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SOME STUDIES ON NEONATAL CALF DIARRHEA IN EGYPT

Part 1: Causative agents and some epidemiological aspects

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ETUDES SUR LA DIARHEE DU VEAU NEONATAL EN EGYPTE

1ère Partie : Les agents causals et quelques aspects épidémiologiques

Résumé

L'examen clinique de 1200 veaux néonatals dans trois fermes situées dans différentes localités en Egypte a révélé que 200 veaux souffraient à des degrés divers de diarrhée et de déshydratation. Les veaux étaient classés en 4 groupes selon leur âge : 1er groupe (1 – 7 jours) ; 2ème groupe (8 – 14 jours) ; 3ème groupe (15 – 21 jours) et 4ème groupe (22 – 30 jours).

A partir de l'isolement et de l'identification du virus par le Test de neutralisation du sérum utilisant le sérum de référence connu, 53 échantillons fécaux sur 200 ont produit un effet cytopathique sur la culture de tissus. Des virus *Rota* étaient isolés de 38 échantillons, des virus *Corona* de 25 échantillons. L'infection mixte entre les virus *Rota* et *Corona* était observée dans 10 échantillons et l'infection mixte entre les virus *Rota* et le virus BVD dans 5 échantillons. Le taux le plus élevé d'isolement de virus s'est produit dans le 2ème groupe.

E-coli spp était isolé de 45 échantillons et *Salmonella spp* de 10 échantillons ; un échantillon avait une infection mixte. Le taux le plus élevé d'isolement d'*E-coli* et de *Salmonella* s'est produit dans le 1er groupe (30%) et dans le 2ème groupe (8,6%) respectivement.

Clostridium perferingens spp était isolé de 10,5% des échantillons et le taux le plus élevé s'est produit dans le 4ème groupe (13,3%).

Soixante échantillons sur 200 étaient infectés par l'ovocyste *Cryptosporidium parvum* et l'on a constaté le taux d'infection le plus élevé dans le 2ème groupe (42,8%).

Mots-clés : Diarrhée du veau néonatal, virus *Rota*, virus *Corona, E-coli spp., Salmonella spp., Clostridium perferingens spp., Cryptosporidium parvum.*

Summary

The clinical examination on 1200 neonatal calves in three different farms in various localities in Egypt revealed that 200 calves suffered from variable degrees of diarrhea and dehydration. The calves were classified according to their ages into 4 groups: 1st group: 1-7 day; 2nd group: 8-14 day; 3rd group: 15-21 days and 4th group: 22-30 days.

From the viral isolation and identification by Serum neutralization Test (SNT) using known reference serum, 53 out of 200 fecal samples gave Cytopathic Effect (CPE) on tissue culture: *Rota* viruses were isolated from 38 samples, *Corona* viruses from 25

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samples, the mixed infection between *Rota* and *Corona* viruses was observed in 10 samples and the mixed infection between *Rota* viruses and *BVD* virus in 5 samples. The highest rate of viral isolation occurred in 2nd group.

E- coli spp was isolated from 45 samples and *Salmonella spp.* isolated from 10 samples and one sample had mixed infection. The highest isolation rate of *E-coli* and of *Salmonella* occurred in 1st age group of (30 %) and in 2nd age group (8.6 %).

Clostridium Perferingens spp. were isolated from 10.5 % of the samples, the highest rate occurred in 4th age group (13.3%).

Sixty out of 200 samples were infected with *Cryptosporidium parvum* oocyst. The highest infection rate was found in 2nd group of age (42.8 %).

The study indicated the effects of age and location (management) on neonatal diarrhoeic infection in cattle in three randomly selected farms in Egypt. The data confirms the principal causal agent in neonatal calf diarrhoea in Egypt as *Escherichia coli, Rota*virus, *Corona* virus, *Clostridium perfringens, Salmonella spp* and Cryptosporidium spp as a single or mixed infection by the isolated agents. In addition, diet, management and sanitation are also significant contributory factors in the aetiology of neonatal calf diarrhoea in Egypt.

Key - word: Neonatal calf diarrhea, *Rota* virus, *Corona* virus, *E-coli spp., Salmonella spp., Clostridium Perferingens spp., Cryptosporidium parvum.*

Introduction

Newly born calves represent an important source of animal production for either meat or breeding[1] world wide. Diarrhoea is one of the very common disease syndromes in the neonatal calves in different countries and this can have severe impacts both economically and in terms of animal welfare. Neonatal calf diarrhoea is a multifactorial disease which despite decades of research in the topics, remains the most common cause of neonatal calf mortalities. Although major risk factors have been long identified, the severity of calf losses due to diarrhoea is not declining. Surveillance data of the National Animal Health Monitoring System[2] showed that, there was essentially no change from 1995 to 2001 in either the overall mortality of pre-weaned calves or in mortality by specific cause, scours and diarrhea still accounted for the largest percentage of deaths in neonatal calves followed by respiratory problems[3]. Large-scale surveys in the United States report that, diarrhoea accounts for 60.5 % of neonatal calf deaths[2]. Economic losses from enteritis are due to direct deaths in dairy calves younger than three weeks old[4], treatment costs and time spent in care, as well as subsequent chronic illnesses and poor growth.

While it may be convenient to focus on the principal infectious causes of calf diarrhoea, it is generally recognized to be the result of interaction between a numbers of related risk factors[5, 6, 7]. These risk factors involved in etiology of neonatal calf diarrhoea include diet, colostrum deprivation, management, sanitation, over crowding, ambient temperature, humidity, stress and infectious agents (bacterial, viral and protozoal)[8].

Several enteropathogens are implicated in neonatal calf diarrhoea and

their relative prevalence varies geographically but the most common prevalent infections in most areas are *Escherichia coli, Rotavirus,* and *Corona virus, Clostridium Perfringens, Salmonella* and *Cryptosporidium*. Cases of neonatal calf diarrhoea are commonly associated with more than one of these agents and the cases of most outbreaks are usually multifactorial[9, 10].

Consequently, this study was planned to study some of the epidemiological aspects of neonatal calf diarrhoea and the isolation and identification of the infectious agents involved in neonatal calf diarrhea in Egypt. In addition, observation of clinical signs and their correlation with disease progression are reported.

Material and Methods

1- Farm Animals

This work was carried out from (March 2005 to February 2006) using a total of 1200 neonatal calf of different ages (1-30 days old) examined clinically for diarrhoea. The calves were born in three different farms located in different governorates in Egypt and managed under varied hygienic conditions. Out of these calves, 200 calves were found suffering from different degrees of diarrhoea, dehydration, emaciation and weakness. The distribution of the examined calves in relation to localities, ages and seasons is shown in Table (1).

Laboratory animals

Healthy albino guinea pigs (350-450gm) were used for detection of dermonecrotic reaction for typing of *clostridium perfringens* isolates.

2- Samples

a- For trails of viral isolation and viral antigen detection

Two hundred fecal swabs were collected from non treated diarrhoeic calves on sterile Hank's solution for viral isolation. Collected samples were preserved at -20^{0}C and transported to Rinderpest Like Diseases Department in Veterinary Serum and Vaccine Research Institute, Abbasia for examination.

b- For bacteriological and parasitological examination

Two fecal swabs were collected from the rectum of each non-treated infected calves (n = 200), the swabs were put in screw sterilized caped bottle, transported to Laboratory of Infectious Diseases Faculty of Veterinary Medicine Mansoura University, in cold chamber container within few hours post-collection for immediate bacteriological examination.

- Two hundred fecal samples were collected from non treated diarrheic calves directly from the rectum by using disposable plastic gloves and transferred immediately to the laboratory of Infectious Diseases Faculty of Veterinary Medicine Mansoura University for parasitological examination.

3- Tissue culture

Madin Darby Bovine kidney (MDBK) cell line[11] was used for both viral isolation and serum neutralization test (SNT). The cell line was proved to be free from non-cytopathic BVD virus. It was used for viral isolation and identification.

4- Media and Reagent

a- Growth medium:

Minimal Essential medium (MEM) with Eugle's were used for maintenance of MDBK cells culture after inoculation with the virus.

b- Reagents:

Hank's Balanced Salt Solution (HBSS) was prepared and used for tissue culture and preservation of swabs samples[12].

-Sodium bicarbonate solution (NaHCO3) 4.4% was prepared and used for tissue culture media[13].

Stock antibiotic solution

A stock solution of antibiotics (penicillin and streptomycin) was prepared in Hank's solution and sterilized by filtration. It was dispensed into small quantities in screw capped bottles and kept at –20^{0}C. It was used at the final concentration of 100 iu penicillin and 100 µg streptomycin per ml.

5. Chemicals for parasitological examination

Levitation solution: for specific gravity 1.27, Potassium dichromate 2.5% sol., Modified Zeil-Neelsen stain, Absolute methyl alcohol and Malachite green 5%.

Calves management systems and farm construction.

