OPEN ACCESS

Journal of Veterinary Medicine and Animal Health

September 2018
ISSN: 2141-2529
DOI: 10.5897/JVMAH
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Journal of Veterinary Medicine and Animal Health

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Vol. 10(9), pp. 217-223, September 2018
DOI: 10.5897/JVMAH2018.0688
Article Number: 1B13EE658173
ISSN: 2141-2529

http://www.academicjournals.org/JVMAH

ACADEMIC JOURNALS

Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Association of body weight, scrotal circumference, heart girth and penile development with spermatogenesis in the Nubian bucks

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Received 2 March, 2018: Accepted 16 July, 2018

This study was designed to monitor the morphological development of the reproductive tract of the Nubian bucks in relation to puberty. Thirty-two Nubian male kids were used in this study. Their ages ranged between 1 day and 24 weeks. The study was undertaken to correlate the body weight (BW), heart girth circumference (HG), scrotal circumference (SC), testicular descent into the scrotum and penile separation from prepuce (PS) with age at puberty. Penile separation started slightly at 12 weeks of age and continued with advancing age till it was completely achieved between 22 and 24 weeks of age. Strong correlation has been established between the levels of the reproductive hormones and the morphological maturation of the reproductive tract. The first surge in the levels of these hormones (occurred between weeks 10 and 12) coincided with the increase in the diameters of the seminiferous tubules, the epididymis, ductus deferens and the penis. The first appearance of secondary spermatocytes and initiation of penile separation occurred during this period. The second surge was associated with the first appearance of spermatids, spermatozoa and completion of penile separation, which occurred between 20 and 24 weeks.

Key words: Nubian bucks, spermatogenesis, body measurements.

INTRODUCTION

Sudan is predominantly an agricultural country with the largest livestock population in the Arab World and ranks second to Ethiopia in Africa. Despite this large population, there is a critical shortage in milk supply and other dairy products. This is mainly due to the poor feeding, poor management and prevalence of diseases. Goats play an important economic role in the livelihood of many Sudanese families.

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The local breeds of goats in Sudan embrace the Nubian, Desert, Nilotic dwarf and Taagari. Among these breeds the Nubian goat is recognized as the only specialized milk breed (Hassan and El Derani, 1990).

Goats are used as a representative of large animals for certain experimental purposes like studies on reproduction as the reproductive tracts of the male goat, ram and bull are essentially similar (Goyal, 1985). Among these three species, male goats receive preference because, unlike rams, and bulls, are inexpensive and easier to handle for surgical manoeuvres (Goyal et al., 1999).

The reproductive tract of the male goat consists of testes, epididymis, ductus deferens, penis and the accessory sex glands (seminal vesicles, prostate and bulbo-urethral (Cowper's) gland). The male reproductive tract has been investigated histologically, ultrastructurally and histochemically in several species including bull (Abdel- Raouf, 1960; Mohammed, 2005), camel (Ali et al., 1978; Tingari and Moniem, 1979; Tingari et al., 1984), rat (Pogach et al., 1993) and domestic fowl (Tingari 1972). The goat in general received little or no attention. In spite of the importance of the Nubian goat, there is no reference in the literature dealing with the characteristics pertaining to the morphological and physiological changes of the reproductive tract prior to the age of puberty.

Studies on puberty have been reported for the bull (Abdel Raouf, 1960; Renaville et al., 1993), goat (Nasir et al., 2013), sheep and goat (Louw and Joubert, 1964; Lord et al., 1991), ram (Dun, 1955), camel (Abdel Rahim, 1997), rhesus macaques (Bercovitch, 1993) and man (Martha and Reiter, 1991). Puberty, the culmination of a multitude of developmental processes at the hypothalamic, pituitary and gonadal levels, is essentially manifested by the episodic release of testosterone (Renaville et al., 1983; Schams, Winkler et al., 1988) which serves as a primary regulator for other major physiological changes during this period (Martha and Reiter, 1991). Associated with this increase in plasma testosterone concentrations, a growth hormone (GH) discharge may precede the onset of puberty (Thompson et al., 1972).

Puberty is reached when sexual organs have become fully developed (Abdel-Raouf, 1960), the sexual instincts are prominent and reproduction is possible. The objective of this study is to examine the changes in the morphological parameters in the male reproductive tract of the Nubian bucks prior to the age of puberty.

MATERIALS AND METHODS

This research work was conducted in the period between May, 2001 and May 2002. Samples were obtained from different parts of the male reproductive tract including the testis, epididymis, ductus deferens and penis of 32 Nubian male kids, from birth up to six months of age. Samples were taken at 15 days interval for investigation. The different groups were represented by at least two animals each. The animals were used initially for data collection including; body weight (BW), scrotal circumference (SC), Heart Girth (HG), testicular descent into the scrotum and penile separation from prepuce.

Statistical analysis

A computer package for statistical analysis was used (SPSS version, 11). The data obtained were computed to find correlations between body weight (BW), scrotal circumference (SC), Heart Girth (HG) in association with penile separation and appearance of spermatozoa in the seminiference tubules.

RESULTS

Body weight (BW)

The result showed that body weight in Nubian male kids increased with age in a linear pattern starting with 2.3 kg at week one and ending with 13.5 kg at 24 weeks of age (Figure 1).

Heart girth circumference (HG)

Heart girth measurements increased with age in a linear pattern, reaching higher values at 22 weeks of age. HG showed a strong correlation ($P< 0.1$) with age (Figure 2).

Scrotal circumference (SC)

SC measurement increased with age in a linear pattern reaching the maximum at 22 weeks of age (Figure 3). There were strong correlations between SC and BW ($P< 0.01$) and between SC and HG ($P< 0.01$).

Correlation between the body weight (BW), heart girth circumference (HG), scrotal circumference (SC)

Very strong positive correlations were found between the BW, heart girth circumference (HG) and scrotal circumference (SC) (Tables 1 and 2).

Penile separation from the prepuce

The diameter of the penis was 6988 µm wide at week one. The skin was closely adherent to the underlying tissue in which Meissner's corpuscles were seen. An inner folded band encircled the corpus spongiosum penis and corpus cavernosum penis and consisted of about ten layers, mainly cuboidal cells in the center, covered on either side by columnar cells and overlying layer of connective tissue containing band of smooth muscle fibers and collagen fibers. Penile separation started slightly at 12 weeks of age and continued with age till it

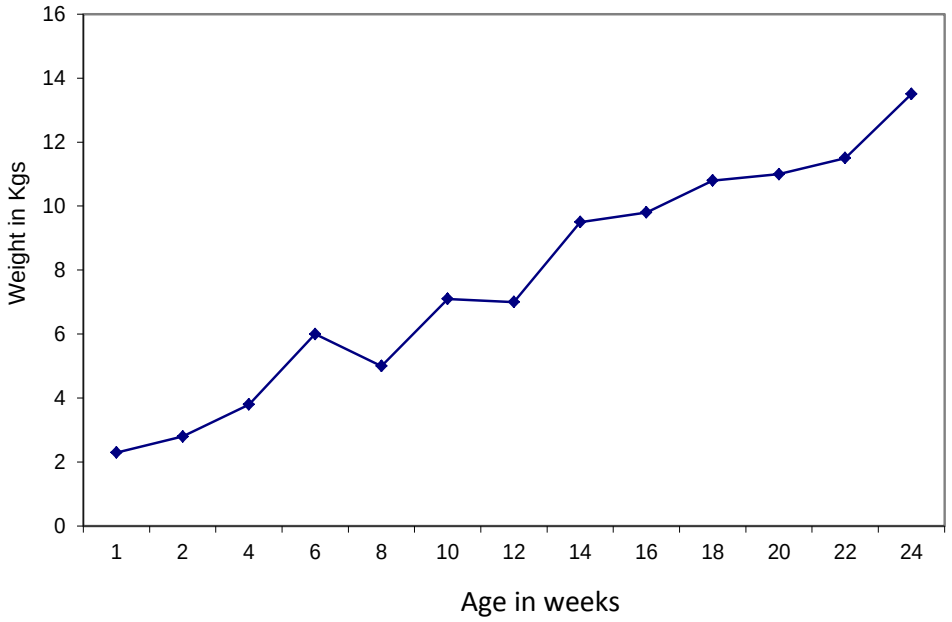

Figure 1. Body weight changes (Kg) with age (weeks) in Nubian male kids.

