the problem, the use of communal watering and grazing sites, improper application of acaricides by non-professionals are the major reasons to induce and augment this endemic situation.

Among the different skin diseases, ringworm (aka Dermatophytosis) ranked to be the first with a prevalence of 6.2%. Dermatophytosis in domestic animals is an infection of keratinized tissues by one of the two genera fungi, Microsporum and Trichophyton (Quinn et al., 2002; Kahn et al., 2005). Although information is lacking in Ethiopia, it is believed to pose the greatest economic and human health consequence in most developed countries (Bradford, 1996). In the present study, the prevalence of the disease was significantly higher ($p<0.01$) in animals less than two years of age (12.4%, OR=7.66, 95% CI=3.34, 17.55) than the older age group (1.8%). This variation in the prevalence can be partly explained by the fact that, dermatophytosis in adult and healthy animals is self-limiting but in young and debilitated animals the infection is wide spread and persistent. Moreover, animal susceptibility is determined largely by immunological status, and hence young animals are most susceptible (Radostits et al., 2000; Quinn et al., 2002).

The overall prevalence of cattle skin parasites (11.6%) was markedly lower than the prevalence reported from southern rangelands (73.3%) by Abebayehu et al. (2011). This could be attributed to the differences in the management practice, level of owners' awareness about the problem, ecology and climate of sampling sites. Ticks require moisture and warm environment for survival and the activity of most ticks commences during spring (Mekonnen et al., 2001). It is admitted that tick infestation, perhaps lice and mange mite infestation too, potentially predispose to other skin diseases (Scott, 2007) and hence even low prevalence could not be underestimated.

The prevalence of pediculosis observed in the present study (6%) was in agreement with previous reports made

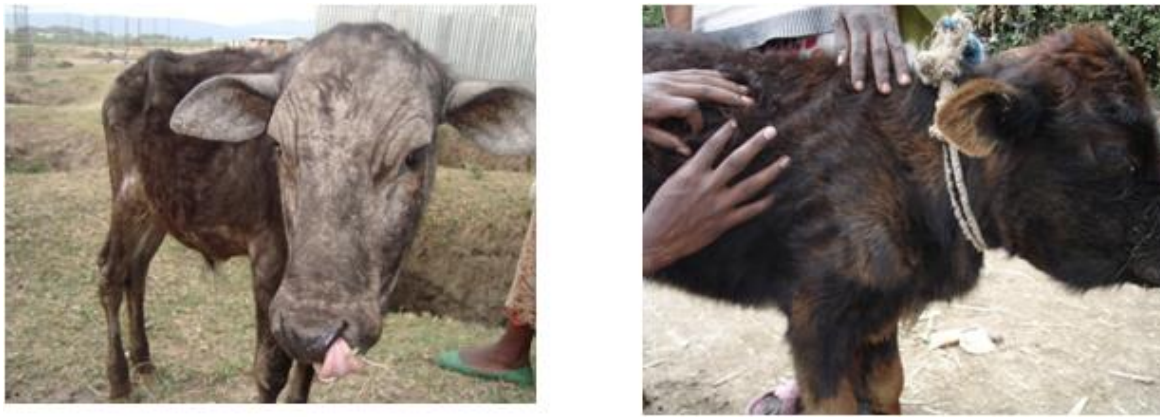
Table 1. Frequency and prevalence of the observed skin problems of cattle (n=662).

Skin problems	№ of positive cases	Prevalence of positive cases (%)	95% CI
Ringworm	41	6.2	(4.4,8)
Lice Infestation	40	6	(4.2,7.9)
Tick infestation	35	5.3	(3.6,7)
Wart/papilloma	29	4.4	(2.8,5.9)
LSD	4	0.6	(0.01,1.2)
Mange mites	2	0.3	(0.1,0.7)
Dermatophilosis	1	0.2	(0.1,0.4)
Overall skin diseases	146	22.05	(18.89,25.22)

CI=Confidence Interval, LSD=Lumpy Skin Disease.