1. Farm (1) located at Aga–Dakahillia governorate, the newly born calves were isolated from their dams in separate wooden floor boxes (1.6 m×1m) immediately after parturition. The calves received colostrum in pails for 3 days then fed on milk from milking cows in the farm three times daily up to 70 days. This farm used Co vaccine 8 to vaccinate pregnant cattle against closteridial diseases and Pneumo-3 vaccine against IBR, BVD, and PI-3 viruses.

2. Farm (2) located at Demiatta El-Gedida road, Demiatta governorate, the management of the calves was essentially similar to that first farm, except that calves were housed in concrete boxes, and there is no vaccination programs with pregnant cattle, calves were fed on milk replacers postpartum .

3. Farm (3) located at El- Khatatiba - Desert road at El-Beharia governorate the management of the calves was similar to that of the first farm but calves were housed in separated boxes on sandy land. One month after birth, calves were placed on milk replacers this farm used Scour Guard 3K in pregnant cattle to protect calves against *Rota* and *Corona* viruses and *E-coli* bacteria, and also used Cattle Master-4 vaccine for protection against IBR, BVD, PI-3 viruses.

4- Media for isolation & identification of bacteria:

Selective enrichment broth such as Selenite F-broth (Difco); Nutrient broth-(Oxiod), were prepared and used according to 14,15.

Selective and differential agar media

MacConkey lactose bile salt agar. (Oxiod); Salmonella Shigella agar (SS agar); Blood agar base; Nutrient agar; - Cooked meat medium[16];Production medium[17]; Egg yolk agar medium[18];

Media for biochemical reaction

Such as Triple sugar Iron (Oxiod); Urea agar base (Lab M); Sugar fermentation media: peptone water containing 1% of one of the following sugar, glucose, maltose, lactose, sucrose, Salicin, Dulcitol; Mannitol,

adintol, inositol and sorbitol were prepared.

7- Reagents Voges proskauer Methyl red solution; Kovacs Urea 40% solution. (Oxiod).

8- Other materials

Physiological saline 0.85%; Gram stain[14] ;Crystal violet;0.5% watery solution; Gram iodine (1gm iodine, 2gm potassium iodide in 200 ml distilled water); Dilute carbol fuschine solution. (1:10); Antisera, polyvalent and monovalent salmonella "O" and "H" sera were used to determine the complete antigenic formula of the isolates; Antisera for serological identification of *E-coli* isolate; *Clostridium. Perfringens diagnostic antisera;* obtained from Burrough Welcome, Research Laborites, Buckingham, London include, Type A, type B, type C and type D *clostridium. perfringens antisera;* Anaerobic jar (BBL); Microbact TM (oxide); Gram negative identification system 12A (12 E) for *Enterobacteraece;*

Methods

1- Clinical Examination:

Clinical examination was carried out on 1200 cattle calves with special reference to diarrhoea[19].

2- Viral isolation and identification

i – Preparation of fecal swabs: Fecal swabs were collected from diseased cattle calves; each swab was inserted into a screw capped bottle containing 3 ml of refrigerated HBSS containing 100 IU of penicillin as well as 100 µg of streptomycin per ml The swabs were squeezed and removed. All samples were then centerifugated at 2000 rpm for 10 minutes in the centrifuge. The supernatant solution was aspirated by a sterile Pasteur pipette and transferred to a sterile bijoux bottle and the samples were preserved at – 20°C till examined.

ii - Titration of Bovine *Corona* Virus and *Bovine Rota Virus:* Serial ten fold dilution of used viruses was prepared in cold HBSS, before inoculation of virus on cell culture; it was treated with 0.1 ml of trypsine for 30 minute at 37^{0}C and 0.1 ml of each dilution treated viruses was inoculated into each of 4 tissue culture tubes[20.] The culture tubes were incubated at 37^{0}C for 2 hours, and then maintenance medium were added. The tubes were incubated at 37^{0}C; the media were changed every 3 days. The culture was observed microscopically for specific cytopathic effect for 10 days. The virus titer was calculated by the method of 21. The titer was expressed as tissue culture infective dose fifty per 0.1 ml of used viruses and they were kept frozen till used for SNT.

iii - Viral isolation on tissue culture

It was adapted from rectal swab samples by inoculation of the prepared supernatant fluid in 2 tubes containing MDBK tissue culture cells per each sample[22], before inoculation of virus on cell culture; it was treated[20] with 0.1 ml of trypsine for 30 minute at 37°C as follows:

1. The growth medium was discarded and the cells were washed by MEM and each tube was inoculated with 0.1 ml of the supernatant fluid.
2. The inoculated cells were left for adsorption at 37^{0}C for one hour.
3. Two milliliters of maintenance medium were added to each tube.
4. The inoculated cells were then incubated at 37^{0}C and observed daily for 3-4 days post inoculation for detection of any cytopathic

effect (CPE).

5. The presence of virus was detected microscopically through appearance of CPE during 3-4 days post inoculation, the suspected samples were kept frozen at –70^{0}C for further propagation and identification.
6. Control non-inoculated cells were involved in the test.

iv - Identification of isolated viruses

The serum neutralization test was performed[23] for identification of isolated viruses. An equal amount of 1:10 dilutions of known specific immune serum was added to 100 TCID50/0.1 ml of isolated virus suspension and were incubated at 37^{0}C in a water bath for ½ hour. Then 0.2 ml of each mixture was inoculated into each 4 tissue culture tubes. Cell control tubes were also used. Tubes were observed for specific cytopathic effect of different viruses for 10–14 days.

3 - Bacteriological Examination

A- Isolation of salmonella.

- 10 grams of fecal samples were inoculated on selective enrichment Selenite F. broth medium
- Inoculated slenite F. broth medium was incubated for 18-24 hrs at 37^{0}C.
- A loopfull of enrichment culture was streaked on to MacConkey agar and S.S agar. All plates were incubated at 37°C for 24-48 hrs.
- Suspected Salmonella colonies (non lactose fermenting medium size colonies) were transferred to triple sugar iron (T.S.I) and urea agar slant.
- Cultures that gave reactions typical of Salmonella (opaque non spreading surface growth on (T.S.I) with an alkaline slant acid button and H_2S production as well as negative urea's reaction were subcultured and kept for further studies.
- Smears for all isolates were stained by Gram's stain[14].

Culture and biochemical behavior:

For initial identification, attempts were made using the criteria as described by[14,24,25] which include the following tests; indole, voges proskauer, methyl red, citrate utilization, urea hydrolysis, H_2S production and fermentation of lactose, sucrose, salicin, glucose, maltose, mannitol, dulcitol, inositol and adonitol.

Serological identification of *salmonella* microorganisms which possessed the culture characters and biochemical reactions was applied for further serotyping using salmonella agglutination antisera[26] by slide agglutinins test using polyvalent O group antiserum then monovalent O and H antisera factors to determine the antigenic structure of the isolated salmonella in (Animal Health Research Institute, Dokki .Giza, Egypt).

B- Isolation of *Escherichia-coli (E-coli)*

- The rectal swabs from each infected calf (200) were inoculated onto 10 ml of nutrient broth and were incubated for 18-24 at 37°C.
- A loopfull of the enriched culture was streaked onto MacConkey agar. All plates were incubated at 37°C for 24-48 hrs. Isolated colonies were purified and

subjected to the following identification tests according to 16.

Morphological characters

- Staining: Films were made from the pure culture of isolated organisms, stained with Gram's stain and examined microscopically.
- Identification of suspected *E-coli* and Salmonella colonies was identified by Microbact TM (Gram negative identification system)[14] .
- Serological identification of *E-coli* microorganisms which possessed the culture character and biochemical reactions were subjected to further serotyping using specific antisera[26].

C- Isolation and characterization of clostridium perfringens

- Fecal swabs were inoculated on cooked meat media and incubated anaerobically for 18-24 at 37°C in anaerobic jar. A loopfull from cooked meat media was streaked on to blood agar. All plates were incubated anaerobically at 37°C for 24-48 hrs.
- Suspected colonies of *clostridium perfringens* surrounded with zone of hemolysis, were stained by Gram's stain, cultured again on cooked meat broth and preserved for further identification.

Identification

o Microscopical examination: Gram's stained smears from suspected colonies were examined for Gm +ve bacilli.

o Culture character: Suspected colonies were subcultured on 10% sheep blood agar and cooked meat medium anaerobically for 24 hours at 37^{0}C. Type of hemolysis on sheep blood agar, characters of colonies, growth on egg yolk agar for lecithenase production and changes in meat particles were recorded.

o Biochemical reactions: The suspected purified colonies were subcultured in cooked meat medium and incubated anaerobically at 37^{0}C for 24 hour, and then they were identified according to scheme of[16]; by using the following tests, nitrate reduction test, indole test, catalase test, nagler reaction. The suspected clostridium colonies were cultured on production media for toxin production.

- Identification and typing of toxogenic strains: The toxin–antitoxin neutralization test was used for typing of the isolated strains and toxins[27, 28], by intradermal inoculation of albino guinea pigs by toxin suspension (active growing culture) with different types of antisera. Dermonecrotic reaction and its neutralization were read after 24 hours.

