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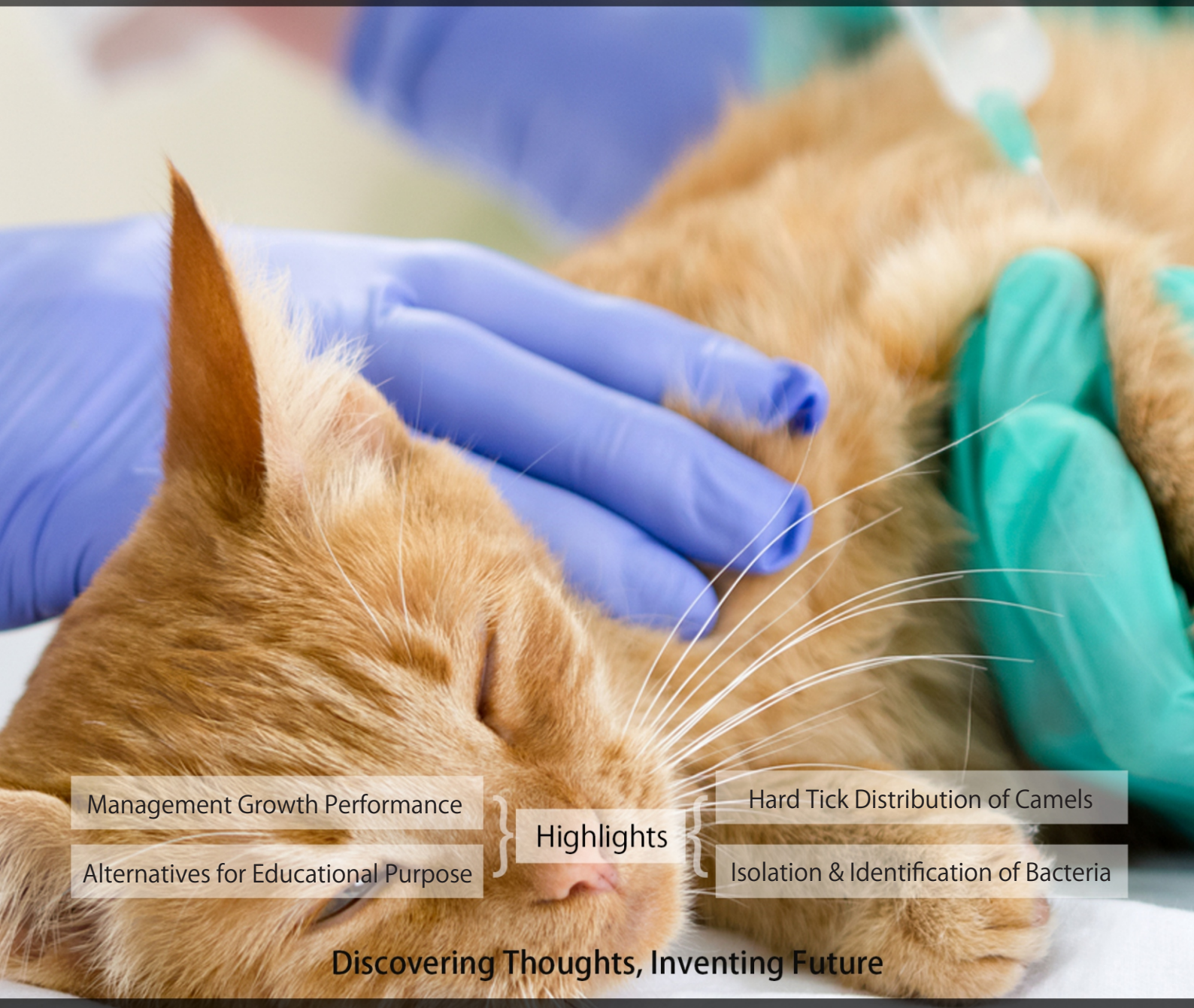

Management Growth Performance

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Discovering Thoughts, Inventing Future

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Management, Growth Performance and Cost Effectiveness of Japanese Quail in Khaza Quail Farm and Hatchery Limited at Chittagong in Bangladesh

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Abstract- An observational study was conducted on 650 Japanese quail chicks from hatching up to marketing age (0-6 wks) in a farm at Moij-jartack, Chittagong in Bangladesh to evaluate their management, growth and productive performance under litter floor rearing system. The total study period was 70 days. The chicks were hatched in the own hatchery farm where the hatchability rate was 71.42%. The daily amount of supplied ration was fixed and varied in different ages. The birds were reared for 6 wks (marketing age) and were sold when 120-130 gm weight was achieved. The feed conversion ratio (FCR) in 1st wk was 1.4, but from (2-6) wks it was increased to 3.1, 5.24, 5.6, 5.6 and 6.37 respectively. The average weight was 130 gms at 6 wks with an average feed conversion ratio of 4.55: 1. Mortality rate decreased with age (2.42%). The farming was also profitable in terms of production per birds. It may be inferred that Japanese quail performs well under litter floor rearing in Bangladesh.

Keywords: *Japanese quail, hatchability, FCR, mortality rate.*

GJMR-G Classification: *NLMC Code: QW 70*

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Jotan Kar [α], Tapas Roy Barman [σ], Arup Sen [ρ] & Sabuj Kanti Nath [ω]

Abstract- An observational study was conducted on 650 Japanese quail chicks from hatching up to marketing age (0-6 wks) in a farm at Moij-jartack, Chittagong in Bangladesh to evaluate their management, growth and productive performance under litter floor rearing system. The total study period was 70 days. The chicks were hatched in the own hatchery farm where the hatchability rate was 71.42%. The daily amount of supplied ration was fixed and varied in different ages. The birds were reared for 6 wks (marketing age) and were sold when 120-130 gm weight was achieved. The feed conversion ratio (FCR) in 1st wk was 1.4, but from (2-6) wks it was increased to 3.1, 5.24, 5.6, 5.6 and 6.37 respectively. The average weight was 130 gms at 6 wks with an average feed conversion ratio of 4.55: 1. Mortality rate decreased with age (2.42%). The farming was also profitable in terms of production per birds. It may be inferred that Japanese quail performs well under litter floor rearing in Bangladesh.

Keywords: Japanese quail, hatchability, FCR, mortality rate.

I. Introduction

Quail is the smallest and latest domesticated poultry species. There are about 131 species and 17 to 18 varieties of wild quail found all over the world, of which Japanese, Bobwhite, King and Stable quail are most important. Japanese quails are the natural inhabitant of Japan. Quails are reared in Japan from the time immemorial. The scientific name of Japanese quail is *Coturnix coturnix japonica* under the class aves and family Phasianoidea (Hashanuzzaman, 2013). Poultry eggs and meat provide approximately 38% of the total animal protein in Bangladesh. As compared to other countries of the world the protein consumption in Bangladesh from animal origin is significantly lower. The annual Avg. a deficit of chicken egg is 6939 million numbers and the annual average deficit of meat is 3.81 million metric ton (Andrew, 2003). With the rapid increase in total population, the demand for poultry products has been increasing. To meet up the growing demand for poultry products, the development of poultry industry is very important. The popularity of quail husbandry is increasing all over the world. In Bangladesh quail was introduced for the first time in 1988 (Das, 2004). Quail farming for egg and meat is quite popular in Japan, Hongkong, Korea, China, Singapore, India, Thailand, Malaysia, Indonesia, France, Italy, Germany, Britain, and Russia. Only Bobwhite quail and Japanese quail have been domesticated for commercial purposes and in Bangladesh, these two are commercially available. Besides, scientists developed many quail lines e.g. white egg shell line, meat line etc. Japanese quail, a recently introduced economic avian species is ideally suited for meat and egg under intensive management due to their low maintenance cost, early sexual maturity, higher exponential growth, higher heat tolerance, fitness for higher density rearing, higher disease resistance and higher egg production than that of other poultry species. Short generation interval and quick business return and the requirement of low investment attracting people to rear them. It appears that quail rearing may be important to the chicken when chicken survived in hostile climates and also for havoc like avian influenza and salmonellosis. The climate and natural condition of Bangladesh are very suitable for quail rearing. Quail can be reared in this country throughout the year with a good performance in meat and egg production. It has a shorter life cycle and its production requires less capital and land (Vali *et al*., 2005). Being an agricultural country the government of Bangladesh has shifted policy emphasis on poultry rearing. Quail supplies meats, eggs, and extra income also. The quail farming has the unique advantage of tapping the growing market demand for poultry products as a supplement of chicken and duck farming (Sultana *et al*., 2007). Nowadays a large number of quail farms have been established in Bangladesh to supply quail meats in hotels, shops, and household consumption as its demand is increasing day by day. Japanese quail is the smallest avian species farmed for meat production (Vali, 2008). The meat from broiler quail is very delicate and tasty. It is considered as a superior item in different restaurant and homes. One five-week-old broiler quail

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attains 140-150 gm body weight within 5 weeks of age and yields 72.5 % carcass for consumption (Das, 2004). Success in poultry farming depends on scientific breeding, feeding, management and disease control of the flocks. There is a relationship of Japanese quails (heavy body weight) line to dietary energy levels and graded essential amino acid levels on growth performance and immunocompetence (Kaur *et al.,* 2008).

The profitability in quail farming is possible by better management due to the above reasons. Reports on quail growth and body composition are numerous. The better growth performance and meat quality of broiler quail (Japanese quail) are supported by the findings of (Kaur *et al*., 2008 and Vali, 2008). The findings of their study clearly indicate that quail farming is a promising sector in poultry meat production. In public sector limited scope is given to a large number of educated people are looking for self-employment. The time has now come for creating alternative employment opportunities for these educated people. The self-employment scheme is one probable answer and quail farming seems to be a promising enterprise in this direction. It is hoped that quail farming will be recognized as popular poultry sector one day in our country.

The objectives of the study were to determine the management, growth, production performance and cost effectiveness for the rearing of Japanese quail under controlled housing system.

II. Materials and Methods

a) Location and duration of the experiment

The study was conducted at a local Quail farm & hatchery known as Khaza quail farm & hatchery Ltd., Moij-jartack, potia, Chittagong. It is a small local quail farm which includes three rearing units and one hatchery. During observation, the total numbers of broiler quails were 650.

b) Housing and management

The housing system is most important. In the case of quail farming, housing system should be wire floor or battery or cage system. In that farm,

i. *Floor space*

The floor management for rearing broiler was 55 ft long and 20 ft wide. The floor space for day old chicks up to 3 wks was 100 sq. cm/ bird and from 3 weeks up to 6 wks (marketing age) it was 170 sq. cm/bird.

ii. *Litter materials*

Wood shaving was used as litter material at the depth of 7 cm over the floor.

iii. *Pre-incubation care of egg*

Eggs were collected from the own hatchery, stored at a temperature 15°C and were fumigated after they are collected. Fumigation was done by using 25gm of potassium permanganate and 35 ml of formalin (40%) for each cubic meter of incubator space.

iv. *Incubation and hatching*

The incubation period for quail is 17-18 days, depending on the strain and the incubation procedures. Successful hatches depend upon a good understanding of incubator controls. A still-air incubator was used. The incubation temperature was 38.3°C (101°F) which did not exceed 39.5°C (103°F) temperature until hatching was completed. The temperature was measured at the top of the eggs. Humidity was less than 70%. The eggs were turned by hand at least three, and preferably five, times in a day. A pencil mark on the side of each egg helped to ensure proper turning. The eggs were hatched at 17th to 18th day of incubation.

v. *Brooding*

The chicks were brooded under continuous lighting for the first two weeks and were kept within a case. Papers were used as litter and were changed every day. During brooding 100 sq. cm space was maintained per bird. 95°F temperature was maintained for 24 hrs as brooding temperature from the day of hatching up to 2 wks.

vi. *Lighting management*

During brooding period (0-2 wks) 24 hours lighting was ensured. After the brooding period (0-2 wks), lighting program normally changed depending on the purpose of production. As those birds were reared for meat production so they were exposed to 23 hours lighting with 1-hour darkness.

vii. *Temperature schedule*

During brooding period (0-2 wks), 95°F temperature and from 3 wks to marketing age (6 wks) 75°F temperature was maintained.

viii. *Feeder and watered*

About 1.25-2.5 cm of feeder space were supplied for adult quail. Ample feeds were supplied. Clean, fresh water was provided at all times with a minimum of 0.6 cm of trough space per quail. Nipple drinkers and cups were supplied for adult quail. One nipple or cup was provided for every 5 birds.