(a) (b)

Plate 1. (a) A case of Wart (bovine cutaneous papilloma): extensive proliferative and keratinized growth on the lateral aspect of the neck, 1year-old, zebu heifer; (b) A case of Dermatophilosis: Crusted and mated hairs on the back of the animal, non-pruritic. Inset: hard, horny and confluent scab.



(a) **(b)**

Plate 2. (a) A severe form of Dermatophytosis complicated with mange mite infection: started as ring worm but lately complicated and become prururitic and lichenified. Alopecia, scales, and crusts on the entire body of the animal particularly on the anterior part; (b) Severe pediculosis: a calf with severe lice infestation, faded hairs in normally black hair coat, emaciation and bottle jaw.

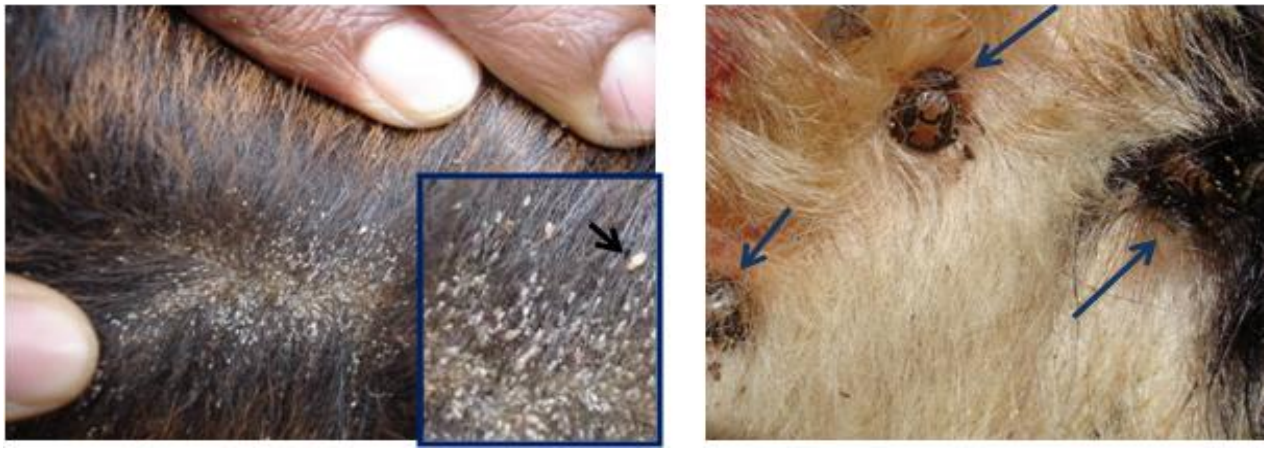


Plate 3. (a) Closer view of the previous case (Plate 2b). Inset showing several lice (black arrow) and nits; (b) Amblyomma species found attached on the axillary region of a cross bred cattle, look the hyperemia around the attachment sites (arrow).

by Yacob et al. (2008) and Regasa and Abebe (2008), who reported 3.94 and 5.13%, respectively. As previously reported (Regasa and Abebe, 2008; Yacob et al., 2008), no significant sexual predisposition was noted ($p>0.05$) in the prevalence of lice infestation. Usually mild cases are not considered as, being having any pathogenic effect, but heavy infestations are associated with extensive hide damage and blood loss (Urqhart et al., 1996). All age groups of cattle can be infested with lice, but the heaviest infestations are usually seen on calves, yearlings or in older unthrifty animal. In line with these, young animals were frequently affected (OR=4.77, 95% CI=2.27,10.04) than older animals, this is perhaps because they possess a higher ratio of accessible surface to body volume, inefficient grooming behavior and other defense capabilities (Urqhart et al., 1996; Zewdu et al., 2015). Besides, 2 cases of mange (Demodectic and Sarcoptic, one case each) were also observed in this study. The prevalence of mange (0.3%) obtained in this study was in agreement with previous report made by Regasa and Abebe (2008).