4 - Parasitological Examination

Macroscopic examination

The examination of feces was carried out in the laboratory of Infectious Diseases Fac. Vet. Med. Mans. University and includes color, odor, consistency, presence of blood and mucus.

Identification of cryptosporidium oocyst Concentration floatation technique[29] .

- Fecal samples were examined for the presence of *cryptosporidium oocyst* by dichromate solution using flotation technique as following: 3 ml of fecal dichromate solution was placed in screw capped centrifuge tube; and mixed with 5ml of levitation solution (prepared by dissolving 545 gm granulated sugar in 355 ml distilled water in addition to 6.7 ml liquid phenol. five ml of distilled water were added, tube containing fecal solution mixture was inverted 10 minutes to mix the

contents then centrifuged at 1500 rpm for 10 minutes. The meniscus was removed by placing loop full of liquid on a glass slide then covered by cover glass and examined microscopically (using high power X40).

Staining Method

The modified Zheil-Neelson Stain[31] was used for staining of fecal smears either directly or after the application of flotation technique. Thin smear were prepared, all glass slides were left to be air dried and then fixed with methanol for 10 minutes, then they were immersed in concentrated cold carbol fuschin (1.0 gm carbol fuschin, 10 ml ethanol and 90 ml of 5% phenol) for five minutes. Decolorized with 10% sulfuric acid for 30 second was made and then rinsed with tap water for two minutes. Counter staining with 5% malachite green (5gm of malachite green in 100 ml of 10% ethanol) for one minute was carried out, then rinsed again with tap water; dried in the air and examined microscopically by oil immersion lens (X100).

Results and Discussion

The present study was carried out on 1200 neonatal calves, among them 200 neonatal calves (16.66%) showed variable degrees of diarrhoea which varied from mild to profuse watery feces, its color varied from whitish yellow to greenish color, in some cases tinged with blood or mucus. Calves were suffering from different degrees of dehydration, weakness, unable to stand, in some cases there is rise in body temperature and some cases were discarded from the farms. The highest rates of diarrhea were observed in 1st group of age (1-7 days) 24% , Table (2) followed by 2nd group (8–14 days) 17.5%, then 3rd group (15–

Table 1: Distribution of clinically examined calves in relation to localities in Egypt, ages and season.

1st group 1-7 days (250)					2nd group 8-14 days (400)					3rd group 15-21 days (300)					4th group 22-30 days (250)					Total
Winter	Spring	Summer	Total		Autumn	Winter	Spring	Summer	Total	Autumn	Winter	Spring	Summer	Total	Autumn	Winter	Spring	Summer	Total	
40	20	40	**130**		60	70	30	20	**180**	30	50	20	50	**150**	10	5	15	10	**40**	**500**
10	25	5	**60**		30	20	10	20	**80**	20	20	20	30	**90**	20	30	10	10	**70**	**300**
30	15	5	**60**		30	10	40	60	**140**	30	10	10	10	**60**	30	45	45	20	**140**	**400**
80	60	50	**250**		120	100	80	100	**400**	80	80	50	90	**300**	60	80	70	40	**250**	**1200**

Table 2: Prevalence of neonatal calves diarrhea according to age.

Age group	Total examined calves	Clinically diseased calves	%
1st group	250	60	24
2nd group	400	70	17.5
3rd group	300	40	13.33
4th group	250	30	12
Total	**1200**	**200**	**16.66**

1st group: calves aged 1-7 days 2nd group: calves aged 8- 14 days
3rd group: calves aged 15- 21 days 4th group calves aged 22 – 30 days

Table 3: Prevalence of neonatal calves diarrhea according to season.

Season	Total examined calves	Clinically diseased calves	%
Autumn	320	40	12.5
Winter	340	80	23.55
Spring	260	50	19.23
Summer	280	30	10.71
Total	**1200**	**200**	**16.66**

Table 4: Prevalence of neonatal calves diarrhea in different localities.

Farm	Locality	Total examined calves	Clinically diseased calves	%
Farm 1	El- Dakahilia	500	80	16
Farm 2	Demiatta	300	50	16.66
Farm 3	El- Behara	400	70	17.5
Total		**1200**	**200**	**16.66**

22days) 13.33 % and lastly the 4th group of age (22–30 days) 12%. The results obtained are similar to those obtained Bellinzoni[30] who reported that the incidence rate for diarrhea during neonatal period was 14.6% in south west France. 52 % of diarrhea appears during the 1st week and only 15% after the second week of life, It should be noted that the highest incidence was observed in December and March months by 17.6% and 23.6% respectively. On the other hand, 31 noted that in a survey, which lasted one year, the incidence data of 73 dairy cows with their calves, revealed that neonatal diarrhoea was higher in calves, which were born in winter compared with those born in summer, similarly the results showed that, the highest rate of diarrhoea occurred in the winter months (23.55%), followed by Spring months (19.23%), Autumn months (12.5%), and the low rate of diarrhoea was observed in Summer months (10.71 %) Table 3. The highest rate of diarrhoea occurred in farm (3) in El-Behara (17.5), followed by farm (2) in Demiatta (16.66%) and lastly farm (1) of El-Dakahilia (16%), Table (4). This high occurrence of diarrhoea may be attributed to poor hygienic measures in the farms and overcrowding, moreover 32 reported that most clinical cases of neonatal calves diarrhoea occurs between 1 and 10 days of age, probably because this in the age bracket of highest and maximum of exposure and neonatal susceptibility. In addition, declining levels of colostrally acquired antibodies in the gut

Table 5: Rate of isolation of *Rota, Corona* and *BVD* viruses from clinically diseased calves according to age.

Age group	Clinically diseased calves	*Rota* virus alone		*Corona* virus alone		Mixed *Rota* and *Corona*		Mixed *Rota* and *BVD*	
		No.	%	No.	%	No.	%	No.	%
1st group	60	8	13.3	5	8.3	1	1.7	2	3.3
2nd group	70	20	28.6	14	20	6	8.6	3	4.3
3rd group	40	6	15	4	10	2	5	-	-
4th group	30	4	13.3	2	6.7	1	3.3	-	-
Total	**200**	**38**	**19**	**25**	**12.5**	**10**	**5**	**5**	**2.5**

Table 6: Rate of isolation of *Rota, Corona* and *BVD* viruses from clinically diseased calves according to season.

Season	Clinically diseased calves	*Rota* virus alone		*Corona* virus alone		Mixed *Rota* and *Corona*		Mixed *Rota* and *BVD*	
		No.	%	No.	%	No.	%	No.	%
Autumn	40	8	20	4	10	-	-	2	5
Winter	80	21	26.3	13	16.3	5	6.3	2	2.5
Spring	50	6	12	5	10	3	6	1	2
Summer	30	3	10	3	10	2	6.7	-	-
Total	**200**	**38**	**19**	**25**	**12.5**	**10**	**5**	**5**	**2.5**

lumen at 4-6 days of age may contribute to the effect of age distribution, occasionally older calves are affected. The incidence risks of diarrhoea in calves <30 days old reported by several studies varies between 15 and 20% with a mortality risk of 1.5 – 8%[33, 34].

Several enteropathogens are implicated in neonatal diarrhoea with varying geographically relative prevalence varies but the most common prevalent infections in most areas are *Escherichia coli, Rota*virus, and *Corona* virus*, Clostridium Perfringens, Salmonella* and *Cryptosporidium. Rota* and *Corona* viruses are the most important causative agents of gastroenteritis in calves. They either act alone or combined together with other pathogens. Bovine *Rota*virus was first described as Nebraska calf diarrhoea virus isolated in united states, while bovine *Corona virus* was isolated from diarrheic neonatal calves[35].

Trails of viral isolation from rectal swabs on MDBK tissue culture revealed that (Table 5

Table 7: Rate of isolation of *Rota, Corona* and *BVD* viruses from clinically diseased calves according to locality.

Farm	Locality	Clinically diseased calves	*Rota* virus		*Corona* virus		Mixed *Rota* and *Corona*		Mixed *Rota* and *BVD*	
			No.	%	No.	%	No.	%	No.	%
Farm 1	**El- Dakahilia**	80	19	23.8	12	15	3	3.8	2	2.5
Farm 2	**Demiatta**	50	6	12	5	10	2	4	1	2
Farm 3	**El- Behara**	70	13	18.6	8	11.4	5	7.1	2	2.9
Total		**200**	**38**	**19**	**25**	**12.5**	**10**	**5**	**5**	**2.5**

Fig 1: Rate of isolation of *Rota, Corona* and *BVD* viruses from clinically diseased calves according to age.