Table 1: The following feeder and watered spaces were available in the farm

Items	Brooding period (0-2 wks)	Growing period (3-6 wks)
1. Feeder	1 linear cm/ bird	1 round feeder/ 25 birds
2. Plastic drinker	0.5 linear cm/ bird	1 plastic drinker/ 30 birds

ix. *Feeding and nutrition*

In the farm, controlled packaged ready feed was provided to birds. Per chicks were supplied with 2 gm feed daily on Avg. in (0-1) wks. However, 18.75 gm feed per bird daily were supplied to birds on Avg. from 3 wks to the marketing age (6 wks).

Table 2: Nutritional level of feed

Type of ration	ME(Kcal/kg)		CP%		Ca%		Avg. P%	
	SD. value	PGT. pellet feed	SD. value	PGT. pellet feed	SD. value	PGT. Pellet feed	SD. value	PGT. pellet feed
Starter (0-2 wks)	3200	3000	25	22	0.95	1.15	0.45	0.50
Finisher(3-6 wks)	3200	3050	20.5	20	0.95	1.05	0.42	0.44

SD. =Standard value, PGT. =Progoti feed company, CP=Crude protein, Ca=Calcium, P=Phosphorus.

c) *Source of the standard value: (Larbier and Leclercq, 1994)*

The table shows that the ME of starter (0-2 wks) and finisher feed (3-6 wks) was 3000 and 3050 Kcal/kg respectively which was lower than the standard ME requirement. Likewise, the supplied CP in the starter feed was 22% and in the finisher, it was decreased to 20% which was also lower than the standard CP% (25% & 20.5% respectively). But the Ca% & P% of supplied feed was slightly higher than the standard levels.

i. *Body weight, weight gain and feed conversion ratio*

a. *Body weight gain*

Average daily gains (ADG) were estimated using the formula

ADG = (W2−W1)/N
Where W2 is the final weight
W1 is the initial weight
W2 -W1=Live weight gain
N is the number of days taken from initial weight to the present weight.

Live weights of the birds were recorded weekly from 0-6 wks. From this live weight, the live weight gain was calculated. And then, Avg. daily weight gain (ADG) was calculated by dividing the every obtained value per wk. with 7. Such as the hatch weight of chick was 10gm and the weight of 1st week was 20 gm. So, lives weight gain of the 1st week = (live weight of 1st week – hatch weight). Now the ADG at 1st wk. = (live wt. gain at 1st wk. /7). Similarly, live weight gain of the 2nd week= (live weight of 2nd week – live weight of 1st week).

b. *Feed Conversion Ratio (FCR)*

The gain per feed intake was estimated for the first 6 weeks on weekly basis. This was estimated using the formula.
Feed conversion ratio = Feed intake/ Avg. daily weight gain.

Table 3: Medication routine

For the starter (0-2 wks):

Age (days)	Medicines	Amount (in 1 litre water)
1-2	Glucolyte	10-20 gm
3-8	Doxyoxy	5 gm
	Gluco-C	10 gm
9-14	Thiamix-RP	1 gm
	Sancal-P	1 gm
	Vitamina AD3E	1 ml

For the finisher (3-6 wks)

ii. *Data collection*

The farm was regularly visited and data were collected by own observation & interviewing the owner Abu Sadek of the respective farm from 9th September 2016 to 17th November 2016.

III. Results

The farm was a potential commercial farm for quail rearing. The adult birds were sold after 6 weeks of age when the expected weight is acquired i.e. about 120-130 gm. During this study, the average body weight of the targeted batch of 6 weeks aged birds were 130 gm and FCR were 4.55:1. Feed intake was 5 gm per bird daily on average up to 2 weeks and 18.75 gm per bird daily on average from 3-6 wks. Mortality rate was higher at (0-2) wks.

The results of the study are based on the following data:

a) *Hatchability*

The total number of fertile eggs settled in setter was 910.

Total number of chicks hatched after 18th day of incubation was 650

So, the hatchability= (Total no. of chicks hatched / Total no. of fertile eggs settled) × 100
=71.42 %

b) Feed intake

The feed intake was based on daily basis and it was noted for the first 6 weeks. Balanced pellet feed from the Progoti poultry feeds Ltd were supplied to the birds. The Avg. feed intake per bird per day frm 0 wk. to 6 wks. was reported 14.2 gm.

c) Body weight, weight gain and feed conversion ratio

The body weight was calculated by weighing the birds in a weighing tool. The hatch weight (Avg.) was 10 gm. The Avg. body weight and Avg. weight gain at marketing age (6 wks) was 130 gm and 22 gm respectively. The feed conversion ratio (FCR) was reported 4.55:1.

d) Mortality

The percentage mortality was estimated for the first 6 weeks on weekly basis. This was estimated using the formula

Mortality rate = (No. of dead quail over the week/ No. of quail at the beginning of the week) ×100. The Avg. mortality rate up to marketing age (6 wks) was 2.42%.

Table 4: The overall performances of the khaza quail farm & hatchery ltd. in relation to the standard value from literature shown in the following table

Time (WK.)	FI (gm) / day (avg.)		LW (gm)	LWG (gm)	ADG (gm)	FCR (gm)		MY (%)
	SD.	Achieved	Achieved			SD.	Achieved	Achieved
0-1	3-4	2	20	10	1.43	1.33	1.40	4.62
1-2	7-9	8	38	18	2.57	1.93	3.11	4.03
2-3	11-14	15	58	20	2.86	2.34	5.24	2.02
3-4	15-18	20	83	25	3.57	2.93	5.60	1.89
4-5	18-20	20	108	25	3.57	3.44	5.60	1.22
5-6	20-24	20	130	22	3.14	4.01	6.37	0.71
Total- 6 wks.		14.2 (avg.)				2.66 (avg.)	4.55 (avg.)	2.42 (avg.)

FI= Feed intake, LW=Live weight, LWG=Live wt. gain, ADG= Average daily gain, FCR= Feed Conversion Ratio, MY=Mortality, SD. = Standard value, WK. = Week.

Source of the standard feed intake value: **(Das, 2004)** and Standard FCR by: (**Naim, 2012).**

According to the above table, the feed intake of chicks at 1st and 2nd weeks were 2 gm and 8 gm/bird/ day respectively, where at 1st week it was given 2 gm feeds which was below the standard feed intake levels (3-4 gm). Although, the feed intake at 3rd wk. was 15gm but at 4-6 wks and onward in every wk. per birds were given 20 gm (Avg.) feeds/day. The live weight of birds at (0-6) wks was 20 gm, 38 gm, 58 gm, 83 gm, 108 gm and 130 gm respectively and ADG at (0-6) wks were 1.43, 2.57, 2.86, 3.57, 3.57 and 3.14 gm respectively. The table shows that, live weight gain was increasing gradually with the live weight and age up to 5 wks of age. The average weight gain of birds at marketing age (6 wks) was 130 gm. The FCgnR was 1.4, 3.1, 5.24, 5.6, 5.6 & 6.37 at 1st to 6th wks respectively with an Avg. of 4.55:1. The mortality % (4.62 & 4.03) was higher within 0-2 wks of age. After that, the mortality rate was decreased at a decreasing rate with an Avg. mortality of 2.42% after 6 weeks.

e) Cost-effectiveness of per bird rearing

The feed and water cost per bird up to 6th weeks = 29 Tk.

Litter materials= 500 Tk.

Cost of equipment & electricity = 1400 Tk.

Depreciation cost = 500 Tk.

Total medicine cost =1120 Tk.

Total Cost per bird = 34.41 Tk.

The price of a bird at market age = 45 Tk.

Total profit from per bird production = (45 – 34.41) Tk.
= 10.59 Tk. /per bird.

IV. Discussion

From the technical and economic points of view, quail rearing is attractive due to their rapid growth and early onset of lay, high reproduction rates and low feed intake (Seker *et al*., 2004). In an observational study by Dauda *et al*., 2014 it was found 70.48 % hatchability of Japanese quail eggs which was slightly lower than this study of 71.42 % hatchability. The storage of quail eggs at tropical temperature seems to be suitable up to 6 days when hatchability remains 70%. Due to pre-incubation mortality or early embryonic death, there is an increasing rate of unhatched eggs after one week of storage. In the study, the birds were fed formulated diet containing (20-22) % crude protein and (3000 -3050) Kcal/Kg metabolizable energy, both of which were higher than earlier study of Begum and Howlider, 2000 where, 18 % CP & 2800 Kcal/Kg ME

were provided but in the study they found that the value is lesser than the recommendation (3200 Kcal/Kg from 0-6 wks and 25 % ME in 0-2 wks) of (Larbier and Leclercq, 1994). After all, the present findings indicate that Japanese broiler quail needs a diet containing 3200 kcal ME/kg and (24.5-25) % CP during the first two weeks of age to achieve optimum growth performance. Similarly, the dietary level of 3200 kcal ME/ Kg and (20.5-21) % CP should be offered at the finisher (3-6 wks) stages. Birds should also provide an appropriate amount of feed in every wks as mentioned by (Das, 2004). Daily feed intake recorded in the study of control feeding & choice feeding of adult quail was 24.92 gm in control feeding & 24.38 gm in the case of choice feeding (Canogullare *et al*., 2004). Adult Japanese quail eat between 14 to 18 gms of feed per day (Sakunthala *et al*., 2010). In an experimental study, Rahman *et al*., 2010 reported that average daily feed intake of Japanese quails was increased with increasing dietary CP level. Here, although the feed intake (FI) from 4th - 6th wks were 20 gm average, in (0-1) wks it was 2 gm/bird only where, the FI value in 1st wk does not support the recommendation by (Das, 2004) reported that Feed intake was higher in broiler quails than in egg- type quails due to their higher body weight (140-150 gm) as compared to that of egg-type quails (120-140 gm). However, feed intake increased with advancement in age and ranged from 3.1 gm in week 1 to 15.2 gm at the 6th week of age in a study by (Dauda *et al*., 2014). In the study, the average daily gain (ADG) and live weight gain (LWG) in 1st wk. was 1.43 gm & 10 gm respectively and recorded maximum LWG of 25 gm and ADG of 3.57 gm in between (3-5) wks of age. The marketing age was (40-45) days after gaining 130 gm body weight. The average daily gain & live weight gain increased with chronological age up to 5 wks. The study showed that, the Avg. FCR (4.55) was found much higher than the standard FCR value 3:1 for broilers (Das, 2004). Earlier, in a study by Dauda *et al*., 2014 feed conversion ratio estimated 3.01:1 at week 2 resembling my findings (3.1:1) in the same age and 7.08:1 at week 6, which was slightly higher in contrast to my study. Here, feed conversion ratio increased gradually from the initial stage of life (1.4) up to 6th weeks (6.37) of age. The higher FCR may be due to lower intake of energy and CP in the regular feeds and also due to less feed intake (<3 gm) in the early stage supporting the statement of (Hashanuzzaman, 2013). However, an improvement in FCR in growing quails with increasing dietary energy level or increasing dietary energy to protein ratio has been mentioned by (Gheisari *et al*., 2011). The mortality rate decreased with age and was relatively higher in 0-2 wks (4.62, 4.03%), with the average of 2.42 % after 6th wks. Naim, 2012 also found relatively higher mortality in 0-2 wks of age with an Avg. of 2.57 % expressing similarity to my result. The study also showed that total profit from per bird production was more than the cost of production. Therefore, quail farming is profitable. The findings on growth and productive performance of Japanese quails in this study suggest that although the housing and hatchery management is favorable to the birds daily feed intake must follow standard Japanese broiler Quail feeding guideline and ready feeds should be checked for ME and CP for their proper maintenance, optimum growth, and production. Above all, Quail rearing can serve as an alternative source of protein to the populace, thus adequate publicity is required to propagate the production of this bird to increase animal protein intake in Bangladesh.

V. Conclusion

Bangladesh has nearly achieved self-sufficiency in staple food. It is now actual time to make quail farming as a major profession for the growth of livelihood and sustainable development. The policy makers should, therefore, take necessary measures which would encourage the development of quail farming. Thus, this farming site will quickly spread all over the country which will make an example for this sub-continent. By combining mental strength, physical effort with few basic technical knowledge one can easily become a successful quail farmer. It is no doubt, that quail farming will become one of the main poultry industries of our country in the near future. The major advantage of quail rearing is its low investment compared to other poultry farming. The management system and performance of the studied farm is surprising. However, the quail farming is profitable and it may be an income generating source by alleviating unemployment burden, enrich our poultry meat supply and thus will meet the daily protein requirement of the nation.