Among all skin diseases encountered in this study, wart was the fourth main skin disease with prevalence of 4.4%. In the present study, the prevalence of the disease was significantly higher ($p<0.01$, OR=24.72, 95% CI=5.69, 107.34) in animals less than two years of age (9.8%) than the older age group (0.5%). This is because, most virus-induced papilomas are self-limiting and are usually found in young animals (Goldschmidt and Hendrick, 2002), which develop good immunity afterwards.

The prevalence of lumpy skin disease in the current study (0.6%, 4 cases) was lower than the report of Ayelet et al. (2014) and Gari et al. (2010), who reported a prevalence of 13.61% in central Ethiopia (Mojo, Adama, Welenchiti and Wenji) and 8.1% in different agro-climatic zones of Ethiopia, respectively. This difference could be associated with the variation in agro-climatic condition, season and vector population during the study periods. A 6 years retrospective data collected and analyzed by Ayelet et al. (2014) suggest that, outbreaks of lumpy skin disease frequently occur between September and December than in November to June, the period at which the current study was conducted.

In the current study, the prevalence of dermatophilosis was 0.2%. Comparable prevalence ranging from 0.0 to 4.8%, were reported in zebu cattle in different part of the country (Berhanu and Woldemeskel, 1999; Woldemeskel, 2000; Yacob et al., 2008; Meseret and Sefinew, 2011; Teshome and Derso, 2015). This low prevalence could be due to the fact that, the pathogen may not yet established its spore in the premises or the animals were less exposed to the possible predisposing factors such as ticks, insects, thorny bushes, ox-pecker birds, rain, etc. Owing to the difficulty of control and eradication of established dermatophilosis, the current prevalence should not be underestimated. Because of budget and time limitations, this study were conducted mainly during the dry period, hence we couldn't see the effect of season on the prevalence of the different skin diseases including dermatophilosis.

Conclusion

In the study area, different skin diseases were prevalent and among the major diseases affecting cattle production. Compared to the clinical physical examination, assessment at wet blue stage in the tanneries would have indicated more problem/defects that can't be detected while the animal is still alive. The higher prevalence of dermatological problems in

Table 2. Prevalence of different skin diseases in cattle and their variation among/between the categories of the considered risk factors (n=662).

Risk factors		№ of cattle Examined	Ringworm %(№)	Lice %(№)	Ticks %(№)	Dermatophilosis %(№)	Wart %(№)	Mange %(№)	LSD %(№)	Overall %(№)
Sex	Male	213	7(17)	6.6(14)	5.2(11)	0(0)	4.2(9)	0(0)	0.9(2)	23.5(50)
	Female	449	5.3(24)	5.8(26)	5.3(24)	0.2(1)	4.5(20)	0.4(2)	0.4(2)	21.4(96)
Age	Young	275	12.4(34)**	10.9(30)**	6.2(17)	0(0)	9.8(27)**	0.7(2)	0.4(1)	38.5(106)**
	Adult	387	1.8(7)	2.6(10)	4.7(18)	0.3(1)	0.5(2)	0(0)	0.5(3)	10.3(40)
Management	Poor	513	7(36)	7.8(40)**	5.8(30)	0.2(1)	3.1(16)**	0.4(2)	0.4(2)	23.6 (121)
	Medium	149	3.4(5)	0(0)	3.3(5)	0(0)	8.7(13)	0(0)	1.3(2)	16.8(25)
Origin	Chefe	405	6.9(28)	9.1(37)**	6.9(28)	0.2(1)	0.9(4)**	0.5(2)	0.5(2)	24.4(99)
	Dato	50	6(3)	6(3)	4(2)	0(0)	0(0)	0(0)	0(0)	16.0(8)
	Hawassa	207	4.8(10)	0(0)	2.4(5)	0(0)	12.1(25)	0(0)	0.9(2)	18.8(39)

*Statistically significant ($p<0.05$), ** statistically highly significant ($p<0.01$), LSD= Lumpy Skin Disease.

young animals warrant special attentions in the implementation of feasible control and prevention measures at young age categories even before animals reach the age of slaughter.