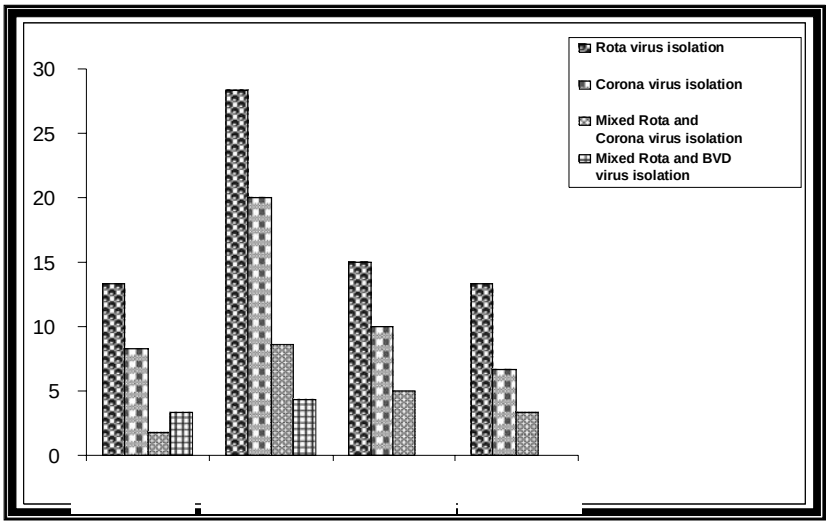


Fig 2: Rate of isolation of *Rota*, *Corona* and *BVD* viruses from clinically diseased calves according to season.

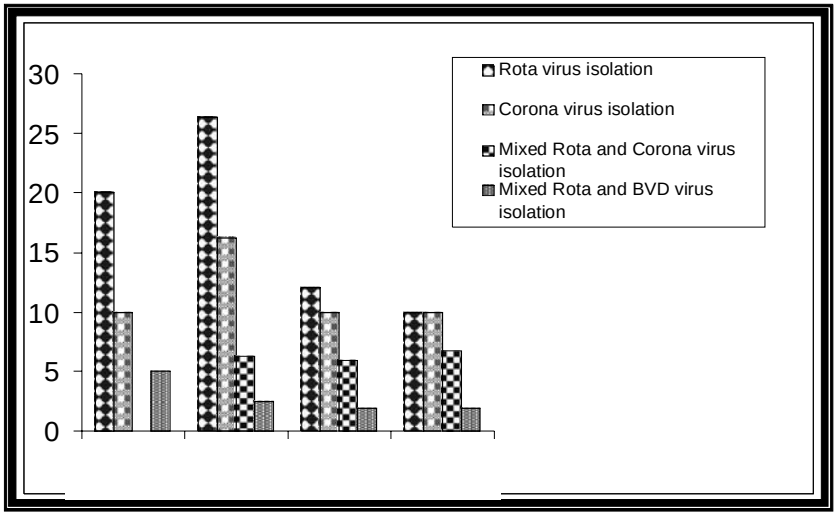
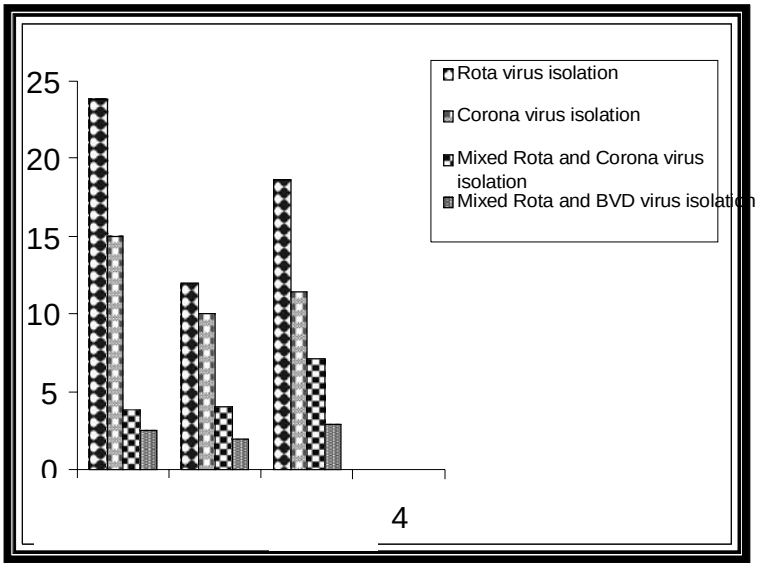


Fig 3: Rate of isolation of Rota , Corona and BVD viruses from clinically diseased calves according to locality



Bacteriological examination.

Table 8: Isolation rate of *Escherichia Coli spp.* and *Salmonella spp.* from clinically diseased calves according to age.

Age group	Clinically diseased calves	*E-Coli Spp.* alone		*Salmonella spp* .alone		Mixed infection	
		No.	%	No.	%	No.	%
1st group	60	18	30	1	1.7	1	1.7
2nd group	70	15	21.4	6	8.6	-	-
3rd group	40	7	17.5	2	5	-	-
4th group	30	6	20	2	6.7	-	-
Total	**200**	**46**	**23**	**11**	**5.5**	**1**	**0.5**

1st group: calves aged 1-7 days 2nd group: calves aged 8- 14 days
3rd group: calves aged 15- 21 days 4th group calves aged 22 – 30 days

Table 9: Isolation rate of *Escherichia Coli spp.* and *Salmonella spp.* from clinically diseased calves according to season.

Season	Clinically diseased calves	*E-Coli Spp.*		*Salmonella spp.*		Mixed infection	
		No.	%	No.	%	No.	%
Autumn	40	6	15	2	5	-	-
Winter	80	25	31.2	7	8.7	1	1.25
Spring	50	11	22	1	2	-	-
Summer	30	4	13.3	1	3.3	-	-
Total	**200**	**46**	**23**	**11**	**5.5**	**1**	**0.5**

Table 10: Isolation rate of *Escherichia Coli spp.* and *Salmonella spp.* from clinically diseased calves according to locality.

Farm	Locality	Clinically diseased calves	*E-Coli pp.*		*Salmonella spp.*		Mixed infection	
			No.	%	No.	%	No.	%
Farm (1)	El- Dakahilia	80	20	25	4	5	1	1.25
Farm (2)	Demiatta	50	10	20	3	6	-	-
Farm (3)	El- Behara	70	16	22.8	4	5.7	-	-
Total		**200**	**46**	**23**	**11**	**5.5**	**1**	**0.5**

Table 11: Serotyping of isolated *Escherichia coli spp.* and *Salmonella spp.*

Bacteria spp.	Total isolate	Serotypes											
		E-coli K_{99}		O_{119}:B_{14}		O_{26}:B_7		O_{111}:B_4		O_{55}:B_5		untyped	
		No	%	No	%	No	%	No.	%	No	%	No	%
E- Coli spp.	46	5	10.9	3	6.5	8	17.4	10	21.7	12	26.1	8	17.4
Salmonella spp.	11	*Salmonella* typhimurum						*Salmonella* enteriditis				untyped	
		No.		%				No.		%		No.	%
		6		54.55				3		27.27		2	18.18

& Fig 1) *Rota viruses* was isolated from 38 samples out of 200 samples (19%), *Corona* virus was isolated from 25 samples out of 200 (12.5%), and the mixed infection of *Rota* and *Corona* viruses was obtained in 10 samples out of 200 examined samples (5%). Mixed infection between *Rota* virus and BVD virus in 5 samples out of 200 (2.5%) was recorded.

When age related incidence of viral isolation was studied, it was found that the highest rate of viral isolation occurred in 2nd group in which *Rota*viruses was isolated by (28.6%) and *Corona* viruses isolated by (20%); followed by 3rd age group, *Rota* viruses was isolated by (15%) and *Corona* virus by (10%), and lastly the 1st and 4th groups, these results are directly correlated with the results of 36 who detected *Rota*virus in 50 fecal samples out of 229 fecal samples examined from diarrheic neonatal calves, and the same result is obtained Fukai[37] who isolated bovine *Rota*virus group A from 28 fecal samples out of 167 fecal samples (16.77%) examined from neonatal calves with diarrhea in Hokkaido in Japan. Also the same finding is recorded by Salem[38] who identified 17 cytopathic *Rota* viral antigens from fifty-seven (57) fecal samples obtained from diarrheic Friesian newborn calves, their ages ranged from one day to two weeks, and 11 *Rota*virus isolates were identified by serological technique. Our results nearly agree with the results obtained by Fejes[39] who examined fecal samples from 63 diarrheic calves, and 232 calves that had died of diarrhoea (the majority were less than 6 days of age) on intensive dairy farms over 2 years period. The result revealed that *Rota*viruses were detected in 11 (3.7%) and *Corona* viruses were detected in 18 (16%). Similarly, Bound[40] isolated *Rota*virus from five out from 10 samples obtained from dairy calves aged 2-7 days, *Corona* virus was isolated from (11.1%), lastly agree Novert[41] who isolated *Rota*viruses with a percent of 19.6% (11 fecal samples out of 56 fecal samples), obtained from untreated diarrheic calves aged from one day up to 3 weeks of age. similar reports were described Fatehia[42] who concluded that 17.6% and 11.8% samples out of 122 collected samples from cattle and buffalo calves were positive for *Corona* and *Rota* viruses, respectively. On the other hand high prevalence of *Rota* and *Corona viral* infection was recorded Abd El-Rahim[43] who reported *Corona virus* infection (29.6%) in buffalo calves in Assuit governorate. Hassan[44] detected *Rota* virus antigen in 10 out of 44 (22.7%) fecal samples, while *Corona virus* antigen was detected in 57 fecal samples out of 87 (65.5 %) fecal samples obtained from calves by different serological techniques, and Hegazy[45] who found that the prevalence of *Rota*virus was 60 % in 1st week, 83 % at 2nd week and 33% in the 2nd month, Chauhan[46] isolated *Rotavirus* from 36 diarrheic calves, (3-10 days old) out of 86 calves from area out Utar Pradeah India. Younis[47] reported that (136) 39 % of examined fecal samples (355) were positive for *Rota* virus by latex agglutination test, 48 recorded that among 450 examined newborn calves in three provinces in Upper Egypt from January 1998 to June 1999, the incidence of *Rota* viral enteritis, *Corona* viral enteritis and mixed, *Rota, Corona* viral enteritis were 32%, 20% and 19.8% respectively by using Dot ELISA. The variation in prevalence of the two viruses may be due to environmental factors or presence of high numbers of carrier animals among susceptible calves or overcrowded condition which increases chance of viral fecal contamination and using different