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Hard Tick Distribution of Camels in and Around Galkaio District, Somalia

By Farah Isse, Ahmed Saed & Mahdi Ali
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Abstract- A cross sectional study aimed to identify available tick species, determine the distribution and assess the risk factors for infestation was conducted from March to May 2017 in Galkaio District, central Somalia. Adult ticks were collected from 384 randomly selected camels and identified to species level. Stereomicroscopic investigation were employed. A total of 576 adult tick species were collected from different body parts. The study revealed that there was high tick infestation in the study area with an overall prevalence of 371 (97%). Two tick species from one genera were identified *Hyalommadromedarii* and *Hyalommatrancatum*. Among the species identified in the study area *Hyalommadromedarii* was the most abundant (56.8%) followed by *Hyalommatrancatum* (43.2%). In the present study, the prevalence of all tick species was higher in female animals than male animals but statistically insignificant ($p>0.05$).

Keywords: *camel; hard tick; distribution; prevalence, galkaio.*

GJMR-G Classification: *NLMC Code: WC 900*

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Farah Isse [α], Ahmed Saed [σ] & Mahdi Ali [ρ]

Abstract- A cross sectional study aimed to identify available tick species, determine the distribution and assess the risk factors for infestation was conducted from March to May 2017 in Galkaio District, central Somalia. Adult ticks were collected from 384 randomly selected camels and identified to species level. Stereomicroscopic investigation were employed. A total of 576 adult tick species were collected from different body parts. The study revealed that there was high tick infestation in the study area with an overall prevalence of 371 (97%). Two tick species from one genera were identified *Hyalommadromedarii* and *Hyalommatrancatum.* Among the species identified in the study area *Hyalommadromedarii* was the most abundant (56.8%) followed by *Hyalommatrancatum* (43.2%). In the present study, the prevalence of all tick species was higher in female animals than male animals but statistically insignificant ($p>0.05$). There was no statistically significant variation ($P>0.05$) in prevalence of *Hyalommadromedarii* and *Hyalommatrancatum* between age categories, but body condition and animal origin showed statistically significant variation ($P<0.05$) and good body conditioned animals were highly infested by ticks compared to poor and medium conditioned animals. Majority of *Hyalommadromedarii* and *Hyalommatrancatum* were attached to premium region. There was statistically significant difference between all tick species and ticks 'predilection site ($p < 0.05$). This study showed high rate of hard tick distribution in the study area and appropriate strategic management and further study is recommended to improve the health and performance of Camel.

Keywords: camel; hard tick; distribution; prevalence, galkaio.

I. Introduction

Camels are the most capable animal species in utilizing marginal areas and in survival and production under harsh environmental conditions that are inhospitable to other domestic animals (Schwartz and Dioli, 1992). Many pastoral groups and communities in diverse ecological zones throughout the world are depending on camels for their livelihood. This dependence consists of utilization of camel meat, milk, leather and wool, exportation of live camels, uses as an important sport, tourism resource and use as animals for packing, transport and riding (Snow et al., 1992). Most importantly, in mixed species the camel feed on plants and parts of plants that are not eaten by other conventional livestock due to its size to browse the highest strata. Thus, reducing competition and enhancing complementariness (Wilson, 1998).

It is an important working animal of the arid and semi-arid ecosystem because of its unique adaptive physiological and anatomical characteristics (Rabana et al., 2011). However, camel production is conversely affected by the occurrence of various diseases, inadequate veterinary services and feed shortage (Bekele, 2010). According to the UN Food and Agriculture Organization (FAO, 1977) estimates, there are approximately 15 million dromedary camels in the world, of which 65% are found in the northeast African states of Somalia, Ethiopia, Sudan and Kenya. The Somali community (in Kenya, Somalia and Ethiopia) has the largest population and highest density of camels in the world, and to the same extent this animal also pervades the Somali culture. Historically, the geographical area that is now Somalia may have been a focal point in the introduction and dispersal of the domesticated dromedary (Abokor, 1993).

Various internal and external parasitic diseases have been reported to be the major problems affecting the health, productivity and performance of camel. Ticks are one of the most important parasites among the factors affecting the health, productivity and performance of camels (Anwar and khan, 1998; Bekele, 2010) by transmitting various disease causing agents, and causing blood loss, irritation, inflammation, hypersensitivity and damage to hide and under (Wall and Shearer, 2011; Walker et al., 2003). Ticks are responsible for losses caused by their attachment to animal hides, by the injection of toxins, and/or by the transmission of diseases that reduce yield. The globalization have ensured that hard ticks can travel to new environments and become established with wide success, and as they travel so can their disease (Walker et al., 2003).

Hard ticks are the most important external parasites of camel in the world and can easily constitute a limiting factor to successful stock farming unless appropriate measures are taken to control them (Howell, Walker &Nevill 1978). The geographic distributions of many original ticks are, however, not related with gross climatic conditions of the world, but rather with factors within the microclimate of the vegetation within their distribution variety. The most endemic tick species in Somalia are *Rhipicephaluspulchelus, Hyalomma*

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Dromedarii, Amblyomma Gamma and *Amblyomma Variegatum* (Pegram el at 1981).

Tick damage hide and skin of livestock including camels. There is no available data about hard tick distribution in the study area and the country in general. Due to unavailability of hard tick information this study aimed to identify and to assess distribution of hard ticks in Galkaio district, Somalia.

II. Materials and Methods

a) Study area

This study was conducted in Galkaio district, Puntland state of Somalia. Galkaio is situated in north-central of Somalia, in the heart of Mudug region. Five villages were included in this study which are under Galkaio district namely Taalla'ad, Jeehdin, Tawakal, Dhagahyo'ad and Halabookhad. Mean annual rainfall is 200-300 mm bimodal, average temperature is 32.7 degree Celsius and altitude is 302. (Muchiri, 2007).

b) Study population and study design

The Study animals were consist indigenous breeds of one hump camels managed under pastoral production system, that allows free grazing always mixed with livestock from other villages, and in that the animals move from feed shortage area to a better grazing area. Galkaio district was purposively selected based on camel population, accessibility and convenience. Cross sectional study was conducted from February to May to estimate distribution of camel hard ticks among the one humped camel herds kept under pastoral management system in Galkaio district.

c) Sample size determination

The desired sample size was determined by assuming 50% expected prevalence of tick distribution at 95% confidence interval and 5% absolute precision. Therefore, the relevant formula for the desired sample size was based on Thrusfield (2005).

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

Where; n = required sample size
Pexp = expected prevalence and
d^2 = desired absolute precision.
Z = constant from normal distribution table at a given confidence level. The calculated sample size was 384.

d) Hard tick collection and laboratory examination

General physical examination was conducted on each camel. Information regarding to age, sex and body condition were recorded in careful manner. The age and body condition of camels were determined based on their dentition and hump structure as described by (Schwartz and Dioli, 1992; CACIA, 1995). After proper restraining hard ticks were collected from tick loving soft areas of the skin such as ear, legs, tail, under tail, around anus, nose, perineum, neck and eyes using hand and steel forceps. The ticks from each animal were separately stored in 35% formalin in a single labeled bottle.

The collected ticks were identified to species level at Parasitology Laboratory of Faculty of Veterinary Medicine in Red Sea University using stereomicroscope. Identification was done using standards recommended by (Hoogstraal, 1956; Walker et al. 2003).

e) Data analysis

All collected data were entered to MS excel sheet and analyzed by using SPSS version 20. The distribution and hypothesized risk factors like age, sex, origin, predilection site and body condition was related using chi-square test (x^2) and $p<0.05$ were considered as statistically significant in all cases.

III. Results

This study was performed in February to May 2017 in 5 villages of Galkaio district, namely; Taalla'ad, Jeehdin, Tawakal, Dhagahyo'ad and Halabookhad. Out of 384 examined camels, 371 (97%) were harboring different species of hard ticks. Total of 576 adult ticks collected, 327 (56.8%) were H. *dromedarii* spp. and 249 (43.2%) were H. *tranctum*spp. Table (1) shows sex distribution of the identified ticks. *HyalommaDromedarii* male was 263(45.7%), *HyalommaDromedarii* female was 64(11.1%), *Hyalommatrancatum* male was 84(14.6%) and *Hyalommatrnacatum* female was 156(28.5%).

Table 1: Distribution and sex category of adult tick species in Galkaio district

Tick species	Male	Female	Total	Percentage dist.
Hyalommadromedarii	263(45.7%)	64(11.1%)	327	56.8%
Hyalommatrancatum	84(14.6%)	156(28.5%)	249	43.2%
Total	347(60.3%)	229(39.7%)	576	100.0%

Table (2) shows tick burden compared with age, sex and body condition categories. Only body condition score showed statistically significant difference ($P<0.05$) where sex and age did not display any significance. Good body conditioned animals showed high burden of both *HyalommaDromedarii* and *Hyalommatrnacatum* compared to those having poor and medium body condition.

Table 2: Tick burden with in sex, age and body condition

	sex		Age		Body condition		
	Male	Female	≤ 5 yrs	> 5 yrs	Poor	Medium	Good
H. dromadarii	46 (8%)	28 (4.9%)	66 (11.5%)	261 (453%)	7 (1.2%)	42 (7.3%)	278 (48.3%)
H. trancatum	281 (48.8%)	221 (38.4%)	42 (7.3%)	207 (35.9%)	4 (0.7%)	10 (1.7%)	235 (40.8%)
Total	74 (12.8%)	502 (87.2%)	327 (56.8%)	468 (81.2%)	11 (1.9%)	52 (9.0%)	513 (89.1%)
P-Value, Chi square	0.316, 1.006		0.312, 1.020		0.001, 13.805		

Hard ticks collected from nine different sites. 31% of the ticks were collected from premium region, 15.8% from the ear, 11.6 from tail, 11.45% from the anus, 10.4% from the nose, 7.8% from the eyes, 5.6% where from under the tail, 3.6% from the neck and the least area was tail with 2.25% (Table.3).

Table 3: Distribution of different tick species in different body parts of animal

Attachment site	Tick species		
	H. dromedarii	*H. tracatum*	Percentage
Ear	58	33	15.8%
Eyes	23	22	7.8%
Nose	35	25	10.4%
Neck	15	4	3.29%
Perineum	104	79	31.8%
Leg	28	39	11.6%
Tail	9	4	2.25%
Under tail	24	8	5.7%
Around anus	31	35	11.45%

Different villages showed statistically significant difference of hard tick distribution ($P<0.05$). Peak number (36.76%) of the hard ticks were collected from Halabookhad village, followed by Dhagaxyo'ad (24%), Tawakal (22.74%), Jeehdin (9%), lastly Taala'ad with smallest number (8.5%).

Table 4: Tick distribution in different Villages of Galkaio district

Area	*Hyalommadromedarii*	*Hyalommatrancatum*	Total	Percentage	Chi square	P –value
Taala'ad	39	10	49	8.5%	26.477	0.000
Jeehdin	31	21	52	9%		
Tawakal	85	46	131	22.74%		
Dhagahyo'ad	58	80	138	24%		
Halabookhad	114	92	206	35.76%		
Total	327	249	576	100%		

IV. Discussion

In this study only two species of hard ticks were identified namely *Hyalommadromedarii* and *Hyalommatrancatum* contrarily other researchers found more hard tick generas and species (Samere *et al.,* 2014; Ayele T. and Mohammed M. 2013; Mohsen *et al*., 2013; van Straten, Jongejan 1993). *Hyalommadromedarii* was the most abundant hard tick in the study area with proportion of 56.8%, this is quite higher when compared to a distribution reported from northern part of Ethiopia (42.7%) by Samere, *et al.,* 2014 and much higher in another research done by (Ayele T. and Mohammed M. 2013) in Dire Dawa town (26.85%). Nonetheless this result is much lower than the studies from other researchers in Iran (Mohsen *et al*., 2013) who reported 90.7% and van Straten Jongejan 1993 in Egypt who as well reported 95.6%.*Hyalommatrancatum*was found to be the second ample hard tick in the area (43.2%) which disagrees and much higher in other researchers (Ayele T. and Mohammed M. 2013) in Dire Dawa whom reported 7.19% and (Samere, *et al.,* 2014) in Tigray region of Ethiopia whom also found 8.9%. this great variation of tick burden in different countries could be climatic difference, production system factors, use of acaracide and Ivermectin, seasonal availability is also another important factor, this study was done in a dry season and the situation in the rainy season is unknown.