In general, unless feasible control measures are timely implemented the encountered skin diseases can have varied and adverse effect on the cattle production, tanning industry and health of the public and concomitantly pose huge economic loss. To design appropriate control strategy, further studies should be carried out on the seasonal occurrence and socio-economic impacts of these diseases, on large sample size and wider study sites. Moreover, regular application of acaricides and timely treatment of clinical infectious cases were suggested to reduce further impact.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Study of porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) frequencies and coinfection in mexican farrow to finish pig farms

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The porcine circovirus type 2 (PCV2) is commonly present along with the porcine reproductive and respiratory syndrome virus (PRRSV). These are the most important pathogens for pig industry worldwide. In this study, we determined frequencies for both viruses, as well as coinfection, in farrow to finish farms from México. For this, pigs from 28 farms in different states were sampled for tonsils, lungs and lymphatic nodes and assayed for PCV2 and PRRSV by polymerase chain reaction (PCR) and reverse transcription-polymerase chain reaction (RT-PCR). Herds detected positive were: 16 PCV2 (57%), 10 PRRSV (36%); and 5 had both viruses (18%). Samples detected positives were: 49% PCV2, and 39% PRRSV. Coinfection frequency observed in this work contrasted those observed in other countries. We think that these data will contribute to a better understanding of both diseases in order to take better measures for preventing and controlling them.

Key words: Laboratory diagnosis, viral frequency, viral coinfection, PCV2, PRRSV, farrow to finish farm.

INTRODUCTION

At the beginning, the porcine circovirus type 2 (PCV2) was reported in high-health porcine farms that were negative for common pig pathogens, including the porcine reproductive and respiratory syndrome virus (PRRSV) (Harding et al, 1998; Ellis et al., 1999). However, recently both diseases have caused great economic losses to the porcine industry (Piñeyro et al., 2015). PCV2 mainly affects animals between 5 and 15 weeks of age, inducing among several signs growth retardation and respiratory diseases (Segalés et al., 2005). In México, PCV2 has been reported since 2001 (Trujano et al., 2001), although a retrospective study done in a collection of sera obtained from 1972 to 2000 showed serological evidences of PCV2 presence since 1973 (Ramírez-Mendoza et al., 2009).

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Today PCV2 is thought to be ubiquitous in porcine from our country, such as has been described in other countries (Segalés et al., 2005). The porcine reproductive and respiratory syndrome (PRRS) is one of the most important infectious diseases affecting the porcine industry, due to its heavy economic national and international impact (Blaha, 2000). PRRS is characterized by causing reproductive signs in sows, respiratory signs in pigs of different ages and severe weight loss in fattening pigs (Benfield et al., 1992). Serological studies in the United States have reported PRRSV occurrence in up to 80% of swine herds (Zimmerman, 2003). In Mexican Republic, serological surveillances performed from 1995 have indicated that PRRSV is thoroughly distributed, and seropositive pigs are often detected in most of the industrialized farms (Weirmersheimer et al., 1997; Diosdado et al., 2004).

In different countries, swine farms have registered very variable coinfection frequencies between PCV2 and PRRSV. In western Canada the frequency of viral coinfection was 20% (Allan and Ellis, 2000); in Spain 48% (Segalés *et al*., 2002); and in the United States up to 60% (Sorden, 2000).

In this study we determined PCV2, PRRSV, and viral coinfection frequencies in pigs with evident growth retardation, from Mexican farrow to finish swine farms, in order to contribute with recent information for our country.

MATERIALS AND METHODS

Ethics statement

Animals used in this study belonged to commercial farms and were slaughtered according to practices specifically regulated by Mexican Official Norm (NORMA Oficial Mexicana NOM-033-SAG/ZOO-2014, 2015).