Fig 4: Isolation rate of *Escherichia Coli spp*. and *Salmonella spp.* from clinically diseased calves according to age

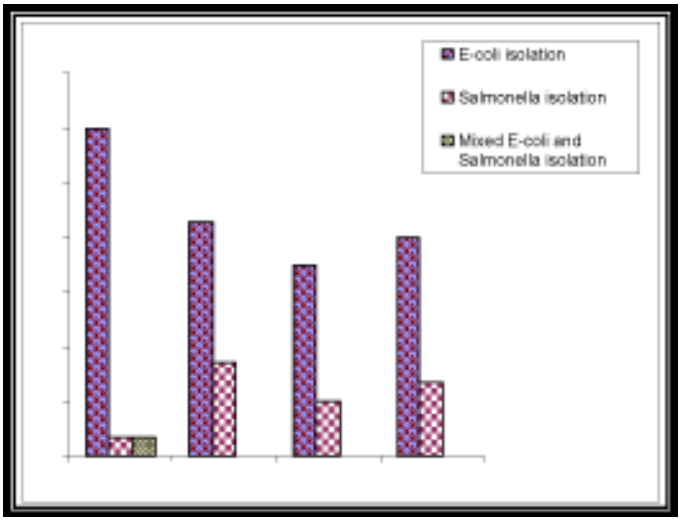


Fig 5: Isolation rate of *Escherichia Coli spp.* and *Salmonella spp.* from clinically diseased calves according to season

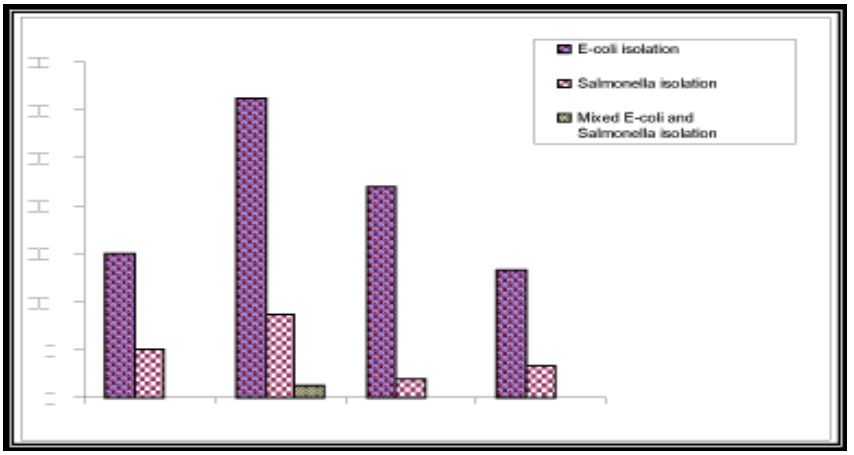
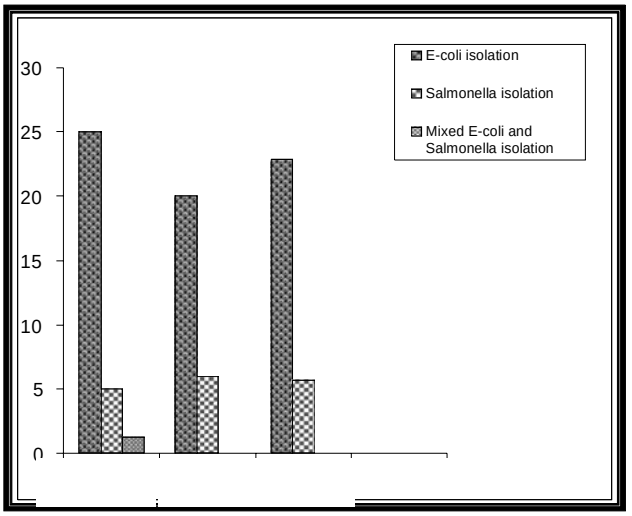


Fig 6: Isolation rate of *Escherichia Coli spp.* and *Salmonella spp.* from clinically diseased calves according to locality



Anaerobic bacteria.

Table 12: Isolation rate of *Clostridium perferingens* isolated from clinically

Age group	Total clinically diseased calves	No. of Isolates	%
1st group	60	7	11.7
2nd group	70	7	10
3rd group	40	3	7.5
4th group	30	4	13.3
Total	**200**	**21**	**10.5**

Table 13: Isolation rate of *Clostridium perferingens spp.* isolated from clinically diseased calves according to season.

Season	Total clinically diseased calves	No. of Isolate	%
Autumn	40	3	7.5
Winter	80	11	13.8
Spring	50	5	10
Summer	30	2	6.7
Total	**200**	**21**	**10.5**

techniques in diagnosis.

When viral infection in calves was investigated in correlation with seasonal prevalence of the disease (Table (6) & Fig 2), it was found that the highest rate of *Rota* and *Corona* viral isolation occurred in winter months; 21 isolates out of 80 samples examined (26.3) for *Rota* virus and 13 isolate for corona virus (16.3), followed by Autumn months in which *Rota* virus isolated by (20%) and *corona* virus by (10%), Spring months and finally Summer months. The obtained results are going in the same way with the results obtained by Faheem[48] who concluded that most of *Rota* viral infection was observed during 1st week of life, *Corona* viral enteritis was mostly observed in calves aged 1-4 weeks. High rate of mixed infection with *Rota* and *Corona* viruses was noticed among calves of 0-2 weeks old, added that high incidence was reported during the winter season and there was no significant difference of infection between male and female calves. Dhanaraj[49] reported that calves in the age group of 0-7 days showed 33% of incidence of *Rota*virus followed by 7-15 days of age (20%) in India. The majority of cases occurred during November and December months, corresponding to winter months.

From spartial perspective (Table (7) & Fig 3) the highest rate of *Rota* and *Corona* viral isolation occurred in farm 1 El–Dakahilia (23.8%) for *Rota*virus and 15% for *corona* virus followed by Farm (3) El-Behara and lastly in Farm (2) Demiatta in which *Rota*virus isolated by 12% and *corona* isolated by 10%. This variation between 3 farms may be due to different hygienic measures, presence of carrier animals and bad management programs.

The role of bacterial agents in neonatal calf diarrhea is more complex because they are associated with many attributes as certain proteins which have toxic and lethal effect on the host cell. The common agent of bacteria involved are numerous, however colibacillosis infection caused by *E-coli* is by far the most common usually manifested by rapid death and most common in calves during the first four days of life. According to Vazquez[50], Salmonellosis is an economically important disease as well as public health problem, infection of calves with various serovars of *Salmonella* can result in serious clinical disease and always constitutes a vat reservoir for infection of animals and humans[51].

The result of bacteriological examination of fecal swabs of calves (Table 8&Fig 4) clearly showed that *E- coli* was isolated from 46 out of 200 examined samples (23 %) and *Salmonella spp.* were isolated from 11 out of 200 examined samples (5.5%) and one sample was found infected with mixed *Salmonella* and *E-coli* infection. According to age the high rate of isolation of bacteria was found in 1st and 2nd group ages followed by 4th group age and at last 3rd group of age (22.5 %). however the highest isolation rate of *E-coli* occurred in 1st group of age (30 %), the highest rate of isolation of *Salmonella* occurred in 2nd group of age (8.6%). The results are agree with the results obtained by El-Hamamy[52] who carried out a study on 40 diarrheic calves belonging to Salhia dairy farm in winter season from December 1998 to March 1999. Calves age ranged from 5-21 days, culture of swab from diarrheic calves revealed that, the predominant isolate was *E-coli* (52.5%), *enterobacter aerogense* 15%, *proteus vulgoris* 12.5 % and *Salmonella* spp. 5%. And with results obtained by Novert[53] who bacteriologically examined a total of 150 fecal samples from diarrheic buffalo calves

Table 14: Isolation rate of *Clostridium perfringens spp.* isolated from clinically diseased calves according to locality.