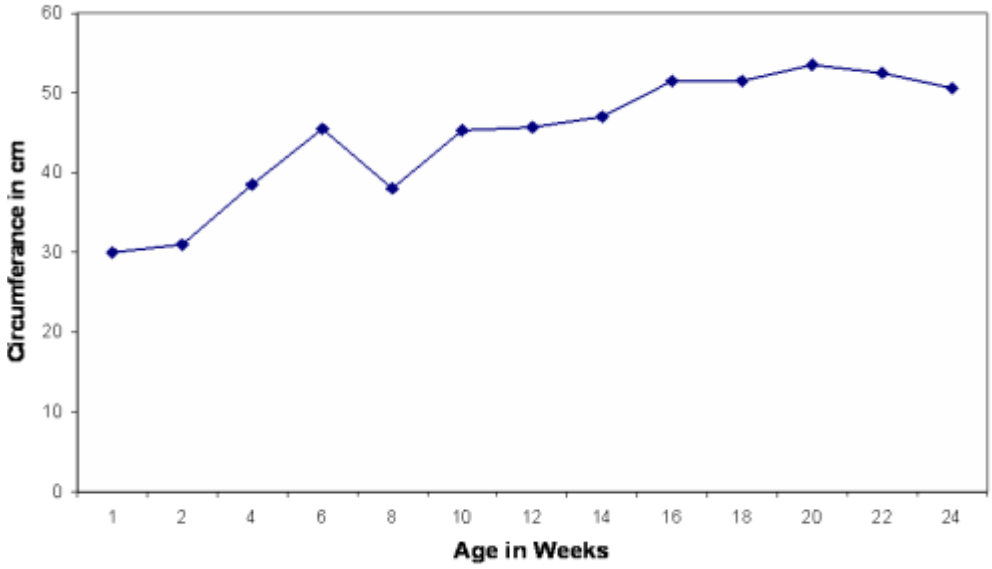

Figure 2. Heart-girth measurements (cm) with age (weeks) in Nubian male kids.

was completely achieved between 22 and 24 weeks of age (Figures 4 and 5).

Puberty

Based on the first appearance of spermatozoa in the seminiferous tubules, puberty was reached in Nubain male kids at the age of 22 weeks. At this age penile separation was completed (Figure 4), body weight was11.5 kg, heart girth was 52.5 cm and scrotal circumference was 13.5 cm.

DISCUSSION

This study was conducted in different seasons and the results were not affected by seasonal changes, since the Nubian goat is a tropical breed and has no pattern of seasonal breeding, (Kurohmaru and Nishida, 1987; Ritar,

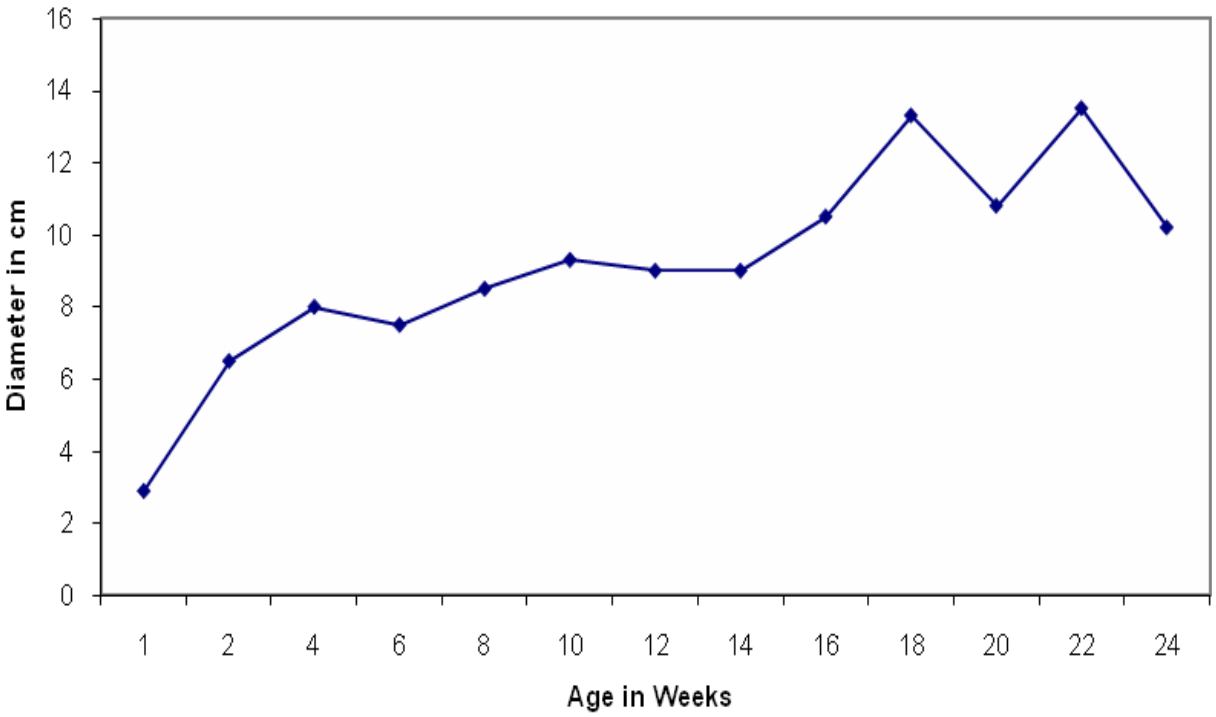

Figure 3. Scrotal circumference (cm) with Age (weeks) in Nubian male kids.

Table 1. Correlations between body weight (kg) heart girth (cm) and scrotal circumference (cm) in Nubian male kids.

	BW	HG	SC
BW	1		
HG	0.962**	1	
SC	0.828**	0.840**	1

** Significant at p<0.01 level (2-tailed).

Table 2. Body weight (kg), heart-girth (cm) and scrotal circumference of nubian male kids.