Both age and sex categories of the host in relation with the hard tick distribution was statistically not significant ($P<0.05$). In sex it disagrees with (Ayele T. and Mohammed M. 2013) who found that female hosts are affected more than male and the possible reason could be female camels reared for milk in pastoral areas

can reduce the number of examined male camels. This study also clearly figured out that tick infestation in adult (> 5 yrs.) was almost similar to that of the young (< 5 yrs.) which is almost comparable with that of Eyerusalem, 2008. This might be due to young animals mostly dwelling around the home and have access to contact with other species of animals; therefore, the chance of getting tick infestation is almost similar to that of adult camels.

According to the body condition of host animals, this study showed statistically significant difference ($P<0.05$) between the good, medium and poor body conditioned animals. Good body conditioned animals where highly affected compared to poor and medium conditioned animals. This result is contrary to that of (Ayele T. and Mohammed M. 2013) and (Eyerusalem, 2008) both found that poor body conditioned animals are much affected. This could be that good conditioned animals attract ticks due to accumulation of fat and skin of the good conditioned animals is much softer then poor and medium conditioned animals.

V. Conclusions

The result of this study indicates that ticks are wide spread throughout the study area and animals are infested with different species of tick. It is well known that ticks cause severe economic loss either by transmitting a variety of disease or by damage to hide and skin. The important and abundant tick species investigated in the study area were*, Hyallomadromedarii and Hyalommatrancatum and the prevalence was* 56.8% and 43.2%. The study indicated that there was high tick prevalence in the study area.

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Conflicts of Interest: The authors declare no conflict of interest.

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Investigation of the Presence of Different Animal Species within Processed Meat and Meat Products using PCR Procedures and Development of Risk Models based on Consumer Health

By Harun Cerit & Ayse Z. Aroguz
Istanbul University

Summary- Fraudulent imitation and adultery of meat and meat products are fooling the consumers, jeopardizing their health, economical situation and potentially causing harm to religious beliefs. The aim of this project was to search for the existence of such fraudulent imitations and adulteries within processed meat products across different sale points (them being markets) found within 11 municipalities of the Marmara Regionusing PCR procedures. According to the findings gathered during the study, 25 of the collected samples (4.54%) contained poultry DNA, 5 of them (0.90%) contained house fly DNA, 6 of them (1.09%) contained sheep DNA, 2 of them (0.36%) contained cockroach DNA, 2 of them (0.36%) contained horse DNA and 4 of them (in chicken sausages/ 0.72%) contained bovine DNA as foreign species. Again our findings showed that, for samples not suitable for human consumption in relation to their Escherichia coli parameter of total coliform bacteria quantity, highest value was found within beef salami and chicken sausage.

Keywords: meat, meat products, adultery, fraud, pathogens, DNA typing, PCR.

GJMR-G Classification: NLMC Code: QW 70

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Harun Cerit [α] & Ayse Z. Aroguz [σ]

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Keywords: meat, meat products, adultery, fraud, pathogens, DNA typing, PCR.

I. Introduction

Having access to sufficient quantities of food which is produced in a high quality and trustworthy environment while guaranteeing its safety is a fundamental right for the well physical, mental and psychological development of every human being. Even though the application of food safety is one of the most prioritized policies of the European Union (EU), when it comes to the management of the quality of meat and meat products throughout the whole process starting at the barn, ending on the table, solely the labeled information cannot actually guarantee the food safety (1). That's why, it's vital for meat and meat products to be checked in order to determine from which animals they are produced from, to validate the labeled information found on their packages, to detect substances that can harm the consumer health(carcass products high in BSE, undesirable fats, illegal addition of animal species into meat products, insect and rodent contamination of the same products because of the lack of proper hygiene, etc.). In the Notification entitled "Instructions for the application of the Notification on meat and meat products" issued in our country on February 2013 (2), the following statement can be found: "Species, as mentioned in its corresponding article in the Notification, can only be mixed with themselves. For example, chicken-turkey mixture or a calf-sheep mixture." which has thus rendered illegal to mix different animal species in meat and meat products.

Listeria monocytogenes is an important gram-positive, facultative anaerobic microorganism that is being frequently isolated from nearly all food products and that can cause sporadic and epidemic infections. As it can live and thrive in active soil, it can survive in vegetables, dairy and dairy products, potable or waste water, as well as poultry meat and poultry products. In turn, this infectious agent can be transmitted to humans or other animals via fecal-oral route (3). Main causes of human listeriosis are pasteurized/non-pasteurized dairy and dairy products, meat and meat products, poultry meat and poultry products, poultry fodders, vegetables and contaminated waters (4). Patients with suppressed immune system because of diseases such as HIV, hepatitis or cancer, as well as pediatric and geriatric cases along with pregnant women form the primary risk group for the human listeriosis.

Escherichia coli are aerobic/facultative aerobic microorganisms that can be found within the normal flora of the intestinal system of humans and warm-blooded animals. Even though some coliform groups as well as some *E.coli* strains are harmless, these

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aforementioned agents can also possess pathogenic strains. Total coliform bacteria quantity and the presence of *E.coli* is being reported as an indicator of poor hygienic conditions and fecal contaminations (5). Among the main sources of the contamination of aforementioned microorganism groups are; willingly or unwillingly introducing foreign animal tissues that weren't subjected to the obligatory food safety inspection system, tissues that come from the same species but shouldn't be put in meat products (such as renal or lung tissues), toilets with poor hygienic conditions and the end consumer or the food production personnel who don't follow the hygienic necessities after using the toilet.

When meat and meat products, all having an important role in human consumption, are acquired from healthy animals and processed within appropriate conditions, they are regarded as microbiologically safe. Unless necessary precautions are taken during elevation and slaughtering, meat and meat products might end up causing serious health problems among the consumers. Also, fraudulent imitation and adulteration done in order to decrease cost and thus increase profit margin may lead to the introduction of undesirable animal species (horse, donkey, pig, etc.) in meat and meat products. Furthermore, in establishments processing than one meat product (mainly establishments processing cattle and poultry meats under the same roof), tissues belonging to foreign animals might unwillingly get introduced into these processed meats. Moreover, in some cases of adultery of meat and meat products, unwanted tissues not coming from a foreign animal (nail, kidney, brain, lung, etc.) might be added willingly or somehow end up unwillingly contaminating these said products.

The aim of this project is to search for the existence of fraudulent imitation and adultery within processed meat products across different sale points (them being markets) found within 11 municipalities of the Marmara Region (Edirne, Tekirdağ, Kırklareli, İstanbul, Kocaeli, Yalova, Sakarya, Bursa, Bilecik, Balıkesir and Çanakkale) using PCR procedures.

II. Materials and Methods

a) Sample collection

Over the course of this study, across 11 different municipalities of the Marmara Region (Edirne, Tekirdağ, Kırklareli, İstanbul, Kocaeli, Yalova, Sakarya, Bursa, Bilecik, Balıkesir and Çanakkale), from a total of 5 different meat product types (Beef Salami, Beef Garlic Flavoured Sausage, Chicken Sausage, Bresaola, Braised Meat), a grand sum of 550 samples were gathered (50 from each municipality, in each municipality 10 samples for each meat product type). The gathered samples, which were put in transportation boxes that were rendered sterile according to the rules of asepsis and antisepsis, were brought to our university inside transportation containers with 4°C inner temperature. Samples were kept at -20°C until the analyses. Detailed information on collected samples is shown on Table 1.

Table 1: Detailed information on the sample collection program.

Tablo 1: Örnek toplama programı hakkında detaylı bilgi

REGION	MUNICIPALITY	SAMPLE NAME	SALE POINT	TOTAL SAMPLE COUNT
Marmara	Edirne	10 of each salami, garlic flavoured sausage, sausage, Bresaola and braised meat sample, 50 samples from each municipality and from all of the municipalities, a total of 550 samples.	Markets	50
Marmara	Tekirdağ			50
Marmara	Kırklareli			50
Marmara	İstanbul			50
Marmara	Kocaeli			50
Marmara	Yalova			50
Marmara	Sakarya			50
Marmara	Bursa			50
Marmara	Bilecik			50
Marmara	Balıkesir			50
Marmara	Çanakkale			50
TOTAL				550

b) Microbiological Analyses

- *E. coli:* From swabsticks containing the growth medium which comes from where the sampling was made, passages have been made, in accordance with asepsis conditions, into TBX agar growth medium that was previously prepared and poured into petri dishes. The petri dishes were then incubated for 24 hours in 44°C. Following this incubation period, typical colonies that formed were counted. About 98% of *E. coli* serotypes contain the enzyme β-D glucuronidase. This

enzyme, rarely found in other bacteria, breaks down its substrate Methylumbilliferyl- β-D glucuronide (MUG), products of which are fluorescent under UV light (6). That's why, while swabbing, a chromogenic growth medium containing MUG (besides TBX Agar) was also used.

- *L. monocytogenes:* 25 gr of the sample was put in 225 ml BLEB, incubated for 4 hours in 30C. Next, selective agents and 25mg/L natamycin were added to the medium and incubated for 48 hours in 35C. At the end of the 48th hour of the incubation, a passage has been made to CLAB, which is one of the numerous selective agars for *L. monocytogenes*. Cultures were purified by making passages from colonies suspected of containing *List. spp.* to a TSA containing Yeast Extract. Suspect isolates were identified according to their following properties: gram staining, catalase, movement, dextrose, maltose, rhamnose, mannitol and xylose fermentation, aesculin fermentation, nitrate oxidation. Furthermore, CAMP test was made using S. aureus in order to detect whether the isolates possess the CAMP factor (6).

c) DNA Extraction

The DNAs of all the isolates were extracted via the commercial DNA extraction kit, in accordance with the kit protocol. The extracts were stored in -20°C to be used later on as target DNA during PCR procedures.

d) PCR

On Table 2 is shown species specific primer sets used during the PCR procedure.

Table 2: Species specific primer sets used during the PCR procedure (7-11).

Tablo 2: PCR prosedüründe kullanılan türe özgü primer setleri (7-11)

Species Name	Primer Direction	Sequence
Pork	Forward	5'-CTTGCAAATCCTAACAGGCCTG-3'
Pork	Reverse	5'-CGTTTGCATGTAGATAGCGAATAAC-3'
Poultry	Forward	5'-TCTGGGCTTAACTCTCATACTCACC-3'
Poultry	Reverse	5'-GGTTACTAGTGGGTTTGCTGGG-3'
Cattle	Forward	5'-CCCGATTCTTCGCTTTCCAT-3'
Cattle	Reverse	5'-CTACGTCTGAGGAAATTCCTGTTG-3'
Sheep	Forward	5'-CCTTATTACACCATTAAAGACATCCTAAGGT-3'
Sheep	Reverse	5'-GGGTCTCCAGTAAGTCAGGC-3'
Horse	Forward	5'-CAGCCAATGCGTATTCGTACTCT-3'
Horse	Reverse	GTGTTCCACTGGCTGTCCG-3'
Donkey	Forward	5'-CATCCTACTAACTATAGCCGTGCTA-3'
Donkey	Reverse	5'-CAGTGTTGGGTTGTACACTAAGATG-3'
Cockroach	Specific	5'-GTGGAACTGGCTGGACTT-3'
Cockroach	Specific	5'-GAGACATGTGTAATCAGG-3'
House fly	Specific	5'-CACAAGGATCGCTTCAAG-
House fly	Specific	5'-TGTTGGTATCATTGTCGG-3'

Besides species specific primers, PCR procedures have been made on colonies that were microbiologically isolated and evaluated as suspicious in order to identify (i) *E. coli*, one of the most important food pathogen which jeopardizes consumer health, (ii) *L. monocytogenes*, which can be isolated and identified in 7 to 10 days and also can be hard to identify due to all the different chemical tests made during its identification process. These two aforementioned food pathogens and the primer sets we have used for them can be found on Table 3.

Table 3: Primer sets designed according to the different serotypes used in our study and their properties (10, 12-16).