Farm	Locality	Total clinically diseased calves	No. of Isolate	%
Farm (1)	El- Dakahilia	80	5	6.3
Farm (2)	Demiatta	50	3	6
Farm (3)	El- Behara	70	13	18.8
Total		**200**	**21**	**10.5**

Table 15: Typing of *Clostridium perfringens spp.* isolated from clinically diseased calves by dermonecrotic reaction in guinea pig.

No. of isolated toxigenic strains	Clostridium perferingens spp.		
	Type A	Type β	Type D
21	15	5	1

Fig 7: Isolation rate of *Clostridium perfringens spp.* isolated from clinically diseased calves according to age.

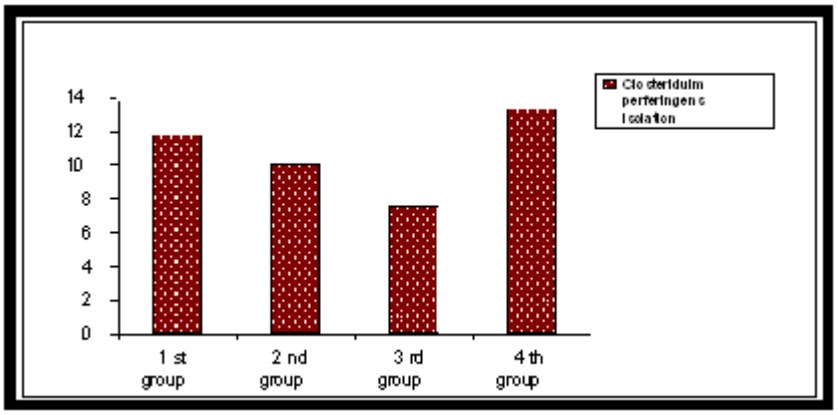


Fig 8: Isolation rate of *Clostridium Perfringens spp.* isolated from clinically diseased calves according to season.

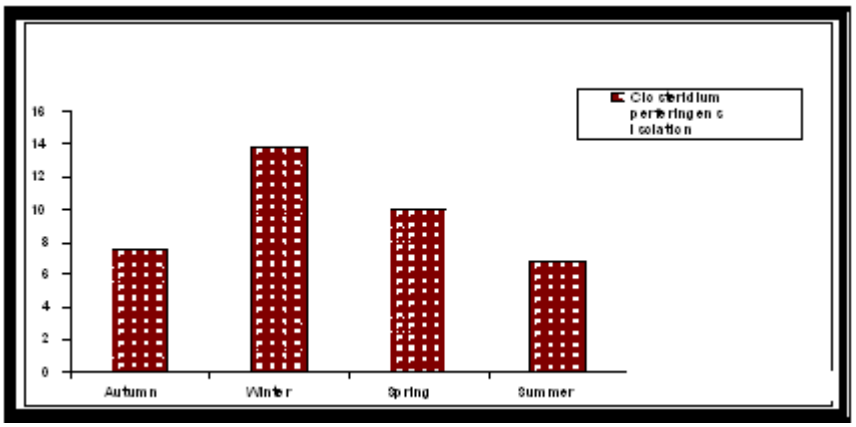
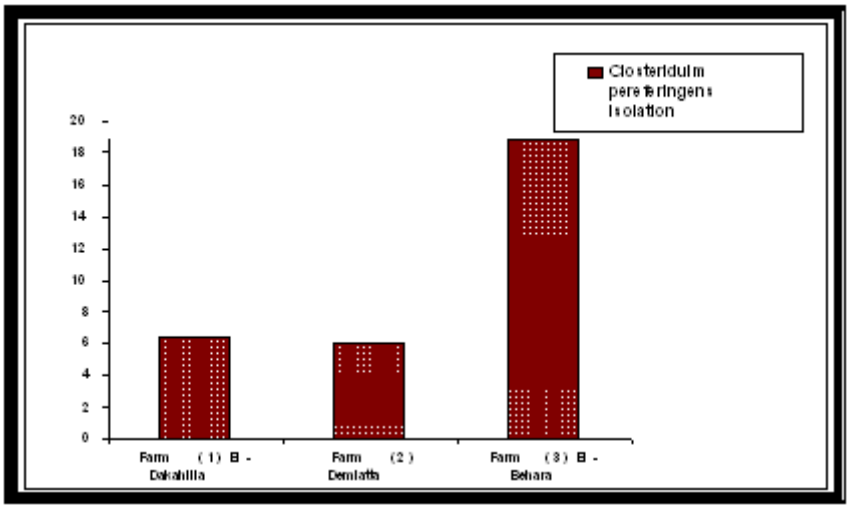


Fig 9: Isolation rate of *Clostridium perfringens spp.* isolated from clinically diseased calves according to locality.



Parasitological examination

Table 16: Detection of *Cryptosporidium parvum oocysts* in fecal samples from clinically diseased calves according to age.

Age group	Total clinically diseased calves	No. of infected samples	%
1st group	60	10	16.6
2nd group	70	30	42.8
3rd group	40	12	30
4th group	30	8	26.6
Total	**200**	**60**	**30**

Table 17: Detection of *Cryptosporidium parvum* oocysts in fecal samples from clinically diseased calves according to season.

Season	Total clinically diseased calves	No. of infected samples	%
Autumn	40	6	15
Winter	80	34	42.5
Spring	50	14	28
Summer	30	6	20
Total	200	60	30

aging from one day up to 2 months old during the period from June 2000 to may 2001, result revealed that 7 samples were positive for *E-coli* with an incidence of 24.66%, *E-coli* K_{99} were isolated from 13 samples, 34 out of 150 samples 22.7% were positive for *combelobacters, Salmonella* were isolated from 22 samples with an incidence of 14.66%. The same results are obtained by41 who detected *E –coli* K_{99} antigen in 13 fecal samples (23.2%) out from 56 fecal samples obtained from untreated diarrheic calves aged from one day up to 3 weeks of age, using the traditional culture method. Also same results supported Bendali[54] who concluded that *E-coli* was isolated from 20.3% diarrheic fecal samples out of 3080 fecal samples obtained from neonatal calves with diarrhea, it appeared during first days of life.

Concerning with seasonal variation the present study showed that (Table 9 & Fig 5) showed that the highest rate of bacterial isolation occurred in winter months in which *Salmonella* isolated by 8.7% and *E-coli* isolated by 31.2 % followed by spring months in which *E-coli* isolated by 22%, autumn months and the low rate of isolation found in summer months 13.3% for *E-coli*. According to locality the high rate of bacterial isolation was occurred in Farm (1) El-Dakahilia 25% for *E-coli* and high rate of *Salmonella* isolation occurs in Farm (2) Demiatta (6%) Table 10 & Fig 6. In serotyping of *E-coli spp.* and *Salmonella spp.* (Table 11) showed that among 46 isolate of *E-coli,* *E-coli* K_{99} antigen was detected serologically in 5 samples by (10.9%) and *E-coli* antigens O119:B14, O26:B7, O111:B4 and O55:B5 were detected in 3, 8, 10 and 12 isolates respectively and 8 isolate were untyped. On the other hand, among 11 *Salmonella spp.* Isolate, 6 isolates were identified serologically as *Salmonella typhimurum* and 3 isolates *Salmonella enteriditis* and 2 isolates were untyped. The obtained results are in agreement with the results obtained by Battisti[55] who isolated 21 *Salmonella* strains from neonatal calves. Nine serotypes were found namely *S. Typhimurum, S. enteritidis, S. dublin, S. newpart, S. gallinarum, S. hader, S. isongi* and *Salmonella* group 0:8(C2). Blake[56] reported that *S. typhimurum* caused severe diarrhea and collapse in two calves (10-14 days old) out of 120 calves suckling cows in UK. In Brazil it was reported that *E-coli* was found in 100% of 51 examined samples from neonatal calves with diarrhea, the serological grouping were configured as 34.2%, 17.8% and 47.9% of the samples belonging to serological groups O8, O11, and O101 respectively, *Salmonella dublin* and *Salmonella typhimurum* were isolated in 5.4% and 6.1% of the examined samples. The same results are obtained John[57] who isolate d EHEC O26 and EHEC O111 from diarrheic and non-diarrheic young calves from 115 different farms. Of the 257 calves with diarrhea, 37 (14.4%) and 32 (12.5%) tested positive EHEC O26 and EHEC O111, respectively. suggesting that EHEC O26 and O111 are possible causes of the disease in infected neonatal calves. The high percentage of bacterial isolation in winter months may be attributed to increased relative humidity which activate microorganisms and increase number of births which facilitate contamination and spread of infection.

Different closteridial species cause intestinal disorders and enterotoxaemia in various animals species including neonatal calves demonstrated that, in neonatal calves enterotoxaemia is defined as a sudden death syndrome with lesions of hemorrhagic enteritis[59], the infectious etiology has not

Table 18: Detection of *Cryptosporidium parvum* oocysts in fecal samples from clinically diseased calves according to locality.