Age (weeks)	Body weight (kg)	Heart-girth (cm)	Scortal circumference (cm)
1	2.3	30.0	2.9
2	2.8	31.0	6.5
4	3.8	38.5	8.0
6	6.0	45.5	7.5
8	5.0	38.0	8.5
10	7.1	45.3	9.3
12	7.0	45.7	9.0
14	9.5	47.0	9.0
16	9.8	51.5	10.5
18	10.8	51.5	13.3
20	11.0	53.5	10.8
22	11.5	52.5	13.5
24	13.5	56.0	10.2

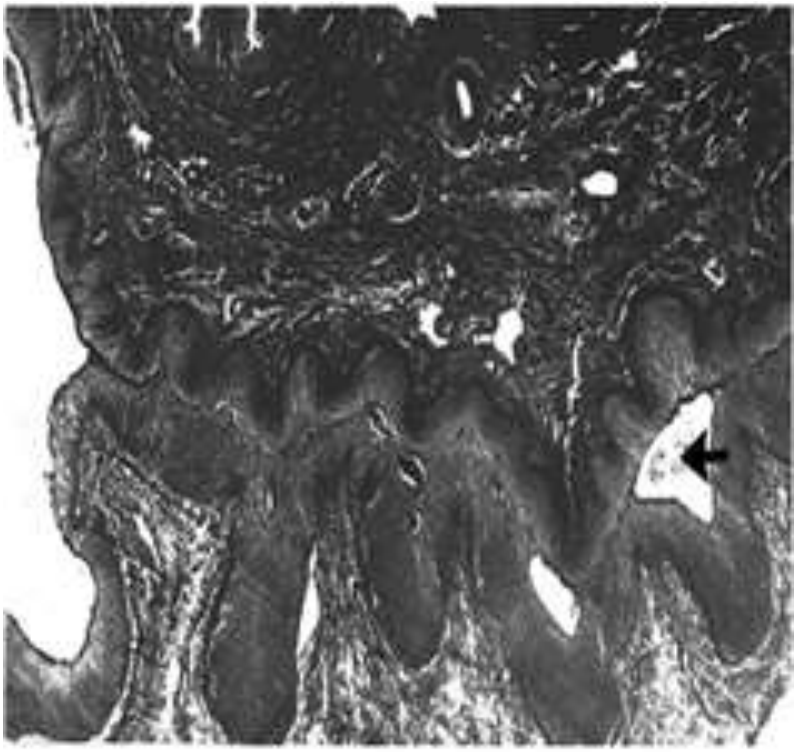

Figure 4. Division into two layers of stratified squamous epithelium has started (arrow) 40000X.

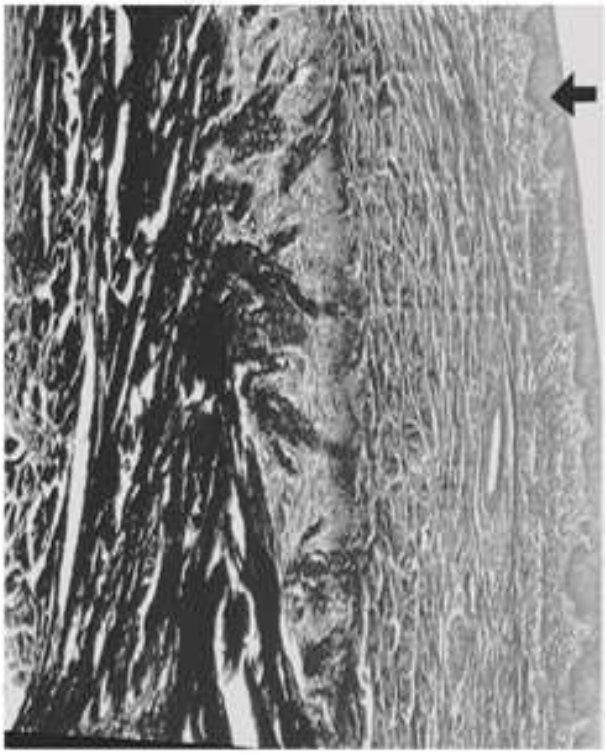

Figure 5. Complete separation of epithelium band has been completed (arrow) 640X.

1991). With regard to data on measurements of scrotal circumference, heart-girth and body weight, good correlations were found between these parameters and age. These body measurements reached their maximum at the age of puberty (22 - 24 weeks). Similar findings were also reported in man (Forest et al., 1976), monkeys (Mann et al., 1994; Lunn et al., 1997), ram (Dun, 1955), and sheep (Louw and Joubert, 1964).

The environment for the bucks used in this study was the same in relation to feeding, management and housing. Thus nutrition was not considered as a variable. However, some researchers focused on nutrition to be the most important factor that can affect body measurements in small domestic animals (Setchel et al., 1965; Martin et al., 1994; Thwaites, 1995a).

For the determination of age of puberty, evidence for commencement of spermatogenesis was considered in this study. Thus, puberty in Nubian bucks was reached at 22 weeks of age (Shaaeldin, 2006). Moreover, penile separation was also completed at the same age. Similar findings have been reported on measurement of scrotal circumference, heart girth, and body weight in other breeds of goat and sheep which were indicative of puberty to be at the age between 18 and 22 Weeks. The maximal levels of plasma protein (PRL), testosterone, LH, FSH was reached between 18-20 weeks (Shaaeldin, 2015), which give further support to pubertal characteristic of goat.

However, other breeds of goat and sheep reach puberty between 16 and 21 Weeks (Louw and Joubert, 1964) and 30 weeks (Nasir et al., 2013). Dyrmundsson (1973) noticed that there were great differences in age of puberty and body weight between ram lambs of various breeds of sheep. Large animals are said to reach puberty at the age between 23-26 weeks (Abdel Rahim, 1997). The present study confirms earlier observations that signs of puberty in the Nubian bucks appeared around 22-24 weeks of age. Maturity would be achieved a few weeks later reaching up to one year of age (Renaville et al., 1993; Adil and Nasir, 2015).

Conclusion

The results obtained revealed the age at puberty for the first time in male kids of Nubian goat depending on the first appearance of spermatozoa in the seminiference tubules. The associated body measurements could be used as simple guide for selection of breeding males at puberty. Nevertheless, age at sexual maturity still needs more time to accomplished, it will be a subject for future research.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

I feel greatly indebted to Prof M.D., Tingari for his keen interest, guidance, encouragement and constructive criticism. I would like to express my sincere thanks to Prof Makawi, Sharaf Eldin, for their helpful guidance. I wish to express my thanks to all the staff member of the Department of Anatomy, Khartoum University for their technical assistance. Thanks and appreciations are extended to Mr. Abdalrhhaman M. Salih for his technical assistance. The work was supported by a grant from the Ahfad University for Women and the Gordon Memorial trust UK.

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Vol. 10(9), pp. 224-230, September 2018
DOI: 10.5897/JVMAH2018.0673
Article Number: C9A8CED58175
ISSN: 2141-2529

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Full Length Research Paper

Assessment on major reproductive health problems of dairy cattle in Boloso Sore, Southern Ethiopia

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Received 2 February, 2018: Accepted 25 July, 2018

The ultimate goal integrated herd health management is to lower calving interval, decrease the number of services per conception thereby increasing reproduction and production but reproductive health disorders affect the reproductive performance of the dairy cows, the number of potential replacement needed to maintain a constant herd size and the longevity of the cow in the herd. There was insufficiency research information in the particular situation in the area for dairy production intensification. Assessments were conducted to identify the major reproductive health problems of dairy cattle and associated risk factors at Boloso Sore, Ethiopia from November 2016 to April 2017. In the present study, smallholder dairy owners were interviewed using local language; data on dairy reproductive performances particularly history of reproductive health problems were collected. A total of 200 respondents were interviewed and the finding revealed that the most frequently encountered reproductive health problems were repeat breeding, dystocia, abortion retained fetal membranes, uterine prolapsed, and still birth: 17,16.5, 14.5, 5.5, 3.5, and 1% respectively were record. Overall, fifty two percent (n=104/200) of dairy owners responded to the presence of either one or more reproductive disorders history in their herd. In the present study the association of history of reproductive problems showed significant difference with respect to breed and parity of dairy cattle thus, Holstein cross breed dairy with increased parity was recorded to have history of reproductive health problem than others in the management system. Further studies should be conducted at different cross blood level, and awareness given to farm owners and attendants to improve dairy management with increased parity.

Key words: Abortion, Boloso sore**,** breed, dairy, parity, reproductive health problem.