Tablo 3: Çalışmamızda kullanılan farklı serotipler için hazırlanmış primer setleri ve onların özellikleri (10, 12-16)

Primer No	Sequence (5' – 3')	Target Gene / Amp (bp)	Target microorganism
1	GCTGATTTAAGAGATAGAGGAACA	*actA* / 827	*L. monocytogenes*
2	TTATGTGGTTATTTGCTGTC	*actA* / 827	*L. monocytogenes*
3	CAATTTTCGTGTCCCCTTCG	*23S* / 450	*Escherichia coli*
4	GTTAATGATAGTGTGTCGAAAC	*23S* / 450	*Escherichia coli*

The real-time PCR procedure is as follows:

- 50-100 mg of tissue from samples were sliced or crushed to bits and then were put in microcentrifuge tubes.
- 400 μL of SH solution was added into the samples in microcentrifuge tubes and mixed via vortex.
- To the homogeneous-looking mixture were added 8 μL of proteinase K and 40 μL of SLS solution. After mixing well enough, the mixture was kept under 60°C for 2 hours for the cells to open up.
- Following the 60°C incubation, 300 μL of SP solution was added to the mixture which was then stirred via vortex for 30 seconds.
- The mixture was centrifuged at 12000 rpm for 30 minutes. The supernatant was then moved into an empty tube.
- 500 μL of isopropanol was added to the supernatant, stirred via vortex and then incubated under -20°C for 1 hour.
- Following the incubation, the mixture was centrifuged at 12000 rpm for 20 minutes. The supernatant was thrown away.
- After adding 0.5 mL of ethanol to the pellet remaining at the bottom of the tube, the pellet was dissolved by gently vortexing and then centrifuging at 12000 rpm for 5 mins.
- The ethanol was thrown away and the sedimenting DNA was left to dry.
- With the ethanol completely evaporated, on the remaining pellet was added 150 μL of SE solution and then it was kept overnight for the DNA to dissolve under room temperature.
- The dissolved DNA was measured via UV spectrophotometer and was diluted to reach a concentration of 50 ng/μL.

Afterwards, the following heat cycle protocol was executed,

1. 95°C for 10 minutes
2. 95°C for 10 seconds
3. 60°C for 15 seconds

2nd and 3rd steps were repeated 35 times in a cycle.

III. Results

a) Foreign species identification and detection of fraud and adultery

In this study, a total of 550 samples of processed meat was collected from different sale points (supermarkets, markets, local bazaars etc. / being local brands, if present), found within 11 municipalities of Marmara Region (Edirne, Tekirdağ, Kırklareli, İstanbul, Kocaeli, Yalova, Sakarya, Bursa, Bilecik, Balıkesir and Çanakkale) and from these collected samples, existence of voluntary and involuntary (in establishments processing meats of different species, improper equipment use/surfaces/personnel borne improper procedure applications…) fraud and adultery was researched using PCR procedures. These aforementioned fraud and adultery applications were analyzed by taking into account 8 different animal species (pork, poultry, cattle, sheep, horse, donkey, cockroach and house fly). Details concerning the collected samples and findings are shown on Table 4.

Table 4: Detailed information on sample collection program.

Tablo 4: Örnek toplama programı hakkında detaylı bilgiler.

REGION	MUNICIPALITY	SAMPLE NAME	SALE POINT	TOTAL SAMPLE COUNT	POSITIVE SAMPLE COUNT	FOREIGN ANIMAL SPECIES
Marmara	Edirne	Beef Salami	Market	10	1 (10%)	Poultry
		Beef Garlic Flavoured Sausage	Market	10	2 (20%)	Poultry [×2]
		Chicken Sausage	Market	10	1 (10%)	House Fly
		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	1 (10%)	Sheep

Marmara	Tekirdağ	Beef Salami	Market	10	2 (20%)	Poultry [×2]
		Beef Garlic Flavoured Sausage	Market	10	0	-----
		Chicken Sausage	Market	10	1 (10%)	Cockroach
		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	1 (10%)	Poultry
Marmara	Kırklareli	Beef Salami	Market	10	0	-----
		Beef Garlic Flavoured Sausage	Market	10	2 (20%)	House Fly,Poultry
		Chicken Sausage	Market	10	1 (10%)	Sheep
		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	0	-----
Marmara	İstanbul	Beef Salami	Market	10	2 (20%)	Poultry, House Fly
		Beef Garlic Flavoured Sausage	Market	10	3 (30%)	Sheep, Poultry [×2]
		Chicken Sausage	Market	10	1 (10%)	Horse
		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	1 (10%)	Cockroach
Marmara	Kocaeli	Beef Salami	Market	10	0	-----
		Beef Garlic Flavoured Sausage	Market	10	1 (10%)	Sheep
		Chicken Sausage	Market	10	1 (10%)	Cattle
		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	0	-----
Marmara	Yalova	Beef Salami	Market	10	0	-----
		Beef Garlic Flavoured Sausage	Market	10	0	-----
		Chicken Sausage	Market	10	0	-----
		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	1 (10%)	Poultry
Marmara	Sakarya	Beef Salami	Market	10	1 (10%)	Poultry
		Beef Garlic Flavoured Sausage	Market	10	2 (10%)	Poultry [×2]
		Chicken Sausage	Market	10	0	-----
		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	0	-----
Marmara	Bursa	Beef Salami	Market	10	3 (30%)	Poultry [×3]
		Beef Garlic Flavoured Sausage	Market	10	2 (20%)	Poultry [×2]
		Chicken Sausage	Market	10	1 (10%)	Cattle
		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	1 (10%)	House Fly
Marmara	Bilecik	Beef Salami	Market	10	2	Sheep
		Beef Garlic Flavoured Sausage	Market	10	0	-----
		Chicken Sausage	Market	10	1	Cattle
		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	1	Poultry
Marmara	Balıkesir	Beef Salami	Market	10	1 (10%)	Poultry
		Beef Garlic Flavoured Sausage	Market	10	2 (20%)	Sheep, Poultry
		Chicken Sausage	Market	10	3 (30%)	Cattle, House Fly [×2]

		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	1 (10%)	Poultry
Marmara	Çanakkale	Beef Salami	Market	10	1	Poultry
		Beef Garlic Flavoured Sausage	Market	10	2	Poultry
		Chicken Sausage	Market	10	1	Horse
		Bresaola	Market	10	0	-----
		Braised Beef	Market	10	1	Poultry
TOTAL				550	48 (8.72%)	

- Brandless or local brand products make up 100% of the adulterated and fraudulent samples.
- None of the samples (0%) were contaminated with pork and donkey meat.
- 25 products (4.54%) had poultry DNA as foreign species.
- 5 products (0.90%) had house fly DNA as foreign species.
- 6 products (1.09%) had sheep DNA as foreign species.
- 2 products (0.36%) had cockroach DNA as foreign species.
- 2 products (0.36%) had horse DNA as foreign species.
- 4 products (0.72%) had cattle DNA as foreign species.

b) Microbiological analyses

All of our samples were analyzed according to 2 food pathogens (*Escherichia coli* and *Listeria monocytogenes*) which can seriously harm consumer health. Table 5 shows analysis details of the collected samples during the study, in relation with the chosen food pathogens.

Table 5: Analysis details of the collected samples in relation with the chosen food pathogens.

Tablo 5: Seçilmiş gıda patojenleri bakımından toplanmış örneklerin analiz bilgileri.

Microbiological parameter	Sample name	Positive sample count (from adulterated/ fraudulent samples)	Positive sample count (from unadulterated/non-fraudulent samples)	Positive sample count (total)
Escherichia coli	Beef salami	12 / 13 (92.3%)	21 / 97 (21.6%)	33 / 110 (30%)
	Beef Garlic Flavoured Sausage	14 / 16 (87.5%)	19 / 94 (20.2%)	35 / 110 (31.8%)
	Chicken Sausage	8 / 11 (72.7%)	9 / 99 (9.1%)	17 / 110 (15.5%)
	Bresaola	0 (0%)	0 (0%)	0 (0%)
	Braised Beef	4 / 8 (50%)	13 / 102 (12.7%)	17 / 110 (15.5%)
Listeria monocytogenes	Beef Salami	0 (0%)	0 (0%)	0 (0%)
	Beef Garlic Flavoured Sausage	0 (0%)	0 (0%)	0 (0%)
	Chicken Sausage	0 (0%)	0 (0%)	0 (0%)
	Bresaola	0 (0%)	0 (0%)	0 (0%)
	Braised Beef	0 (0%)	0 (0%)	0 (0%)

Table 6: Statistical analysis results of the PCR results obtained in our study, in accordance with the ISO 16140 evaluation parameters.

Tablo 6: Çalışmamızda elde edilen PCR sonuçlarının ISO 16140 değerlendirme parametrelerine göre istatistiksel analiz sonuçları

	Relative accuracy (%)	Relative specifity (%)	Relative sensitivity (%)	False negative ratio (%)	False positive Ratio (%)
E. coli	88.90	97.34	97.62	1.18	0.0
L. monocytogenes	-----	-----	-----	-----	-----

- As L. monocytogenes wasn't found in any of the samples, it wasn't evaluated.

Table 7: DNA nano-drop measure details of some of the inspected samples which are positive for foreign species contamination (showing one example for each sample containing foreign species).

Tablo 7: İncelenen örneklerden yabancı tür tespiti pozitif olan örneklerden bazılarının (her bir farklı yabancı tür içeren örnekten birer adet numunenin gösterilmesi olarak) DNA nano-drop ölçüm detayları

DNA type	ng/µl	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
Horse	1822.46	36.952	18. 691	1.76	1.82	50.00	230	19.002	3.499
Sheep	2786.21	51.203	24.266	1.89	1.92	50.00	230	26.782	4.001
Poultry	3893.03	72.089	37.668	2.07	1.99	50.00	230	36.988	3.600
Cattle	3055.92	62.580	31.352	2.09	2.01	50.00	230	31.616	5.900
House Fly	3211.87	66.873	34.002	2.13	1.87	50.00	230	32.043	4.999
Cockroach	3100.21	65.660	33.992	2.12	1.43	50.00	230	31.234	5.203
Pork (negative for all the samples)	3343.455	71.650	37.231	1.72	1.89	50.00	230	36.902	5.453

- *Even though no pork DNA was found within any of the samples of our study, pork nano-drop measures were also included since it is important in our country for religious reasons.*

Table 8: Evaluation of group differences between adulterated and unadulterated products in relation with their negative effects on consumer health, using microbiological parameters (According to Pearson Chi Square method). The results obtained on this table shows the group differences between the total number of confirmed unadulterated products and adulterated meat products.

Tablo 8: Tağşiş yapılan ve tağşiş yapılmayan et ürünleri arasındaki grup farklılıklarının tüketici sağlığını riske etmesi açısından analiz edilen mikrobiyolojik parametreler için sınanması (Pearson Chi Square yöntemine göre). Tablodaki sonuçlar tağşiş yapılmadığı tespit edilmiş tüm örneklerin toplamı ve tağşiş yapılmış et ürünleri arasındaki grup farklılıklarını yansıtmaktadır.

	Microbiological parameter	Related variable	Value	Asymp. Sig
Pearson Chi Sq	*Escherichia coli*	Adultered samples / All of the unadultered samples	9.653	.000
Pearson Chi Sq	*Listeria monocyotgenes*	Adultered samples / All of the unadultered samples	-----	-----

- The microbiological load on adulterated samples is statistically significantly higher than it is on unadulterated samples. For every group (them being adulterated and unadulterated samples), group differences were made according to the samples that are positive on microbiological parameter. For these microbiological parameters, samples which didn't show any growth were omitted.
- As L. monocytogenes wasn't found in any of the samples, it wasn't evaluated in this table.

Table 9: Evaluation of group differences between adulterated and unadulterated products in relation with their negative effects on consumer health, using microbiological parameters (According to Pearson Chi Square method). The results obtained on this table shows within the products not suitable for human consumption the group differences between adulterated and unadulterated meat products.

Tablo 9: Tağşiş yapılan ve tağşiş yapılmayan et ürünleri arasındaki grup farklılıklarının tüketici sağlığını riske etmesi açısından analiz edilen mikrobiyolojik parametreler için sınanması (Pearson Chi Square yöntemine göre). Tablodaki sonuçlar insan tüketimine uygun olmayan tüm örneklerin toplamı içerisinden tağşiş yapılan ve tağşiş yapılmayan ürünler arasındaki grup farklılıklarının sısnanmasını yansıtmaktadır.