Farm	locality	Total clinically diseased calves	No. of infected samples	%
Farm (1)	El- Dakahilia	**80**	28	35
Farm (2)	Demiatta	**50**	10	20
Farm (3)	El- Behara	**70**	22	31.4
Total		**200**	**60**	**30**

Fig (10): Detection of *Cryptosporidium parvum* oocysts in fecal samples from clinically diseased calves according to age.

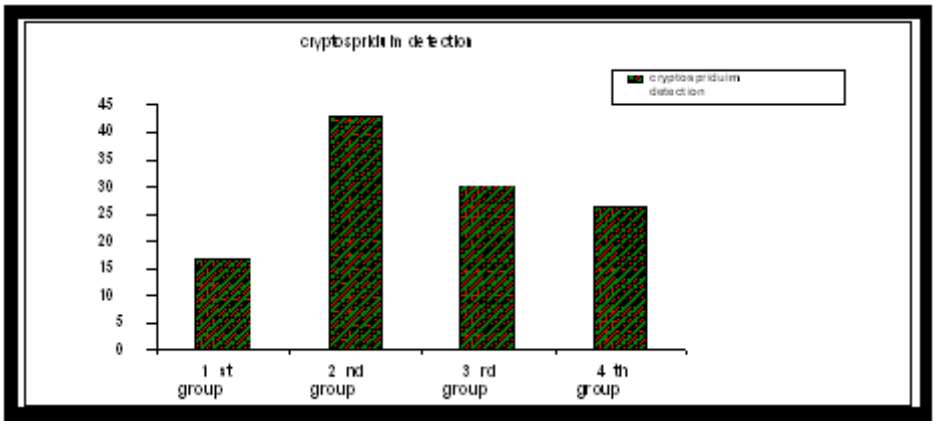
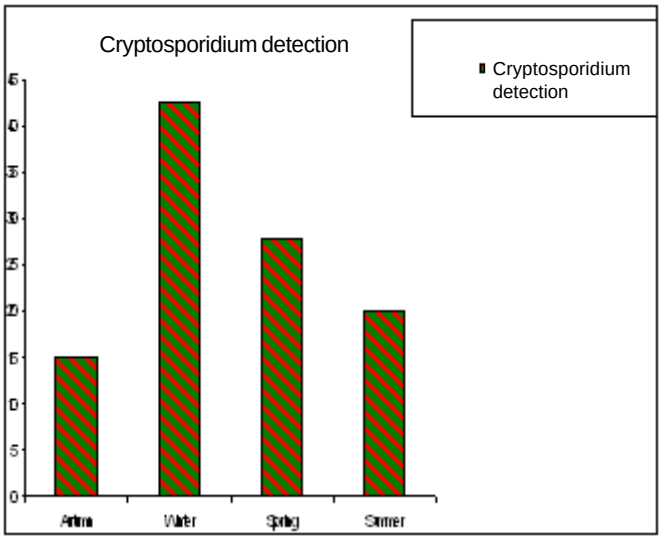
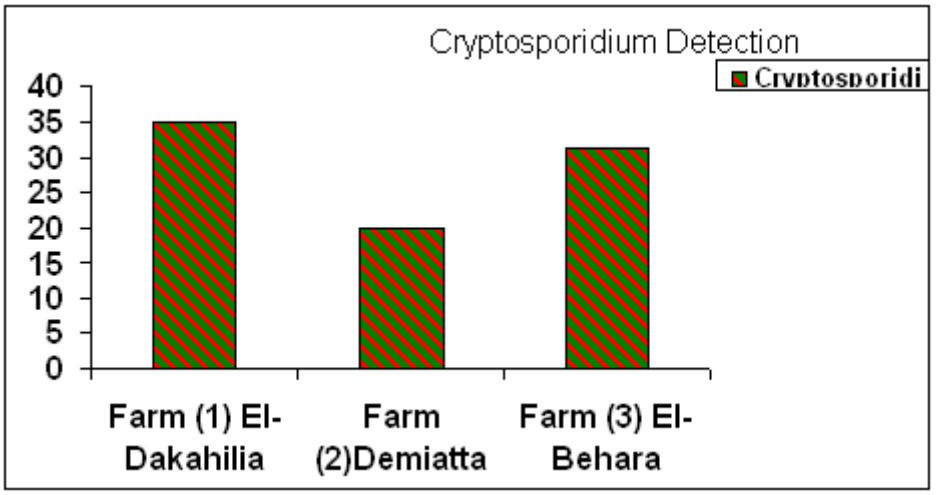


Fig 11: Detection of *Cryptosporidium parvum* oocysts in fecal samples from clinically diseased calves according to season



been identified, although *Clostridium perferingens* is often regarded as responsible[60]. Our bacteriological investigation showed that, 21 samples of 200 samples examined (10.5%) were found positive for isolation of *clostridium perfringens*. the highest rate occurred in 4th age group (13.3) followed by 1st group (11.7%), then 2nd and 3rd age groups (10% & 7.5%) respectively. (Table 12 & Fig 7). The results also demonstrated that, the highest rate of *clostridium perferingens* isolation and toxin detection occurred in winter months in farm (3) El-Behara governorates (13.8% & 18.5 %) followed by spring months and autumn months and summer months by 10% & 7.5 % & 6.5% respectively. (Tables 13 &14 and Figures 8&9). Also our result demonstrated that Typing of toxigenic strains by toxin–antitoxin neutralization by dermonecrtic reaction in guinea pigs revealed that the *closteriduim perferingens* type A is the highest type isolated (15) followed by type B (5) and type D in one samples. Table 15 agrees with the result obtained by Haschek[61] who found that the prevalence of *Clostridium perferingens* was 9.1% in collected feces of 230 calves with and without diarrhoea during the winter period 2004/2005 in 100 Austrian farms (Styria and Lower Austria), a higher prevalence of infection was reported by Ramzy[62] who examined fecal samples taken from 344 calves aging between a day to 4 months old from dairy farms in Fayoum, Kafr El-sheikh and Beharia Governorates, 293 of them were apparently healthy and 51 of them were diarrheic. Microbiological examination revealed that, the isolation of *Clostridium perferingens* was 71.32%, 59.65% and 57.01% from the apparently healthy calves and 96.15%, 88.89% and 87.5% from the diseased (diarrhoeic calves) in Fayoum, Kafr El-sheikh and Beharia Governorates respectively. The highest percentage of isolations was in 1 day–1-week-old calves as it reached 80.61%. Harbby[63] examined fecal swabs collected from 200 calves (150 diarrhoeic, 20 apparently healthy and 30 intestinal samples) aged from one day to 12 weeks old. *Clostridium perfringens* were detected in (66.7%). The differences in

Fig 12: Detection of *Cryptosporidium parvum* oocysts in fecal samples from clinically diseased calves according to locality.



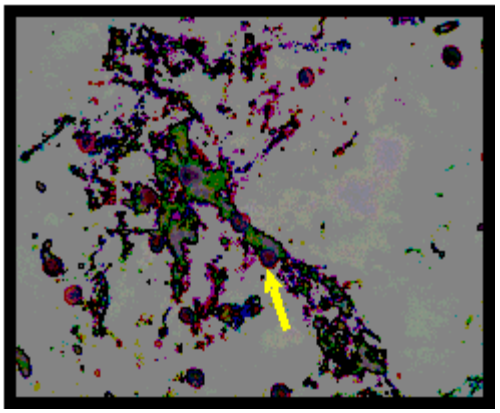


Plate 1: Fecal smear showing severe *cryptosporidium* infection more than 20 oocysts /HPF (modified Zheil – Neelsen)X100.