INTRODUCTION

Ethiopia is believed to have the largest livestock population in Africa. This livestock sector has been contributing considerable portion to the economy of the country and is still promising to contribute to economic development of the country. The Ethiopian total cattle population is estimated to be about 56.71 million. Out of this, the female cattle constitute about 55.45 and 98.66% of the total cattle in the country are local breeds and the remaining are crossbreed and exotic breeds that accounted for about 1.19 and 0.14%, respectively CSA, 2015). The livestock sector plays a vital role as source of food, income, services and foreign exchange to the Ethiopian economy (Ayele et al., 2003). For several years, Ethiopia ranked first in cattle population in Africa.

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(However, the dairy industry is not as developed as that of east African countries; the national milk production remains the lowest in the world even by African standard (Kassa and Lema, 2005).

Milk production in the country is mainly from indigenous cattle breeds, which are kept for multi purposes in the different agro ecology and production systems. The first attempt for the improvement of dairy cattle production in Ethiopia was founded in the late 1960s through the introduction of exotic dairy breeds and use of reproductive technologies (Ahmed et al., 2003). Cow regular breeding depends upon the normal function of the reproductive system. In order to breed regularly, the cow has to have functional ovaries, display estrous behavior, mate, conceive, sustain the embryo through gestation and resume estrus cyclicity and restore uterine function after calving. Each of these aspects of reproductive function can be affected by management, disease and the genetic make-up of the animal. When the function of the reproductive system is impaired, cows fail to produce a calf regularly (Shiferaw et al., 2005).

Reproductive problems have been implicated to cause considerable economic loss to the dairy industry due to slower uterine involution, reduced reproductive rate, prolonged inter-conception period and calving interval, high cost of medication, drop in milk production, reduced calf-crop and early depreciation of potentially useful cows (Bekana et al., 1997). In addition, several studies indicate that reproductive disorders are the most frequent culling reasons (Stevenson et al., 1998). The major problems that have direct impacts on reproductive performance of dairy cow were abortion, dystocia, retained fetal membrane, metritis, prolapse, anestrus and repeat breeding (Lobago et al., 2006). For the production constraints, reproductive health problem (RHP) plays major part but, in the present area there is no study reported thus. The present study was conducted with objective to assess the reproductive health problems and associated risk factors with the reproductive health problem in the area.

MATERIALS AND METHODS

Study area

The study was conducted from November 2016 to March 2017 in and around Boloso Sore District, Southern Ethiopia. Boloso Sore is located in the Wolaita Zone of the Southern Nations, Nationalities, and Peoples' Region, 300 km southwest of the capital city of Ethiopia, Addis Ababa. The area has a latitude and longitude of 7°05′N 37°40′E / 7.083°N 37.667°E and an altitude of 1350-2380 m above sea level. The rainfall pattern is bimodal; a short rainy season runs from March to May and long rainy season runs from June to September with average annual rainfall of 1300 mm and average daily temperature of 20.4°C The district is bordered by Boloso Bombe in the West, Hadaro Tunto of kembata Tembaro North West, Sodo zuria and Damot Sore South and Damota Gale in the East. An administrative center of the district, Areka was establish 1959, and Ethiopian Institute of Agricultural Research opened a research center since 1985 with the mandate as center of excellence with Enset root crop verification, Dorper sheep multiplication and to achieve the national research pillars through commodity base integrations on livestock, crop and natural resource management. Depending on the climatic condition, 80% was Woinadega (mid altitude) the remaining composed of Kola (Lowland) and small proportion was for Dega (Highland). According to Wolaita Zone Livestock And Fishery Resources office 2016 report, the livestock population of Boloso Sore district was estimated as 84,391 cattle, 57,331 ovine, 8,396 caprine, 7,321 equines and 91,375 poultry. The district have a total of 34 district Kebeles, of which four dairy potential kebeles (Dola, Xadisa, Hangada and Puxo) were sampled through multistage system in consultation with zone and district level of Biros of Livestock and Fishery Resources.

Study population

Based on the accessibility and dairy potentials in line with zonal dairy estrous synchronization program, four kebeles were purposively selected and using semi structured questionnaire randomly sampled smallholders were interviewed. The owners were interviewed by local language and data were collected on reproductive health problems, dairy reproductive performance and demographic situation of the smallholder in the area.

Study design and sampling procedures

A cross sectional study was conducted from November 2016 to March 2017 in and around Boloso Sore district. From the study, district through multistage sampling technique, four kebeles namely Dola, Xadisa, Hangada and Puxo were purposively selected based on their accessibility, and existing dairy potential in line with zonal dairy estrus synchronization program. Household's data list who own dairy cattle were captured from the kebeles administrative office, and individual owners for interview were taken by simple random sampling method using lottery system.

Sample size determination

In four kebeles, of the total households, 5080 dairy owners with herd size more than one dairy cows were considered and based on the formula, questionnaire survey sample size was calculated by using the formula given by Arsham (2002) which is: $N= 0.25/SE^2$, Where N= sample size, SE (standard error=5%). The sample size required for the questionnaire survey as per the above formula is 100 for the site. However, to include different risk factors in consideration of kebeles area coverage and large household size, and to increase the precision of the result, the number was increased double across the four kebeles and so a total of 200 individuals were interviewed.

Data analysis

The data were entered and managed in Microsoft Excel. SPSS version 20 software was used for the data analysis. The differences in parameters such as, breed, calving interval, parity and other factors on reproductive problems were analyzed by using $\chi 2$ (Chi-square) test, and the level of significance was set at 95% confidence interval.

RESULTS

In this study, a total of 200 smallholder dairy owners 68.5% male and 31.5% female were interviewed from the selected four kebeles based on smallholder number proportion; 49, 53, 44 and 54 respondents householders were used from Hangad, Puxo, Xadisa and Dola respectively in this data analysis (Table 1). Fifty two percent of the farm owners responded that their cows were affected by either one or more reproductive health problems. The major reproductive disorders in the district were repeat breeding, dystocia, retained fetal membranes, abortions, uterine prolepses and still birth.

Respondent's belongs to adult age group which indicates the consistency of the data generated for average age of 31.12±5 across the kebeles. Concerning the level of education, the highest percentage (54.5%) of the respondents were illiterate, followed by 37.5% which studied in elementary school, and 4.5% of the respondents had attended high school and 3.5% had college diploma education across the kebeles. Average family size in the area was about 4.81±1.75. With regards to the individual, and spouse occupation, 33.5% were actively involved in livestock rearing and the rest (66.5%) were involved in agricultural activities other than livestock keeping due to feed challenge and low initial investment capacity.

Average cattle herd size at individual household level was found; about 3.39±1.8 head of cattle were recorded and the largest portion was local breed accounting for 80% followed by 20%; cross Holstein Friesian (HF) distribution was statistically significant across the kebeles. In this study, average lactation length, calving interval, and average age for heifers at first bull service was 9.2±2.7, 15.21±5.72 and 38.1±6.6 in month's respectively and were statistically significant across the kebeles. An average lactation length of 9.2±2.7 shows that, cows had the second pregnancy which was statistically significant across the kebeles. Actually, there should be about 45-60 open days gap between the calving to the next heat sign unless it will lead to the postpartum complication; this significant record difference indicated the presence of prevalent postpartum complication in the area reported in this study. In the present study, two potential sources for breeding animals origins were identified and overall, 71% of the animals originated through birth and 29% were through market purchase process and the respondent consent across kebeles were not statistically significant, which indicted that reproductive health problems were enzootic and could be mainly management related. For the breeding practice, 62% respondent use conventional bull service followed by 30.5% which use artificial insemination and 7.5% use other alternative and the respondent consent were found to be statistically significant. This could have emanated from inconsistence AI service delivery system, poor heat detection and letting dairy cows free grating in the pasture through mixed herding. For the respondent consent overall, 87.5% respondent had no regular vaccination for the herd for any communicable livestock disease in the area and responded that no vaccination calendar was set by the service delivery system.