	Microbiological parameter	Related variable	Value	Asymp. Sig
Pearson Chi Sq	*Escherichia coli*	Adultered samples / Unadultered samples	11.562	.000
Pearson Chi Sq	*Listeria monocyotgenes*	Adultered samples / Unadultered samples	-----	-----

- Values marked with red are statistically significant since they are lower than $P<0.005$.
- In values marked with red, the positive relationship correlation for adulterated products is positive. Adulterated meat products, compared to unadulterated meat products, are significantly harmful to the consumer health when microbiological parameters are taken into account.
- For every group (them being adulterated and unadulterated samples), group differences were made according to the samples that are positive on microbiological parameter. For these microbiological parameters, samples that didn't show any growth were omitted.
- As L. monocytogenes wasn't found in any of the samples, it wasn't evaluated in this table.

IV. Discussion

Even though the application of food safety is one of the most prioritized policies of the European Union (EU), when it comes to the management of the quality of meat and meat products throughout the whole process starting at the barn, ending on the table, solely the labeled information cannot guarantee the food safety (17, 18). Fraudulent imitation and adultery of meat and meat products are fooling the consumers, jeopardizing their health, economical situation and potentially causing harm to religious beliefs.

According to the findings gathered during the study, 25 of the collected samples (4.54%) contained poultry DNA, 5 of them (0.90%) contained house fly DNA, 6 of them (1.09%) contained sheep DNA, 2 of them (0.36%) contained cockroach DNA, 2 of them (0.36%) contained horse DNA and 4 of them (in chicken sausages / 0.72%) contained bovine DNA as foreign species. No pork DNA was found in the collected samples. 100% of the adulterated or fraudulent samples are made up from openly sold brandless or local brand products. Adultery and fraudulent imitation was not found in samples collected from brands producing and marketing nationwide or internationally. According to the results, it could be seen as a high probability that firms producing meat products either without any brand or under a local brand license are processing more than one species of animals and end up accidentally mixing up tissues belonging to different animal species. Another possible cause would be the staff working at the aforementioned firms lacking anytraining on proper hygiene which leads to the mechanical contamination of meat products due to the lack of training or attention. Another possibility is the thought that these aforementioned firms are willfully executing adultery and fraudulent imitation in order to make profits.

In one study conducted in the United States, Hsieh et al. (19) reported that in 90% of the minced meat samples contained poultry meat introduced willingly or unwillingly and therefore adulterated meat was being marketed. Türkyılmaz et al. (20) found that within 121 meat and meat products analyzed using AGID method, 3 of them (2.5%) contained equidae meat, 2 of them (1.7%) contained pork meat. As a result of the study of 223 samples, Türk et al. (21) has found that 16 of the samples (7.1%) contained pork meat, 12 of them (5.3%) contained equidae meat and 6 of them (2.6%) contained a mixture of pork-equidae meat. Within 410 samples of meat and meat products acquired in Bursa and Istanbul, Günşen et al. (22) has found, using ELISA method, that 14 of these samples (3.41%) contained horse meat. Results in our study are lower in relation to the detected species when compared to the aforementioned studies. In addition to the results obtained by these previously mentioned researchers, in our study, in 2 samples (0.36%) cockroach DNA and in 5 samples (0.90%) house fly DNA was detected. The presence of cockroach and house fly DNA in results makes us think that in their corresponding manufacturers, poor hygiene conditions are present, food safety regulations are not applied and these manufacturers are inefficient when it comes to the general cleaning, disinfection, staff hygiene and self-care.

Throughout literatures in our county and around the world, the causes for the acquisition of different results on this subject would be the different physical

conditions of the sales points along with presence or lack of the application of food safety protocols, deficiencies in processing and/or usage of the same equipment for establishments processing more than one species of animal meat, intentional or unintentional application of adultery and fraudulent imitation and staff's lack of knowledge on applied procedures. It's thought that, at the root of the results obtained in our study lies the deficiencies of the inspection of food safety systems as well as staff's lack of knowledge.

According to the results obtained in this study, 102 of the samples (18.5%) were found to be positive for *E. coli* and therefore not suitable for human consumption. One of the most remarkable findings in our study would be the fact that a significant number of *E. coli* positive samples come from those which were adulterated and fraudulent (Table 5). As explained above, in establishments having really poor hygienic conditions (most of them producing adulterated and fraudulent products), our results show that poor toilet hygiene can also be present. Another possible risk factor is that personnel infected with *E. coli* can easily transmit the bacteria to their surroundings (places such as homes, public transportations, public toilets, local bazaars with lots of people in it, malls, cinemas, schools, etc.).

For samples that weren't "suitable for human consumption" according to the *E. coli* parameter, highest value was 3.7×10^4 cob/gr, whereas lowest was 1.2×10^2 cob/gr. According to the results obtained, for samples not suitable for human consumption in relation to their *E. coli* parameter of total coliform bacteria quantity, highest value was found within beef salami (in which poultry DNA was found) and chicken sausage (in which house fly and cockroach DNA was found). There are studies which report that poultry meat does also contain *E. coli*. In a study conducted in Egypt, Abdul-Raouf et al. (23) studied *E. coli* O157:H7 in various foods. In this study, from samples gathered from slaughterhouses, supermarkets and barns, 3 out of 50 samples (6.0%)of minced bovine meat and 2 out of 50 samples (4.0%) of poultry meat contained *E. coli*. In a study conducted by Doyle and Schoeni (24, 25), from samples gathered from cattle, sheep, pork and chicken meat, *E. coli* O157:H7 was tried to be isolated. At the end of the study, *E. coli* O157:H7 was found in 3.7% of cattle meat, 2% of sheep meat, 1.5% of pork meat and 1.5% of chicken meat. The agent was detected in chicken wing samples and again in another study, within chicken nugget samples, *E. coli* O157:H7 serotype was found (24). One of the main reasons of this difference would be that water activity (a_w) in poultry meat is higher when compared to other butchered meats. It is thought that high water activity levels directly influence the total coliform bacteria and *E. coli* parameters. The results also show us that samples containing cockroach and house fly DNA also contain high amounts of *E. coli*. As mentioned in above paragraphs, flies and cockroaches can transmit, as a primary or secondary contamination source, a high quantity of bacteria, parasite, protozoa and virus to its environment by physical contact.

These insects originating mainly from toilets are thought to transmit *E. coli* to meat products mechanically. Another reason for these aforementioned findings would be the deficiencies in application of hygiene protocols within establishments that produce and sell meat products. Even though during our study, neither establishment hygiene nor critical control points (CCP) within establishments were inspected, in establishments from which samples containing high quantity of *E. coli* and total coliform bacteria were gathered, by external inspection, we can conclude that they are lacking minimum hygiene applications. A different reason for this would be the possibility that these previously mentioned high quantities of *E. coli* and total coliform bacteria were already present inpoultry meat.

In our study, *L. monocytogenes* was one of the investigated parameters. Nevertheless, none of the samples contained *L. monocytogenes*.

Another parameter investigated in our study was the difference in potential risks to the consumer between adulterated/fraudulent products and unadulterated/non-fraudulent products. For this reason, a two-way relationship analysis was done using the Pierson Chi Square method. One of the relationship analyses was made to evaluate the relationship analysis between adulterated/fraudulent products and unadulterated/non-fraudulent products. Another relationship analysis was made to investigate the statistical significance between adulterated/fraudulent products and unadulterated/non-fraudulent products both not suitable for human consumption. According to the results obtained in our study, for both of the relationship analyses, statistically significant differences were found on the basis of *E. coli*. For this microbiological parameter which is significant when it comes to the consumer, possible risks were found in favor of adulterated and fraudulent products (among all the products not suitable for human consumption, adulterated and fraudulent ones were found to contain statistically significantly higher quantities of risk factors on the basis of *E. coli*). Since in none of the samples *L. monocytogenes* was detected, relationship analyses were not done on this factor.

In our country and throughout the world, adultery and fraudulent imitation either occurs willfully and illegally in order to increase profits or accidentally, in establishments processing different species of animal meat, by keeping the production of different animal species on the same space or lack of staff training, poorly executed food safety applications or quality management. Especially, adultery and fraudulent imitation done to increase profits brings with itself

serious microbiological risks that can endanger consumer health. Since such willful adultery and fraudulent imitation is executed illegally, inspection and control procedures don't work on them which can create innumerous microbiologically critical control points during processing. Furthermore, no ante-mortem or post-mortem inspections are done on foreign animal borne meats as well as slaughtered animals. Additionally, control over the processes of extraction of internal organs, meat mincing, packaging and transportation remains impossible. Not identifying microbiological, parasitic, chemical risks throughout the whole process of the arrival of meats to customers can end up creating innumerable risk factors. In our study, *L. monocytogenes* was in none of the adulterated or fraudulent meats. When it comes to *E. coli*, it's found in significantly more adulterated/fraudulent meats than unadulterated/non-fraudulent meats. Our findings show significant differences between unadulterated/non-fraudulent products that are not suitable for human consumption and adulterated/fraudulent products, in terms of microbiological risks that can be brought upon the consumer. In the light of these findings, it can be said that adultery and fraudulent imitation can end upseriously jeopardizingthe consumer health.

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Plastination of Major Organs of Miniature Pigs: Alternatives for Educational Purpose

By Jong-In Kim, Young Ah Lee, Jae Won Lee, Yoon Ju Cho, Sang Cheol Kim & Jin Soo Han

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Abstract- Veterinary science have been influenced by the increasing demand for veterinary schools or research facilities in animal welfare. Thus, there have been new approaches to veterinary education based on 'Three Rs' (3Rs; Replacement, Reduction and Refinement). Plastination, one of the new approaches, is certainly potential method in respect of learning tool for gross anatomy.

We performed the plastination of miniature pig's major organs(liver, heart, lung, kidney, and pacreas) for alternative in anatomic instruction. Silicone impregmented method keeps thoroughly dissected specimens from deteriorating and maintained many of the important surrounding structures. After entire process, organs became dry, odorless, and durable specimens that could be handled without gloves and kept without any special storage conditions or care.

Keywords: *plastination, miniature pig, alternative.*

GJMR-G Classification: *NLMC Code: WA 360*

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Plastination of Major Organs of Miniature Pigs: Alternatives for Educational Purpose

Jong-In Kim [α], Young Ah Lee [σ], Jae Won Lee [ρ], Yoon Ju Cho [Ω], Sang Cheol Kim [¥] & Jin Soo Han [§]

Abstract- Veterinary science have been influenced by the increasing demand for veterinary schools or research facilities in animal welfare. Thus, there have been new approaches to veterinary education based on 'Three Rs' (3Rs; Replacement, Reduction and Refinement). Plastination, one of the new approaches, is certainly potential method in respect of learning tool for gross anatomy.

We performed the plastination of miniature pig's major organs(liver, heart, lung, kidney, and pacreas) for alternative in anatomic instruction. Silicone impregmented method keeps thoroughly dissected specimens from deteriorating and maintained many of the important surrounding structures. After entire process, organs became dry, odorless, and durable specimens that could be handled without gloves and kept without any special storage conditions or care.

This study suggests plastinated organs of miniature pig are valuable alternative material of cadaver on anatomic instruction. Furthermore, it is considered that this non-living specimen largely contribute to humane veterinary education and research.

Keywords: plastination, miniature pig, alternative.