Selection and screening of herds

A convenience transversal sampling of herds from industrialized farrow to finish farms from different states of México was made with the consent of each producer. For this, a total of 28 farms were sampled as follows: Guanajuato, 12; Queretaro, 10; Puebla, 3; Veracruz, 2; and Michoacan, 1.

Specimen collection

For each herd from one to two piglets between three to four months of age, and presenting clinical signs compatible with PCV2 or PRRSV infection, were slaughtered according to NOM-033-SAG/ZOO-2014 regulation. From each pig tonsil, lung and lymphatic node samples were taken. Samples were stored at -70°C until processed.

Detection of viral genomes by PCR and RT-PCR

Extraction of nucleic acids

From each animal equivalent quantities of tonsil, lung and lymphatic node tissues were pooled up to one gram, macerated with sterile sand in 5 ml of sterile physiologic saline, and centrifuged at 14,000 g for 5 min; 200 µl of supernatant were used to extract nucleic acids, using the High Pure PCR Template Kit (Roche), and following the manufacturer's protocol, with the exception of finally eluting the nucleic acids in 50 µl of sterile water for injection.

Amplification and detection of PCV2 DNA

In the polymerase chain reaction (PCR) to amplify the PCV2 genome primers used were: forward 5'-CAGCAACATGCCCAGCAAGAAGAAT-3' and reverse 5'-TCGATCACACAGTCTCAGTAG-3' (Ogawa *et al.*, 2009), which flank a fragment of 703 bp from the PCV2 ORF V1. The components of the reaction mix were: PCR buffer 1x to make a final volume of 25 µl, $MgCl_2$ 1.5 mM, nucleotide mix, d'NTPs 0.2 mM, each primer 20 pmol, Taq Gold DNA polymerase 1.25 U, and sample DNA 2 µl (0.1-50 ng). Amplifications were carried out according to the following profile: an initial step of 4 min at 94°C; 35 cycles of 30 s at 94°C, 30 s at 54°C, and 90 s at 68°C; and one final elongation cycle of 3 min at 68°C. Products were analyzed on 1.5% agarose gels using the TAE (Tris-acetate-EDTA) buffer system, and visualized by the addition of ethidium bromide.

Detection of the PRRSV RNA by one-step RT-PCR

For the reverse transcription and specific amplification of the PRRSV RNA in a single tube, primers used were forward 5'-CCA GCC AGT CAA TCA RCT GTG-3' and reverse 5'-GCG AAT CAG GCG CAC WGT ATG-3' (Donadeu *et al*., 1999), which amplify a ~300 bp from the viral ORF7. The components of the reaction mix were: PCR buffer 1x to make a final volume of 25 µl, $MgCl_2$ 2.5 mM, dNTPs 0.4 mM, primers 20 pmol, Taq Gold DNA polymerase 1.25 U, reverse transcriptase (MuMLv) 12 U, RNase inhibitor 5 U, bovine serum albumin 3 µg, and RNA template 1 µl (0.01 ng-100 ng total RNA). The cycling protocol consisted of reverse transcription 30 min at 48°C; initial denaturation 10 min at 94°C; amplification 35 cycles at 94°C for 1 min, 58°C for 1 min, 72°C for 1 min; and then 7 min at 72°C. Amplification products were analyzed as indicated above. The one-step RT-PCR reduces the time taken to complete the assays, and the possibility of errors and contaminations.

RESULTS AND DISCUSSION

In our hands, PCR used for detection of PCV2 DNA efficiently worked on pooled samples taken at industrialized Mexican swine farms, producing respective amplicons 703 bp in length (Figure 1). Similarly, under previously established conditions for RT-PCR, a 300 bp amplicon from PRRSV ORF7 was obtained (Figure 2).

For detection of PCV2, a total of 28 farrow to finish farms were sampled in five states of Mexico; 16 (57%) of the 28 swine farms were positive. A total of 37 (49%) of 76 tissue pool samples resulted positive by PCR. All animals studied in the states sampled were positive for PCV2 DNA. The RT-PCR assay showed that a total of 10 (36%) of 28 industrialized farms, and 30 (39%) of 76 tissue pools were positive for PRRSV RNA. Five farms (18%) were positive for both viruses (Table 1).