In the present study, the history of reproductive health problem at household herd level were 52% (n=104/200) and the respondent consent were statistically significant across the kebeles. Based on the respondent consent, animal with increased parity stage (2.6±1.6) showed history reproductive disorder and to the type of reproductive health problem identified repeated, breeder syndrome, dystocia, abortion, retained fetal membrane, uterine prolapsed, mixed type and still birth were 17, 16.5, 14.5, 5.5, 3.5, 1.5 and 1% respectively based on their importance. 52% of the respondent knew no identified root cause on the occurrence of the reproductive health problem in the herd and the consent recorded statistically significant in the kebeles. Based on the consent, the herd level history abortion occurs during second half of gestation (5.78±1.72) and the responses were statistically significant for kebeles.

From Tables 2 and 3, herd size, parity stages, dairy breed and breeding service type were used to assess the association with the occurrence of the reproductive problems. In the present study, with the increase of herd size, the respondent consent indicate there was association of history of reproductive health problem occurrence in the herd by 66.7, 42.3 and 36% for larger, small and medium herd size respectively which was not significant statistically. Respondent consent for the association of breed to the history of reproductive health problem occurrence were found higher in cross breed than local by 59.1 and 34.5% respectively and were statistically significant (Table 4). Similarly, with the increase in number of parity have a significant influence the occurrence of reproductive problems that, the effect increased progressively from primiparous to multiparious (37% and 75.4% respectively) since postpartum complication and owner attitude towards dairy management are inversely related that, the production system was purely traditional and with the increase in exotic blood level condition like postpartum complication were increase.

DISCUSSION

Based on the overall respondents consent, 52% (n=104/200) herd level history of reproductive health problems were recorded which was higher when compared with 39.5% found by Wagari and Shiferaw (2016), and 24.8% by Bitew and Prasad (2011), Wujira and Nibret (2016), Madot and Nibret (2015) and Abebaw et al. (2009) who reported 35.5, 29 and 33.59% in and around Horro Guduru Wollega, Bedelle zone, Wolaita sodo, Jimma and Gondar town respectively. The differences were due to difference in dairy management and agro ecological. In the present study, repeat breeder

Table 1. Socio-demographic data of characteristics of households in the study district (Mean± SD, frequency and Chi-square values).

Parameter (%)	Study district kebels				Overall (N=200 hh)	χ^2
	Hangada (N=49 hh)	Puxo (N=53 hh)	Xadisa (N=44 hh)	Dola (N=54 hh)		
Sex of respondents						
Male	26	48	31	32	68.5	19.587**
Female	23	5	13	22	31.5	
Average age of respondents (year)	33.4±4	31.8±6	28.73±6	30.1±5	31.12±5	23.46 **
Marital status						
Single		1	4	6	5.5	8.546 ^{NS}
Married	49	52	40	48	94.5	
Family size of respondents (persons)	5.2±1.8	4.3±1.49	4.8±1.87	4.89±1.74	4.81±1.75	2.987 ^{NS}
Educational profile of respondents						
Illiterate	31	22	30	26	54.5	
Elementary school	14	31	9	21	37.5	31.654 **
High school	2		1	6	4.5	
College and other	2		4	1	3.5	
Respondent occupation						
Livestock rearing	18	25	3	21	33.5	
Agricultural activity other than livestock rearing	31	28	41	33	66.5	19.44 **

hh; =interviewed households; x^2=chi-square; Significant p<0.05**; non-significant; P >0.05

Table 2. Dairy reproductive and productive performance (Mean± SD, frequency and Chi-square values).

Parameter (%)	Study district kebels				Overall (N=200 hh)	χ^2
	Hangada (N=49 hh)	Puxo (N=53 hh)	Xadisa (N=44 hh)	Dola (N=54 hh)		
Average herd size	3.22±1.7	3.3±0.9	3.22±1.4	3.75±2.6	3.39±1.8	3.231 ^{NS}
Average milking cows number in the herd	0.72±0.74	0.58±0.57	0.91±0.68	0.96±1.15	0.79±0.83	16.571 ^{NS}
Breed of dairy cows						
Local	35	46	42	37	80	14.7986**
Cross HF	14	7	2	17	20	
Average lactation length (LL/month)	8.92±2.03	9.1±1.8	8.4±2.9	10.1±3.5	9.2±2.7	53.319**
Average Calving interval (CI/month)	12.34±2.6	14.6±4.9	17.2±6.3	16.8±6.9	15.21±5.72	40.637**
Average age for heifer at first bull service (month)	38.6±5.6	38.5±7.2	37.8±4.6	38.13±6.64	38.1±6.6	42.280**

RFM= retained fetal membrane; RHP=reproductive health problem; hh = interviewed households; $\chi 2$ = chi square; significant P<0.05**; non-significant; P >0.05.

Table 3. Reproductive health problems in the study area (Mean± SD, frequency and Chi-square values).

Parameter (%)	Study district kebeles				Overall (N=200 hh)	χ^2
	Hangada (N=49 hh)	Puxo (N=53 hh)	Xadisa (N=44 hh)	Dola (N=54 hh)		
History of RHP						
No	28.6	50.9	61.4	51.9	48	11.063**
Yes	71.4	49.1	38.6	48.1	52	
Parity stage with history of RHP	3.4±1.2	3.1±1.3	2.4±1.6	2.1±1.7	2.6±1.6	25.817^{NS}
Record history on major RHP type						
Dystocia record	-	7.5	27.3	31.5	16.5	25.269**
Abortion record	18.4	24.5	6.8	7.4	14.5	9.176**
Uterine prolepses record	-	1.9	9.1	3.7	3.5	6.264^{NS}
RFM record	4.1	5.7	4.5	7.4	5.5	3.439^{NS}
Still birth	-	-	2.3	1.9	1	2.146^{NS}
Repeated breeder syndrome (RBS)	6.1	11.3	20.5	29.6	17	11.797**
Mixed	-	-	-	5.6	1.5	11.768^{NS}
Respondent consent on identifying cause of RHP						
Yes	14	27	27	28	48	11.063**
No	35	26	17	26	52	
Abortion history and gestation length (month)	5.82±1.84	7.34±1.51	4.72±1.85	5.24±1.56	5.78±1.72	14.026**

RFM= retained fetal membrane; RHP=reproductive health problem; hh = interviewed households; $\chi 2$ = chi square; significant P<0.05**; NS, non-significant; P >0.05.

syndromes, dystocia, abortion, RFM and uterine prolapse were found to be the major reproductive health problem identified in the area. The higher occurrence for repeated breeding syndrome (RBS) by 17% were factored by use of sub fertile bulls, management practices like insemination timing, faulty heat detection and inconsistence breeding services delivery system which agree with the repot in the country by Ararsa and Wubishet (2014); Adane et al. (2014); 10.3 and 13.08% respectively in and around Borena Oromia and Hossana town are also contributing factors and the portion of dystocia, may be due to parity of the dam as well as the breed of the sire (Arthur et al., 2001). Also, the present record for RBS was higher than the study report in the country, 3.87% by Dawit and Ahmed (2013); 2.9% by Bitew and Prasad (2011) and 1.3% by Abebaw et al. (2009); this high report was due to the synchronization program intervention by individual cow's physiological response difference to prostaglandin injection.