I. Introduction

Plastination is the most important technique that enables biological specimens to be completely preserved without deteriorating. Plastination process is consists of slowly replacing tissue fluids with reactive plastics under specialized vacuum.[1] Plastinated specimens are clean, dry, odorless and graspable, therefore, they are convenient to access the interior of the body in any circumstance. [2-4] The potential value of this technique is in the application of a various fields of anatomical specimens for education, in particular, plastination has been considered a most important tool for instruction of gross anatomy.[1] In addition, plastinated specimens are permanently reusable, thus, the demand of real bodies for cadavers could be reduced. Indeed, practical application of plastination has been influenced on research and education areas in many ways.[4]

We performed the plastination of miniature pigs. Miniature pigs have been proposed for suitable candidate source animal for clinical xenotransplantation.[5] Shortages in the availability of suitable human donors have limited the possibilities of being transplanted, which has stimulated interest in the possibility of animal-to-human transplantation.[6] Particularly, Xenotransplantation using miniature pig has been considered that could potentially overcome limitations in organ transplantation resulting from insufficiency of human organs.[7] Unlike domestic pigs, miniature pigs have a great majority in organ size similar to human organ. [8] In addition, Miniature pigs are similar to human in physiological features and provide several breeding, handling, and reproduction-related advantages compared to non-human primates. Hence, miniature pigs are already extensively used in bio-medical research, and it is anticipated that miniature pigs will be more used notably in teaching and research areas, along with advancement of genetically engineering technique. [9]

However, increasing needs of miniature pigs for educational objects have had a strong possibility of leading to sacrifice it in increasing numbers. This problem is not correlated with recent trend in animal use. In recent decades, the use of animals in veterinary education has show a significant tendency to decline. Consideration for laboratory animals, the *Animal Protection Act*, and growing availability of alternative resources have enabled this valuable trend to be proceeded.[10] Also, the use of live animals in education requires a protocol review and permission by Institutional Animal Care and Use Committee (IACUC) for reducing and replacing the sacrificed animals. In several countries including Korea, however, not a few veterinary schools have showed the not enough try to comply with the regulations strictly, and the lack of interests and efforts.[11] By contrast, human medical schools have been utilized outstanding alternative methods such like plastination more effectively.[4] Hence, for more successful fulfillment of 3Rs in veterinary education, feasible alternatives are requested for that supplant animal realistically. Accordingly, we attempted plastination of miniature pigs, growing need for education on which has been required. This study suggests plastinated organs of miniature pig are valuable alternative material of cadaver on anatomic instruction. Furthermore, it is considered that this non-living specimen largely contribute to humane veterinary education and research.

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II. Meterials and Method

Two male miniature pigs (Micro-pig, PWG Genetics Korea) scheduled to be sacrificed at the termination of the other research, were used in this study. Application of the platination process to these miniature pigs was approved by the Institutional Animal Care and Use Committee of Konkuk University. Pigs were deeply anesthetized by Telazol(Zoletil50®, Virbac) and Xylazine (Rompun®, Bayer). Warm saline and 10% neutral buffered formalin (NBF) are injected through carotid artery in sequence for perfusion and fixation, respectively.

After fixation, major organs of miniature pigs (lung, heart, liver, pancreas and kidney) are trimmed to remain the connected anatomic structures and carefully removed from the body. The connection among trachea, bronchi and lung lobes was remained. Heart was maintained leaving the vessels related the systemic and pulmonary circulation. Caudal vena cava, portal vein, diaphragm, gall bladder and bile duct were also remained with connected liver. Pancreas was dissected including duodenum to maintain pancreatic ducts between pancreas and duodenum. Bilateral kidneys were remained containing descending aorta, caudal vena cava, renal artery, renal vein and ureter.

Until initiation of the plastination process, trimmed organs were stored in 10% NBF. Plastination was carried out in four main stages. (Table 1) After embalming with formalin, the body fluids and soluble fats were dissolved from the organs by placing it into the acetone bath. During forced impregnation, vacuum chamber removed the acetone from the organs, and liquid silicone replaced acetone that had been placed in organs. In the final step, organs were hardened with heat. For visualization of internal structure of organs, each one heart and kidney among the organs were sliced into four sheets and two sheets, respectively.

III. Results

Plastinated specimens made in our study are shown in Figures 1–5. Silicone impregmented method keeps thoroughly dissected specimens from deteriorating and maintained many of the important surrounding structures. After entire process, organs became dry, odorless, and durable specimens that could be handled without gloves and kept without any special storage conditions or care.

a) Heart and kidney

External structure of heart was completely preserved. (Figure 1) Ventricles, artriums, auricles, and grooves were well discriminated, and vascular structures including aortic arch, brachiocephalic trunk, pulmonary artery and vein, and vena cava were distinctly exhibited. In sliced specimens, internal structures containing valves, chorda tendinaes, papillary muscles, septums and coronary artery were clearly identified. Besides, the properties of inner, outer, and sectioned surface of vascular tissues and myocardium were ascertained.

Bilateral kidneys were kept in the connection state with blood-vessels. Renal arteries were maintained to the both side kidneys from the descending aorta, and connection between caudal vena cava and renal veins was also maintained. (Figure 2) In addition, adrenal glands as well as ureter from the renal pelvis were easily shown. In sliced specimens, the discrimination among the renal medulla, cortex and pelvis structure was obvious.

b) Lung, liver, and pancreas

Each lobes and the overall shape of the lung were soundly maintained. Trachea and its bifurcation to both side lobes were shown, and lymph node positioned in bifurcation site was identified. (Figure 3) In the dorsal aspect of the platinated liver, gall bladder, portal vein, hepatic artery and caudal vena cava were maintained without deformation. (Figure 4) Particulary, pancreatic ducts was clearly shown, because the connection between duodenum and pancreas was completely preserved. (Figure 5)

Table 1: Outline of plastination

	Days
Fixation	7
Dehydration	8
Forced impregnation	2
Curing	6

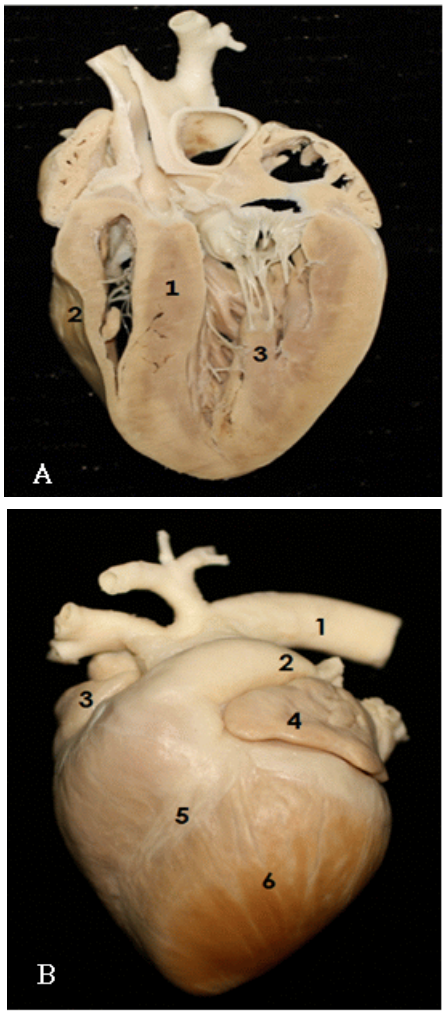

Fig.1: Heart

A. 1: interventricular septum. 2. right ventricle. 3: papillary muscles.
B. 1: aortic arch. 2: pulmonary artery. 3: right auricle. 4: left auricle. 5: paraconal groove. 6: left ventricle.

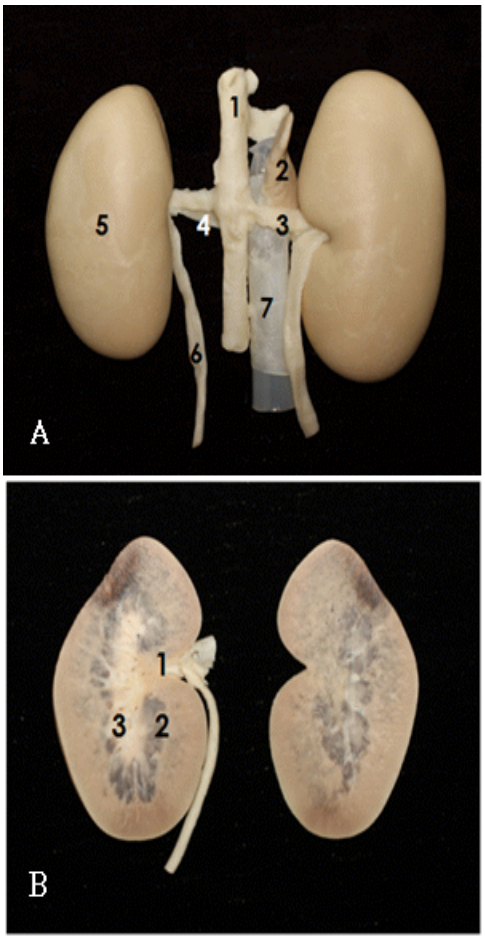

Fig.2: Kidney

A. 1: decending aorta. 2: adrenal gland. 3: renal artery. 4: renal vein. 5: kidney. 6: ureter. 7: caudal vena cava.
B. 1: renal pelvis. 2: renal cortex. 3: renal medulla.

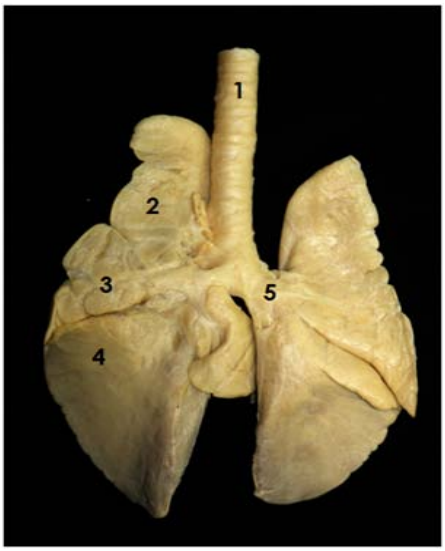

Fig.3: Lung

1: trachea. 2: cranial lobe. 3: middle lobe. 4: caudal lobe. 5: tracheal bronchus.

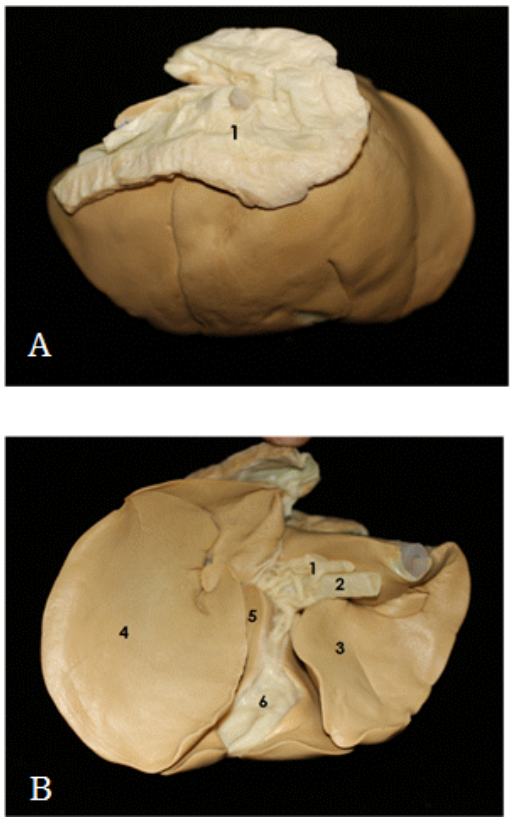

Fig.4: Liver

A (upper). 1: diaphragm.
B (lower). 1: hepatic artery. 2: portal vein. 3: right lobe. 4: left lateral lobe. 5: quadrate lobe. 6: gall bladder.

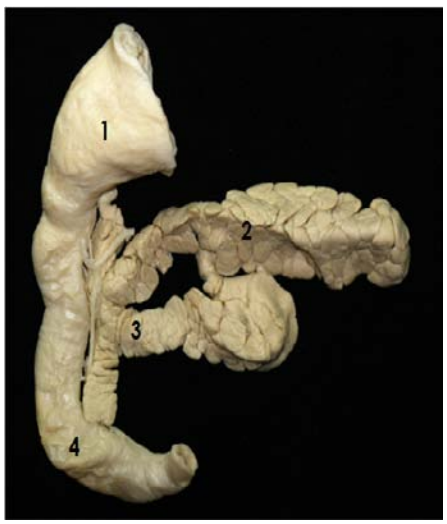

Fig.5: Pancreas

1: pylorus. 2: left lobe of pancreas. 3: right lobe of pancreas. 4: duodenum.