In this study we successfully applied PCR and RT-PCR assays to tissue samples collected from suspected Mexican pigs aged between 3 to 4 months for detection of PCV2 and PRRS. PCV2 frequencies reported over last

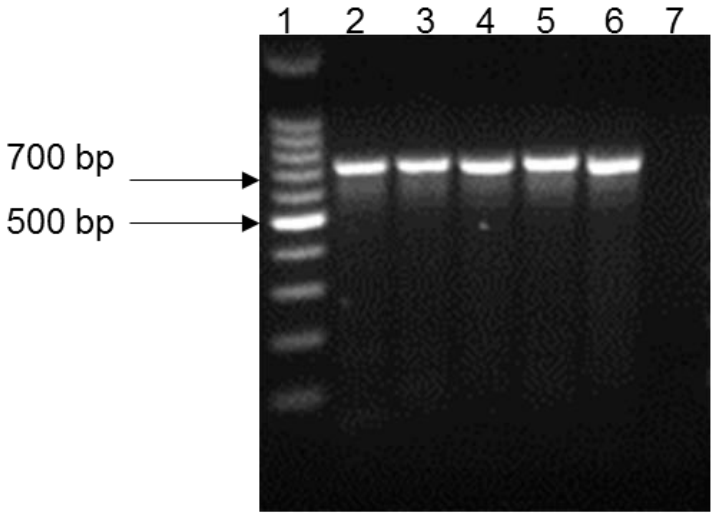

Figure 1. Detection of PCV2 by pcr in tissue samples. Lane 1, 100 bp DNA ladder; lanes 2 to 5, farms pigs from Veracruz state; lane 6, positive control; lane 7, negative control.

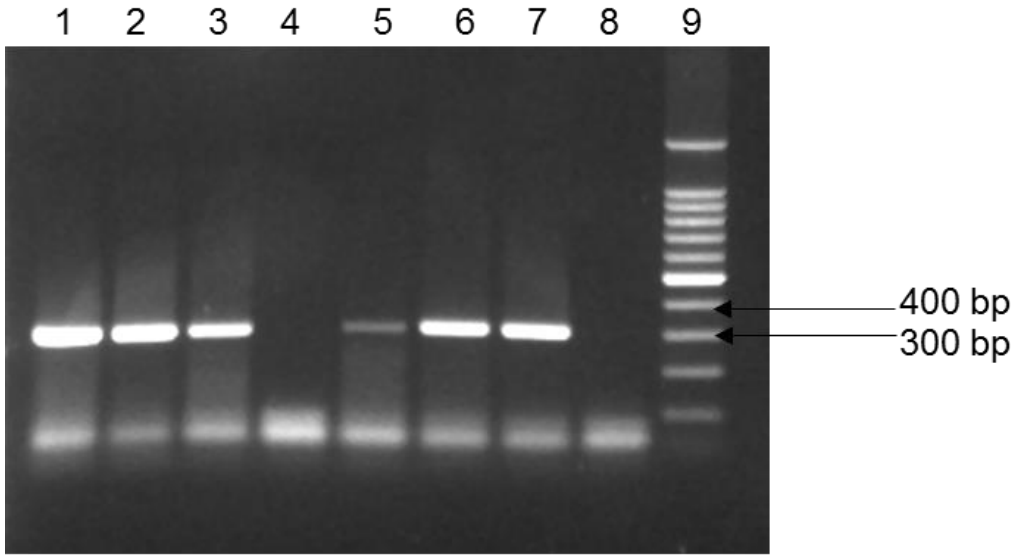

Figure 2. Detection of PRRSV by RT-PCR in the tissue samples. Lanes 1 to 3, Farm pigs (A) from Guanajuato state; (B); lanes 4 to 6, farm form Guanajuato(B); lane 7, positive control; lane 8, negative control; lane 9, 100 bp dna ladder.