In this study, an abortion history most commonly occurs during their second half of gestation which is 5.78±1.72 in month and was statistically significant across kebeles. The occurrence of abortion in this study was about 14.5%, which strongly agrees with the findings of Kifle and Moges (2016) who reported 19.7% in and around Gondar town and Adane et al. (2014) who reported 13.08% in and around Hosanna town. This study shows that abortion in dairy cattle was found to be more common in pluriparous cows at advanced pregnancy stage. The possible predisposing condition of abortion was identified as both mechanical and infectious cause but, need further investigations to identify the etiology. The record for retained fetal membrane (RFM) (5.5%) agrees with the report of Ayana and Gudeta (2015) in selected sites of central zone of Tigrai region, northern Ethiopia reporting an occurrence of 8.3 and 7.18% in Hossan town

Table 4. Association between history of RHP and putative risk factors (Frequency and Chi-square values).

Risk factors (%)	History of RHP		χ^2
	Yes	No	
Herd size			
Small herd size(1-3)	42.3	57.7	1.108^{NS}
Medium herd size(4-10)	36	64	
Large herd size(>10)	66.7	33.3	
Breed of dairy cows			
Local	34.5	65.5	3.96^{**}
Cross HF	59.1	40.9	
Service type			
Artificial insemination	50	50	1.868^{NS}
Bull	34.8	65.2	
Parity type			
Single parity (primiparous)	37	63	6.988^{**}
Multiple parity (multiparious)	75.4	24.6	

RHP=reproductive health problem; χ^2 = chi square; significant P<0.05**; NS, non-significant; P >0.05.

(Adane et al., 2014). The much incidence of RFM might be linked to the low incidence of abortion a known predisposing factor for RFM. Other factors such as year of calving, season of calving, parity of dam, calving difficulty and fetal presentation have all been shown to affect the incidence of RFM (Arthur et al., 2001).

The higher occurrences of reproductive problems in crossbred cattle (59.1%) than local breed (34.5%) may be due to the fact that European breeds are less adapted to tropical conditions of high temperature and humidity, disease and low feed quality than zebu cattle Mukasa-Mugerwa (1989) making them more susceptible than indigenous zebu. Another reason may also be due to the fact that, cross breeds require more elaborated management, feeding and better health care than the indigenous zebu to get better reproductive performance and productivity in the tropics (Tekelye et al., 1991).

Significantly higher occurrence of reproductive health problems observed in multiparous cows (75.4%) in this work is in agreement with those of previous findings by Micheal (2003) which is possibly due to the repeated exposure of the genital tract of pluriparus cows to environmental risk factors that can impart uterine infection. It can also be due to older cattle are not as such good in feed intake capacity and decrement in natural immunity as age increases. Even though the service type (AI or natural mating) of the study animals did not influence the occurrence of reproductive health problems in the present study, that is statistically non-significant, It was high in those which use artificial insemination (50%) than (34.5%).

Conclusions

The present study revealed a high occurrence of reproductive health problems, of which repeat breeder, dystocia, abortion and retained fetal membrane were found the most common problems of dairy cows identified in the area. Also, this study indicates that the association of history of reproductive problems showed significant difference with respect to breed and parity of dairy cattle thus; Holstein cross breed dairy with increased parity was recorded to have history of reproductive health problem than others in the management system. Further studies should be conducted at different cross breed level, and awareness should be creation to farm owners and attendants to improve dairy management with increased parity. Many small holders' farms are run as a sideline business and are often victimized with improper management thus; putative risk factors responsible for the occurrence of reproductive health problems were breed, service type and parity stage. Therefore, improving management like herd health care, heat detection and proper selection of bulls for breeding will minimize the problems and hence increase reproductive efficiency of dairy cows in the area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Vol. 10(9), pp. 231-236, September 2018
DOI: 10.5897/JVMAH2018.0681
Article Number: EF70AEE58177
ISSN: 2141-2529

http://www.academicjournals.org/JVMAH

ACADEMIC JOURNALS

Journal of Toxicology and Environmental Health Sciences

Full Length Research Paper

Prevalence and clinical pathology caused by infectious bronchitis virus in poultry birds at Sindh, Pakistan

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Received 27 March, 2018: Accepted 17 April, 2018

Poultry is a major meat and eggs producing sector that generates high income throughout the world. Despite worldwide growth of poultry, it is influenced by various infectious pathogens especially infectious bronchitis virus (IBV). As a result of heavy economic losses through high morbidity, mortality, deficient performance, low weight gain, abnormal colour and misshapen eggs, this study is designed on identification and quantification of infectious bronchitis virus. A total 4000 tissue specimen (trachea, kidney and swabs) were collected from flocks with respiratory illness, from various areas of Sindh. The specimens were cultured in 10 days old specific pathogen free (SPF) embryonated chicken eggs and amnio-allantoic fluid (AAF) was harvested. Hemagglutination assay (HA) with trypsin showed slightly higher prevalence of IBV in layers (≥61.2%) than in broilers (≥ 52%). ELISA revealed that 84.4% samples were positive, while 14.6% samples were negative for IBV. It is concluded that IBV is highly prevalent in various parts of Sindh province of Pakistan, therefore time to time vaccination is required to prevent heavy economic losses.

Key words: Enzyme linked immunosorbent assay (ELISA), hemagglutination assay (HA), infectious bronchitis virus (IBV), infectious bronchitis virus, poultry, prevalence.

INTRODUCTION

Poultry is a rapid growing sector throughout the world. It has been found that market share poultry meat is about 2 to 2.5 in 1971 that has been increase to 25% in 2010 (GOP, 2013). It is economical to stockholders due to large farming size, quick output, genetic improvement and improvement in feed stuff (Martinez, 2002; Morrison et al., 2004). Despite worldwide growth of poultry, it is influenced by various infectious pathogens including infectious bronchitis virus (IBV) (Sandhu et al., 2009).

Infectious bronchitis virus (IBV) is an acute and highly contagious virus (Bourogâa et al., 2009, Abdel-Moneim et al., 2012) causing respiratory disease in poultry. It is associated with sneezing, gasping, tracheal rales, coughing, puffy swollen eyes and inflamed sinus with poor weight gain (Sediek, 2010). The virus severely damage epithelium of trachea which leads to tracheal

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Table 1. Sample collection for IBV isolation.

Location	Type of flock	Age of flock	Number of sample	Collection date
Karachi			400	10-15/11/2015
Thatta			400	15-25/11/2015
Mirpur khas			400	25-30/11/2015
Larkana	Broiler	5-7 weeks	400	01-10/12/2015
Sukkur			400	10-20/12/2015
Karachi			400	20-30/12/2015
Thatta			400	01-05/12/2015
Mirpur khas			400	05/10/01/2016
Larkana	Layers	35-67 weeks	400	10-20/01/2016
Sukkur			400	20-30/01/2016

haemorrhages, congested lungs, air sacs and seromucus exudates on lungs as well as on air sacs (Sediek, 2005). Drop in egg production, poor quality eggs, misshapen, broken and weak shelled eggs (Seidek, 2010) followed by reduction in egg production up to 50% (Biswas, 2004) and loss shell colour (Cook and Huggins, 1986). The infection is responsible for causing morphological and histopathological changes in oviduct which influence laying of eggs (Jane et al., 2012). Prevalence of infectious bronchitis virus has been increased that lead heavy morbidity and mortality. The prevalence of infectious virus in commercial poultry is about 82.43% (Hadipour et al., 2011).

Outbreaks of IBV have been increased in last few decades in countries such as Tunisia, Egypt and Asia that caused heavy economical losses (Yu et al., 2001). Keeping in view the high morbidity and mortality due to recent outbreaks of Taiwan Group I CK/CH/LDL/97I strains of IBV (Chen et al., 2010; Jinling et al., 2012), this study is designed to investigate the prevalence and clinical pathologies caused by infectious bronchitis virus (IBV) in Pakistan.