IV. Discussion

Veterinary science have been influenced by the increasing demand for veterinary schools or research facilities in animal welfare. Thus, there have been new approaches to veterinary education based on 'Three Rs'(3Rs; Replacement, Reduction and Refinement). Plastination, one of the new approaches, is certainly potential method in respect of learning tool for gross anatomy.[3]

We performed the plastination of major organs of miniature pig for alternative in anatomic instruction. The organ structures and spatial relationships of the surrounding tissues were not altered during the plastination. In addition, fine structures including vessels or ducts were also kept thoroughly, enough to replace the embalmed cadavers. The method using silicone as impregnant we performed, is largely used on dissected specimens. [3] Organ specimens plastinated with silicone had merits of being resilient and flexible, and above all, these organs were suitable for teaching

The miniature pig has stood out as the most suitable donor for animal-to-human xenotransplantation because of the similar size and physiological capacity of its organs.[9] This study is designed to suggest that the plastination of miniature pig could become an alternative for its actual use on veterinary anatomic teaching and xenotransplantation surgery instruction. Even though it was difficult to evaluate the impact of practical application accurately, through the literature search, we could predict that the properties of these plastinated specimens accommodate needs of student and researcher at various levels.

We believe that the plastination model can be can be applied to other laboratory animals, including rats, guinea pigs, and rabbits. We also believe this set of data will be of great help in education about the miniature pig and make significant contributions towards progress of research on xenotransplantation and the miniature pig.

This study suggests plastinated organs of miniature pig are valuable alternative material of cadaver on anatomic instruction. Furthermore, it is considered that this non-living specimen largely contribute to humane veterinary education and research.

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Isolation and Identification of Bacteria from Lung of Apparently Health Camels Slaughtered in Jigjiga Municipality Abattoir, Somali Region, Ethiopia

By Ahmed Ziad Abdulahi & Abdi Hassan Sufi
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Abstract- The study was conducted at Jijiga from October 2009 to May 2010 to isolate and identify the bacterial species from the lung of apparently healthy camels slaughtered in Jijiga Municipality Abattoir. Samples were collected aseptically from the lung for bacteriological examination. Standard microbiological techniques were used for the isolation and identification of the bacterial species. A total of 65 lung samples were examined bacteriologically and the most common lung lesions encountered were pneumonic (64.60%), emphysematous (20%) and 15.40% of necrotic foci. The bacterial species that were isolated include Coagulase negative staphylococci (48.7%), Streptococcus pneumonae (20.5%), Escherichia coli (12.8%), Rhodococcus equi (5.1%), Manhaemia hemolytica (7.7%), Corynebacterium kutcheri (2.6%) and Corynebacterium pseudotuberculosis (2.6%). Result of present study showed that Coagulase Negative Staphylacocci, Streptococcus pneumonae, Escherichia coli and Manhaemia hemolytica were the most important bacteria inhabiting the respiratory tract of camels and therefore these bacteria may cause respiratory diseases when associated with stress factors.

Keywords: *camel, lung lesion, bacteria, microbiological techniques, jijiga.*

GJMR-G Classification: *NLMC Code: QW 70*

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Keywords: camel, lung lesion, bacteria, microbiological techniques, jijiga.

I. Introduction

In Ethiopia, camel is found in eastern, south eastern and north eastern arid and semi-arid areas of the country mainly Borana, Ogaden and Afar regions which are believed to account for about one third of the total surface area of the country (Teka, 1991). The camel population of Ethiopia is estimated to 2.3 million of which two third is found in eastern lowlands (CSA, 2004). Several infectious agents can commonly be isolated from the respiratory tract of clinically sick and health animals. Most of the infectious agents which cause respiratory diseases are ubiquitous in the environment and are present as normal residents in the nasal cavities of normal animals. So these often create difficulty in the interpretation of the microbiological findings in the outbreaks of respiratory diseases (Radostitis *et al.*, 1994).

In Ethiopia few studies were conducted on the extent of respiratory problems of camels as compared to other species of livestock. Generally camel respiratory problem has received little consideration even though it is becoming one of the emerging diseases causing considerable loss of production and death (Tesfaye, 1996).

Depending on the background information and the need for identifying the causes of respiratory diseases of camels the present study was conducted with the following objectives.

- To isolate and identify bacterial species involved with the lung of apparently health camels.
- To assess the correlation of different bacteria isolated from different lung lesions.

II. Materail and Methods

a) Description of the Study area

The study was conducted at Jijiga from October 2009 to May 2010 which is located 636Kms east of Addis Ababa. It is situated 09^0 58'N and 42^0 46' E. The mean annual temperature and rainfall are 18-27^0 and 410-820 respectively. The distribution pattern of the rainfall is bimodal and variable from year to year.

b) Experimental Study animals, Study methodology and sampling strategy

The study was conducted on 65 camels that were slaughtered in Jijiga Municipality abattoir for meat purpose. Most of these camels were from Fafen, Gursum, kebribeyah, Degahbur, babile and around Jijiga town.

A post-mortem examination was made by visualization, palpation and incision of the lung, lung airways and the corresponding bronchial lymphnodes for the presence of lesions. The gross appearance and lesion type was also recorded.

c) Sample collection and transportation

From the slaughtered camels, a piece of lung lesion was removed by using sterile scalpel blade and forceps and then put in to sterile screw capped universal

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bottle. The sample was identified and the tissue specimens were placed in icebox containing icepacks and transported to Jijiga Regional Veterinary Laboratory. After isolation it was transported to National Veterinary Institute (NVI) at Debre Zeit for father identification of the bacteria up to the species level.

d) Bacteriological examination and Identification of bacteria

The tissue samples were collected from the sterilized surface area with the help of sterile forceps, scalpel blade and micropipette and then inoculated in to sterile screw capped test tube with 5ml of tryptose broth and incubated at 37^{0}c for 24 hours. After 24 hours of incubation, a loop full of the broth culture was planted on to sheep blood agar and incubated aerobically at 37^{0}c for 24 hours. After 24 hours of incubation, the isolated colonies were sub cultured on blood agar and Mac Conkey agar and incubated at 37^{0}c for 24 hours to 48 hours. Then the single colonies were sub cultured on tryptose agar and slants and incubated for 24 hours at 37^{0}c. The slants were preserved at refrigerator temperature for further use.

e) Data Analysis

The parameters were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of the statistical analysis system (SAS, 1999). Descriptive statistics were used to summarize the data generated from the study and analyzed using percentage.

III. Results

The present study was undertaken to isolate and identify the bacterial species from the lung of apparently healthy camels slaughtered in Jijiga Municipality Abattoir. The total sample was 65 lung samples examined bacteriologically, out of this, 45 samples showed bacterial growth where as 20 samples showed no bacterial growth. The total bacterial isolates were 48 of which 9 isolates were missed due to transportation problem. The isolates include Coagulase negative staphylococci (48.7%), *Streptococcus pneumonae* (20.5%), *Escherichia coli* (12.8%), *Rhodococcus equi* (5.1%), *Manhaemia hemolytica* (7.7%), *Corynebacterium kutcheri* (2.6%) and *Corynebacterium pseudotuberculosis* (2.6%) (Table 1). Shows the frequency of isolation of bacterial species from lung of camels

Table 1: Frequency of isolation of bacterial species from lung of camels

Types of bacteria	Isolated number	Percentages
Coagulase negative staphylococci	19	48.7%
Streptococcus pneumonae	8	20.5%
E.coli	5	12.8%
Rhodococcus equi	2	5.1%
Manhaemia hemolytica	3	7.7%
Corynebacterium kutcheri	1	2.6%
Corynebacterium pseudotuberculosis	1	2.6%
Total	39	100%

Table 2: The percentages of isolated bacteria and their correlation to the different lung lesions

Types of bacteria	Pneumonic	Emphysematous	Necrotic foci
Coagulase negative staphylococci	11 (57.9%)	5 (26.3%)	3 (15.8%)
Streptococcus pneumonae	8 (100%)	-	-
E.coli	3 (60%)	2 (40%)	-
Rhodococcus equi	1 (50%)	1 (50%)	-
Manhaemia hemolytica	3 (100%)	-	-
Corynebacterium kutcheri	1 (100%)	-	-
Corynebacterium pseudotuberculosis	1 (100%)	-	-

Table 3: Biochemical characterization of some *Corynebacterium* species and *Rhodococcus equi*

Tests	*C. kutcheri*	*C.pseudotuberculosis*	*Rh.equi*
Nitrate reduction	+ve	-ve	+ve
Urease	+ve	+ve	+ve
Maltose	+ve	+ve	-ve
Sucrose	+ve	-ve	-ve
O/F	F	F	Unreactive
Catalase	+ve	+ve	+ve
Oxidase	-ve	-ve	-ve

+ve = Positive; -ve = Negative; F= Fermentative; C= Corynebacterium; Rh= Rhodococcus.

The most lung lesions that were encountered during sample collection were pneumonic (64.60%), emphysematous (20%) and 15.40% of necrotic foci (figure 1)

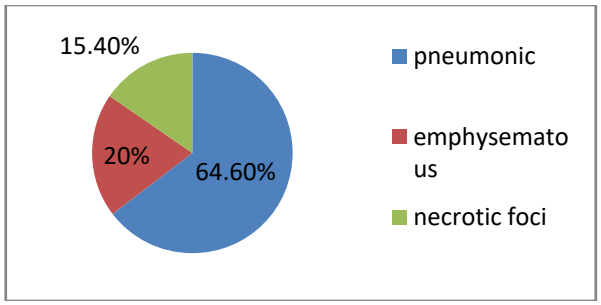

Figure 1: Frequency of lung lesions

IV. Discussion

This study was conducted in the lung of apparently healthy camels that were slaughtered in Jijiga Municipality Abattoir of Somali Regional State. The Coagulase negative staphylococci (CNS) were the predominant species (48.7%). The rate of isolation was agreed with that of Shemsedin (2002) who reported 46.4% and higher than that of Shigidi (1973) who also reported an isolation rate of 18.8%. The difference might be due to geographical and climatic conditions which favors the survival and maintenance of these bacterial species.

Streptococcus pneumonae was the second prevalent organism (20.5%). The rate of isolation was higher than that of Shemsedin (2002) who reported 1.45% and comparable with that of Tekleselassie (2005) who reported an isolation rate of 18.5% from pneumonic lung of goats.

Escherichia coli had an isolation rate of 12.8%. The rate of isolation was agreed with that of Al-Ani *et al*. (1998) who reported 12.5%. *Rhodococcus equi* was isolated at rate of 5.1%. The rate of isolation was agreed with that of Shemsedin (2002) who reported an isolation rate of 5.8%. The report *Rhodococcus equi* from camel respiratory tract in this study could also be due to inhalation of the organism from the soil.

Mannheimia haemolytica was isolated at rate of 7.7%. The isolation rate was agreed with that of Shemsedin (2002) and Al-Tarazi (2001) who reported 8.7% and 6.6% respectively, but it was much lower than that of Al-Ani *et al*. (1998) who reported 56.3%. The difference might be due to that he studied only pneumonic lungs.

Corynebacterium kutcheri had an isolation rate of 2.6%. The rate of isolation was agreed with that of Shemsedin (2002) who reported an isolation rate of 2.9%, which is more similar with finding of the recent study. *Corynebacterium pseudotuberculosis* was isolated at the rate of 2.6%. The rate of isolation was agreed with that of Shemsedin (2002) who reported an isolation rate of 2.9%.

V. Conclutions and Recommendations

The most important lung lesions that were encountered were pneumonic, emphysematous and necrotic foci in which the pneumonic lungs were the predominant lesions. The results in this study indicated that a variety of bacterial species isolated from lung of camel in which Coagulase Negative Staphylacocci (CNS) were the most prevalent bacterial species followed by *Streptococcus pneumonae*, *Escherichia coli* and *Manhaemia hemolytica*. Other important bacterial species isolated include *Rhodococcus equi*, *Corynebacterium kutcheri* and *Corynebacterium pseudotuberculosis*. Thus the present study showed that a large variety of bacterial species that live in the respiratory tract of camels which might impede the health, productivity and performance of camels particularly when the animals are stressed. Therefore, based on the above conclusive remarks the following points are recommended:

- Further investigation as to the isolation and characterization of bacteria as well as their role in the respiratory diseases complex of camels should be carried out; therefore, emphasis should be given to camel diseases especially of respiratory problems.

VI. Acknowledgment

The authors' heartfelt appreciation goes to the Ethiopian Somali Region, livestock and pastoral development (LPDB) for fully sponsoring this study and Addis Ababa University for provision of research facilities.

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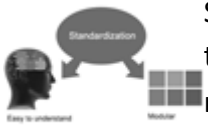

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- Fundamental goal
- To the point depiction of the research
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- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
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Approach:

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- If use of a definite type of tools.
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The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

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	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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