Table 1. Frequencies of PCV2, PRRSV, and coinfection occurrence in pigs from Mexican industrialized herds.

State	Sampled herds	Herds positive for PCV2/Total	Herds positive for PRRSV/Total	Herds positive for coinfection
Guanajuato	12	7/12	5/12	1
Queretaro	10	3/10	1/10	0
Puebla	3	3/3	3/3	3
Veracruz	2	2/2	0/2	0
Michoacan	1	1/1	1/1	1
Total	28	16/28	10/28	5/28

years in different countries have been variable; a study in China found 47% of positive tissue samples from domestic pigs (age not defined) (Sun et al., 2015), while another work, in Japan found 80% of positive tonsil samples collected from pigs aged 6 months (Saekhow et al., 2015). Our results showed that PCV2 was detected in all states, with a frequency of 57% of positive herds, and 49% of positive animals, in five European countries 42% of fecal samples taken from both healthy and diarrheic pigs were positive and the percentage was higher in healthy compared to diseased animals (Zhou et al., 2016), which suggest that most PCV2 infections were subclinical. In our study only samples from diseased animals were included; it could produce very interesting results to include also healthy animals to determine total and subclinical frequencies.

With regard to PRRSV, our data indicate frequencies of 36% of positive herds, and 39% of positive animals, which is consistent with serological data reported since 1997 in different Mexican states (Weirmersheimer et al., 1997; Diosdado et al., 2004).

Queretaro state had lesser frequency of infected herds for both PCV2 and PRRSV. This is likely due to a lesser density of pigs per farm, and longer distances separating sampled farms, in comparison with other states, Guanajuato for instance.

Our study found swine herds positive for PCV2, PRRSV, and coinfections with percentages of 57%, 36%, and 18% respectively. Coinfection frequencies for herds contrast with reports for other countries such as Canada with 20% (Allan and Ellis, 2000); Spain, 48% (Segalés et al., 2002); US, 60% (Sorden, 2000); and Netherlands, 83% (Wellenberg et al., 2004). In our study, we had expected higher coinfection frequencies, since sampling was targeted at pigs with evidence of growth retardation and suggestive signs for the PCV2 and PRRSV presence.

Conclusion

In this study, PCV2 and PRRSV could be detected on pooled samples from several tissues by molecular amplification. PCV2 frequency observed for industrialized Mexican farms was 57%, different from that reported for other countries, while PRRSV frequency was 36%, similar to those reported for previous years in the Mexican Republic. This study, in which sampling was targeted at animals with evidences of clinical signs, were reports of PCV2/PRRSV coinfection in 18% of farms. We consider a priority to design a study more complete for a broader region, to include a greater number of Mexican states, as well as a bigger sample number. This was aimed to determine with a greater confidence the frequency of PCV2/PRRSV coinfections in Mexican porcine herds.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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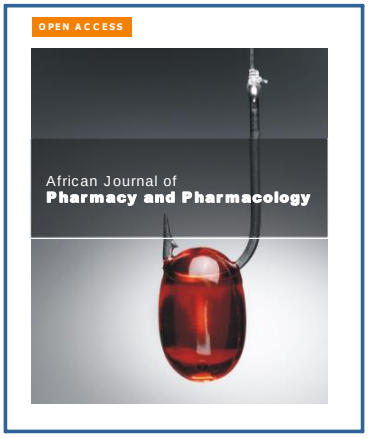

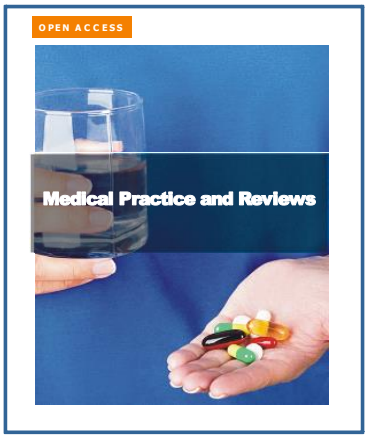

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