MATERIALS AND METHODS

Sample collection and transportation

A total of 4000 tissue specimens (trachea, kidney and tracheal swabs) were collected from flocks with respiratory illness from various districts of Sindh (Karachi, Thatta, Mirpur khas, Larkana and Sukkur), from November 2015 to January 2016. In order to investigate prevalence and quantification of IBV samples were taken from 200 farms (broiler and layers with 1:1 shown in Table 1). Samples were transferred in test tubes containing sterilized phosphate-buffered saline (PBS) supplemented with penicillin (10,000 IU per ml), streptomycin (10,000 ug ml^{-1}) and nystatin sulphate (1000 IU ml^{-1}) as described by Mahmood et al. (2004). The samples were shifted to research and development laboratory and preserved at -80°C.

Virus isolation

One gram of sample was homogenised in 700 ul of PBS, after adding 35 ul of antibiotics. The supernatant was injected to 10 day-old embryonated chicken eggs, amnio-allantoic fluid was harvested 72 h post inoculation and it was subjected to rapid HA test as described by Doherty (1967). A volume of 125 ul of samples with negative rapid HA was treated with 25 ul of trypsin then micro HA was performed (Mahmood et al., 2004).

ELISA

An antigen ELISA kit was used to detect IBV that contained antibody coated microtiter plate wells. A volume of 40 ul of tissue homogenate missed with 10 ul of sample diluent, poured in wells and incubated for 30 min. After the addition of horseradish peroxidase (HRP) conjugate antigen and antibody complex was generated. Chromogen dye followed by stop solution was added the blue color changed to yellow color after incubation in dark room, optical density (OD) value was calculated at wavelength of 450 nm by using automatic ELISA reader after that (S/P) ratio (absorbency of sample / absorbency of positive control) was determined as described by Wang et al. (2002) and Chen et al. (2003). The samples with S/P ratio ≥ 0.2 were considered positive for IBV.

RESULTS

Results explored that Infectious bronchitis virus (IBV) is characterised by general respiratory signs such as severe conjunctivitis, lacrimation, gasping, sneezing, watery eyes, tracheal rales and coughing. Post-mortemlesions include damaged epithelium of trachea, tracheal haemorrhages, sero-mucus exudate in trachea and congested lungs with prominent infiltration of sero-mucus exudates (Figure 1a to d).

Additionally, it has been investigated that kidneys were severely congested, inflamed, pale and distended with prominent urates leading peri hepatitis and aracialities. Moreover, proximal tubules and convoluted tubules were inflamed (Figure 2).

Hemagglutination assay (HA) with trypsin in broiler flocks have found that IBV is highly prevalent at Thatta (61%), lowest at Sukkur (41%), Karachi (55%) and Larkana (45%) (Figure 3a).

Similarly, HA titers with trypsin in layer flocks have

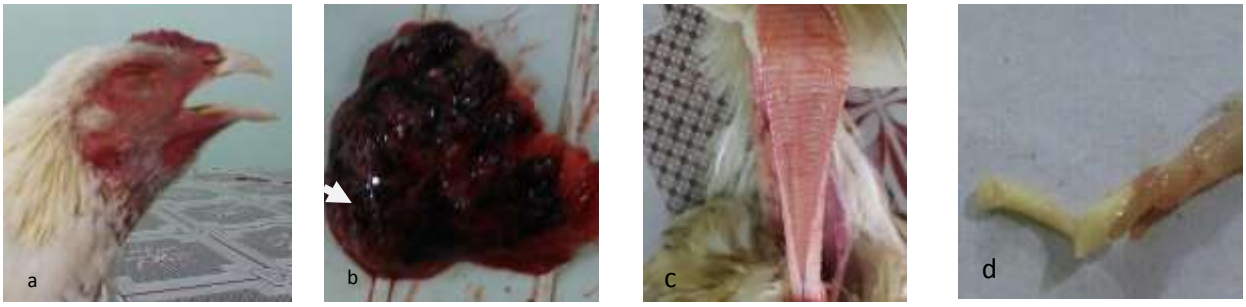

Figure 1. IBV infected birds. (a) Tracheal rales with respiratory distress. (b) Congested lungs with severe infiltration of sero-mucus exudate

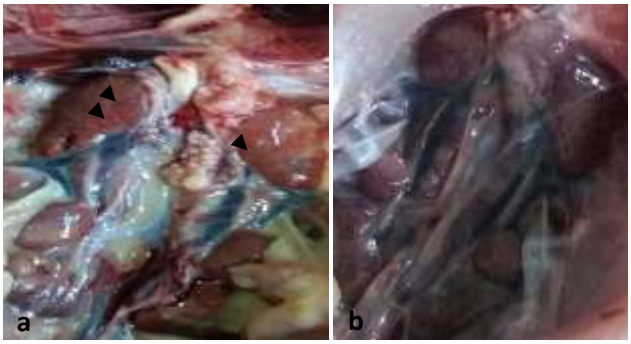

Figure 2. Kidney of IBV infected laying hen. (a) Deposition of uprated in kidney and tubules. (b) IBV infected kidney bulged from renal cavity.

shown that IBV is higher in Thatta (69%) followed by Karachi, Mirpurkhas, Sukkur and Larkana (67, 62, 57 and 51%) as shown in Figure 3b.

Results have found that IBV reduced egg production; poor quality eggs, weak shelled eggs, misshaped eggs and cracked egg (Figure 4a to d). Similarly, it damaged the ovary as a result egg yolk is evenly found in abdominal cavity (Figure 4b).

Enzyme linked immunosorbent assay (ELISA) of 90 randomly selected samples showed that IBV were strongly positive 4/90 (4.44%), positive 46/90 (11.11%), weak positive 33/90 (36.66%) and negative 7/90 (7.77%) as shown in Figure 5.

DISCUSSION

Despite expeditious growth of poultry in Pakistan, this is the first study on overall prevalence of IBV throughout Sindh. The current study had determined that IBV is a major respiratory pathogen that causes catastrophic morbidity; mortality leads to heavy economic loss. The study investigated that IBV infected birds characterized by signs, that is, severe conjunctivitis, lacrimation, gasping, sneezing, watery eyes, severe tracheal rales and cough (Figure 1a to d). Correspondingly, severe conjunctivitis, lacrimation, sneezing, mild tracheal rales and cough have been reported in 2013 in Egypt (Sediek and Awad, 2014). Additionally, IBV infected birds were depressed, lethargic, reluctant to move and take feed these finding was in agreement with (Terregino et al., 2008)

Main lesions in respiratory tract were reddish streaks ranging from mild to severe, increased concentration of mucin in trachea with accumulation sero-mucusexudates in trachea and bronchi (Figure 1c). These findings are correlated with Terregino et al. (2008) and Sediek (2010). However, lungs were congested, discoloured, infiltrated with mucus leading to pneumonia (Figure 1b) that were similar with previous reports (Sediek, 2010).It has been found that sero-mucus exudates in trachea is due to degeneration of cilia by viropexin enzyme produced by IBV (Ashraf et al., 2010). Similarly, infiltration of inflammatory cells in the lamina propria and submucosa, activation of goblet cells, oedema in the submucosa, epithelial lymphoid infiltration, epithelial hyperplasia in trachea have been reported (Cavanagh and Naqi, 2003; Terregino et al., 2008; Sediek, 2010). IBV severely damaged urogenital system especially renal abnormalities (nephritis, pale and enlarged kidneys).

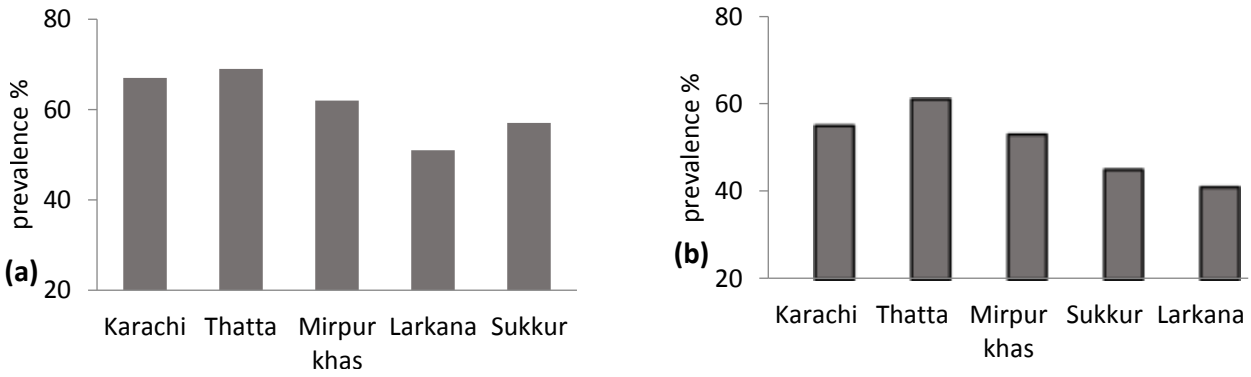

Figure 3. Prevalence % of IBV in broiler birds (a) and layer birds (b) at various districts of Sindh.

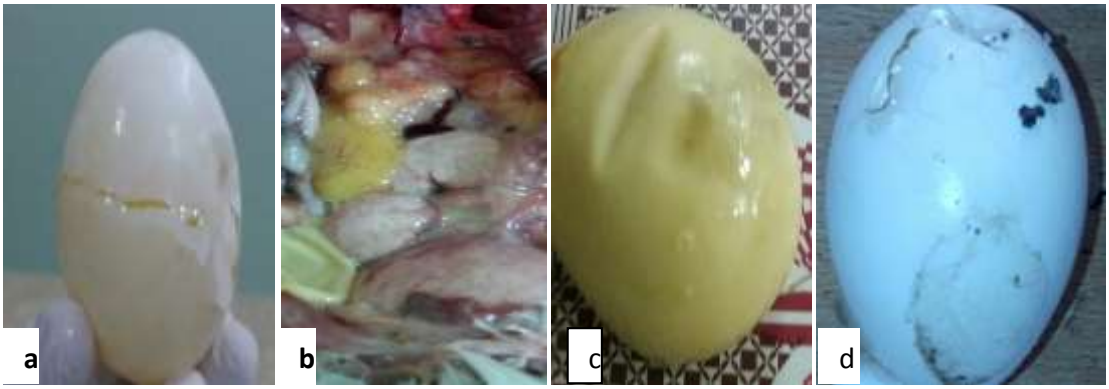

Figure 4. (a) Poor quality egg with ruptured shell; (b) Egg yolk distributed in abdominal cavity shown with single arrow head and suppressed shell shown with double arrow. (c) Weak eggs shell with dimples shown by arrow heads. (D) Cracked egg shell.

Moreover, kidney was bulged from renal cavity, inflamed and distended with prominent urates (Figure 2a and b). Correspondingly, Sediek (2005), Sediek and Awad , (2014) and Abdel-Moneim et al. (2002) found that IBV caused severe congestion, pale and congested kidney with prominent urates, peri hepatitis and airsaculitis. On the contrary, nephritis is seen in naturally infected flocks were due to Nephritis Nephrosis Syndrome (NNS) (El-Sisi and Eid-Amal., 2004). The results of the study differ from that of Ashraf et al. (2010) as they reported that IBV isolate 22 does not produce renal lesions in artificial infection.

Results have found that the severity and prevalence percentage of IBV varies with age and type of birds like IBV produce more severe infection in broiler than layer flocks but prevalence percentage is more in layer than broiler that could be due to repeated exposure of layer to same pathogen. Additionally, IBV adversely affect performance of layers and quality of eggs (misshapen, weak shelled, cracked shell, missing of shell with accumulation of yolk in abdomen) (Figure 4a to d). These results are in agreement with previous studies that IBV infected birds have poor laying percentage along with bad quality eggs (Sediek and Awad, 2014). Interestingly, IBV during growing period appears to have minor effect on the ability of hen to produce eggs of normal quality (Jane et al., 2012).

Hemagglutination assay with trypsin shows that IBV is highly prevalent in layer flocks than broiler, that is, data of layer flocks showed the prevalence inThatta (69%) to be highest followed by Karachi (67%), Mirpurkhas (62%), Sukkur (57) and Larkana (51%)(Figure 3b) while in broiler flocks at Thatta as 61% and lowest at Sukkur (41%) while at Karachi and Larkana was 55 and 45%, respectively (Figure 3a). Similarly Uddin et al. (2016) found through RT-PCR that IBV was higher in broilers (62.5%) and lower in layer breeders (52.94%).

A total of 90 randomly selected samples were subjected to ELISA, and found IBV strong positive 4/90 (4.44%), positive 46/90 (11.11%), weak positive 33/90 (36.66%) and negative 7/90 (7.77%) (Figure 5). The Findings of this study correlated with that of Hadipour et al. (2011) which showed that infectious bronchitis virus is highly prevalent in poultry with broiler as 64%, layer as 53% and broiler breeder flocks as 54.54% leading to heavy economic losses.

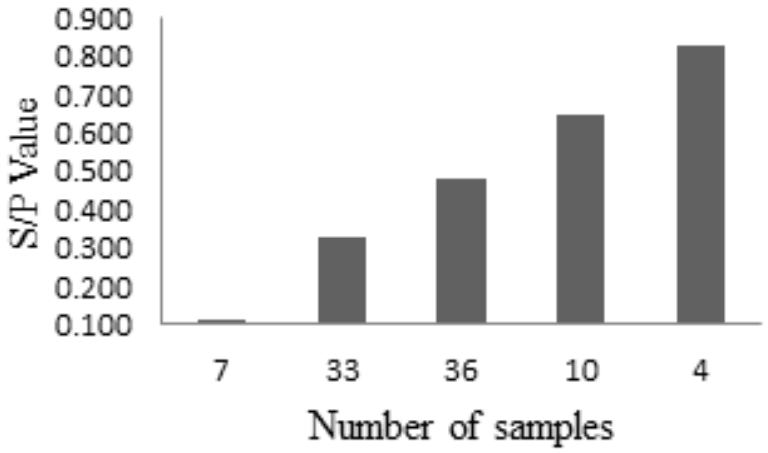

Figure 5. Detection of IBV through ELISA. Samples with S/P value ≥ 0.2 were considered as positive while samples with S/P value ≤ 0.2 were considered as negative

Conclusion

Infectious bronchitis virus is an acute and highly contagious disease of poultry. It causes respiratory distress, heavy morbidity and mortality. Prevalence of IBV is slightly higher in layers (61.2%) than broilers (52 %). Therefore, studies must be conducted on the prevalence, isolation and mechanism of infection and preparation of vaccine from local isolates in-order to prevent economic losses.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The project is supported by Sindh Government under ADP Scheme (Surveillance and characterization of circulating pathogens of poultry in Sindh including Avian Influenza containment program)

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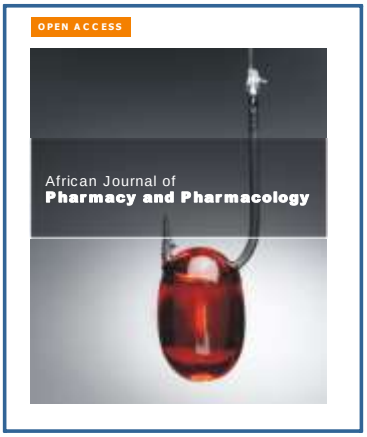

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