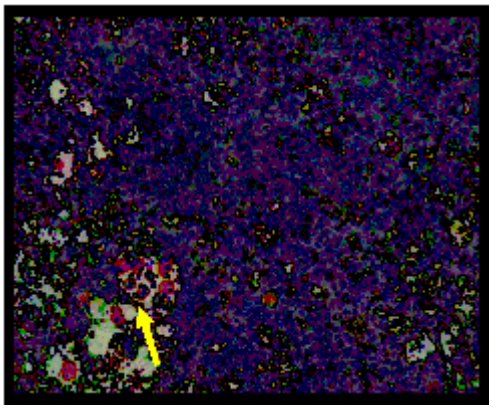


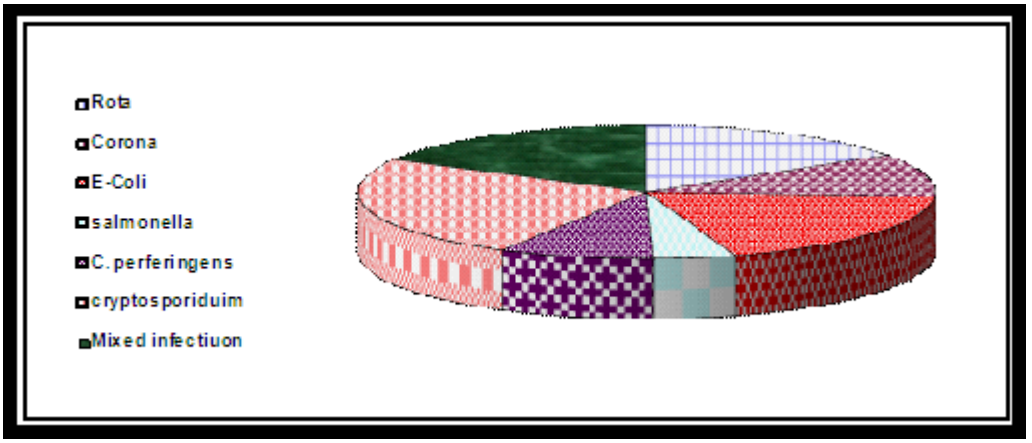
Plate 2: Fecal smear showing moderate *cryptosporidium* infection 6 - 20 oocysts / HPF (modified Zheil – Neelsen)X100.

results may be attributed to feed changes, in the form of irregular feeding, could have accounted for the hungry animals drinking an excess of milk when they had the opportunity to do so. Drinking an excess of milk has been suggested to be responsible for promoting an overgrowth of *C. perfringens* in addition, the lack of colostrum and poor nutrition were most likely responsible for low immunity and stress.

The participation of *Cryptosporidium spp.* in the neonatal calf diarrhoea is also important as this protozoon is hard to treat and can be found in animals with or without diarrhoea, making its control even more difficult. Parasitological examination revealed that, *Cryptosporidium parvum* oocysts were detected in 60 fecal samples out of 200 samples examined 30% by floatation and staining method by modified Zheil Neelson stain[31], the infection ranged from mild to severe. Plates 1 & 2.

According to prevalence of age, the highest rate of infection was found in 2nd group of age 42.8% Table 16 & Fig 10. Followed by 3rd group 30% and fourth group 26.6 % the obtained results are congruent with the results obtained by Surumay[64] who examined 75 fecal samples from young cattle aged between 2 to 20 weeks, collected from four western dairy farms of Venezuela for the presence of *Cryptosporidium oocysts.* It was observed that in 22 animals 29.3%, Lisse[65]. that *Cryptosporidium parvum* infection in 203 40.6% of 500 Ontario dairy calves aged 7-21 days, on a convenience sample of 51 farms in Canada with a history of calf diarrhoea. Balbir[66] detected *cryptosporidial parvum* oocyst infection in fecal samples of 40 from 80 diarrhoeic calves 50% in dairy farms in Punjab, India and added that the prevalence of infection peaked in young calves between 1 and 30 days of age. In Cairo and Giza governorates[67] recorded a higher incidence 33.3% in diarrheic calves aged from one day to one month. Otify[68] mentioned that a high rate of *Cryptosporidium* infection 28.75% was recorded in calves 10–20 days old while Abdel-Salam[69] recorded that the highest rate of infection 51.88% in calves was from one day to one month. It was noticed that the profile of age associated shedding and the

Fig 13 Total infectious agent isolated from diseased neonatal calf with diarrhea



age at which the point prevalence of shedding reached maximum (15 days of age in this study) was very similar to previous work[70,71,72] this observation is consistent with the hypothesis that dairy calves become infected with *Cryptosporidium parvum* either from the post parturient dam while in the materny pen or from hutches contaminated with oocysts from a previous calf. This position supports Maldonaldo[73] who examined 131 dairy farms in three states in central Mexico and found that the overall point prevalence was 25 %, with a maximum risk of shedding at 15 days of age. Ryan[74] recorded that prevalence of cryptosporidiosis were high in 20 Holstein calves in a single farm, the mean age at which *cryptosporidiosis* oocysts were first detected was 16 days.

Also the results demonstrated that the highest rate of infection was detected in winter months (42.5%), followed by spring 28% and summer months 20%. Table 17 & Fig 11. According to locality the higher rate of infection were observed in farm (1) El-Dakahilia 28 samples (35%) followed by farm (3) in El-Behara 22 samples (31.4%) and at last Farm (2) in Demiatta 10 samples (20%) Table 18 & Fig 12 our results are in agreement with the results obtained by EL-Khodery[75] who reported that the high rate of infection with *Cryptosporidium* was observed in winter (46.09%) followed by spring 31.61%, autumn 26.04% and summer (9.37%). Conversely, Olsen[76] found that the highest prevalence of *cryptosporidial* infection was found in summer and autumn months. In Egypt the present result is partially different with those reported by Khalil[67] and Abdel-Salam[69]; who reported that the highest rate of infection was in summer and autumn, these differences could be attributed to local weather (humidity, temperature), the lowest rate of infection was in summer this may be attributed to dryness at the first consideration and high environmental temperature.

The infectious causes of neonatal calf diarrhea are numerous, which may be present either singly or in combination, on the other hand the non-infectious causes, which are not discussed in details here, may also be important; these include improper diet or feeding practices, or poor quality milk replacer. In our investigation the infectious causes of neonatal calf diarrhoea in this study represent 80% Table 19 & Fig 13, in which the infectious causes were detected in 160 samples out of 200 samples examined, while the non

infectious causes represented in 40 samples (20%) this may be due to other factors as overfeeding or change in milk diet or irregular feeding. No enteropathogen were detected in 20% of the neonatal calves, the ratio of negative results is similar to or lower than that reported in other surveys involving diarrhoeic calves[77,78], negative results may be explained as some cases of diarrhea may not be associated with infectious agents as nutritional or management factors, or because other non investigating pathogens were involved, many other enteric bacteria and viruses have also been associated with the disease, but their role as causative agents of calf diarrhea or their relative importance is not well defined.

The rate of mixed infection in our study with two or more enteropathogens was 20.5%, it was detected in 41 samples out of 200 samples examined and their distribution shown in (Table 20 & Fig 14). These results nearly agree with the results that obtained Fuentine[78] who recorded that mixed infection was much more commonly detected in diarrheic calves (28%) suggesting that the presence of more than one enteropathogen may be one of the factors determining whether infection results in a clinical or subclinical presentation, on the other hand, mixed infection may be associated with more severe disease. This percent of mixed infection is higher than that reported by others in surveys involving diarrhea in calves carried out in different countries which ranged from (5–20%)[30, 79].

The high detection rate of mixed infection in these studies may be due to the biased submission of severe diarrhea, the most common mixed infection was *Rota*virus, *Cryptosporidium* as more than

Fig 14: Distribution of mixed infection in neonatal calf diarrhea

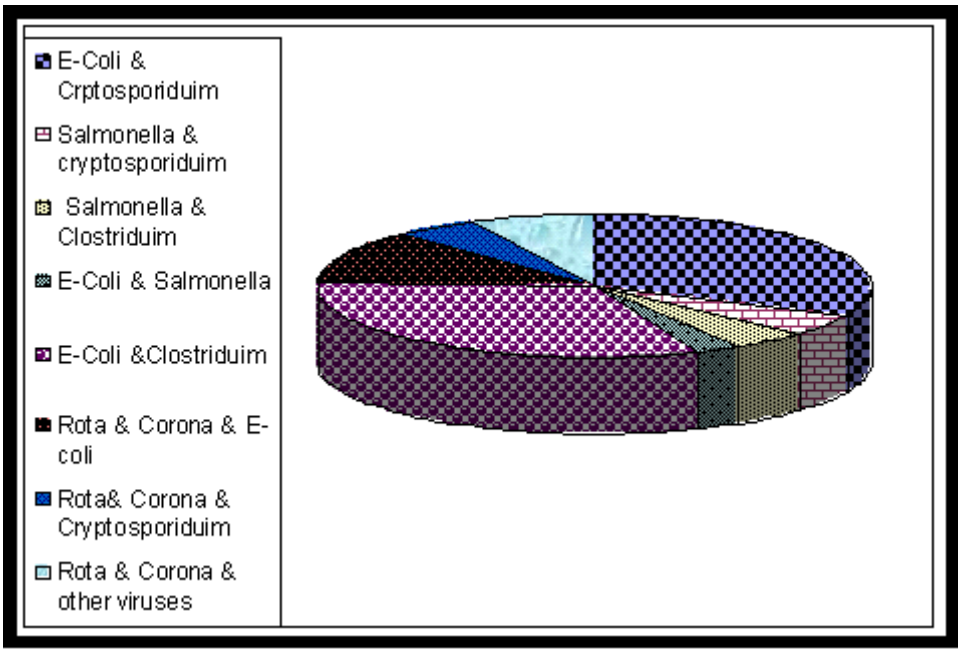


Table 19: Total infectious agents isolated from diseased neonatal calves with diarrhea

Clinically diseased calves	No of infectious cases		Non infectious cases		Distribution of infectious agent													
					Rota V.		*Corona V.*		*E.Coli*		*Salmonella*		*Clostridium perferingens*		*Cryptosporidium parvum*		*Mixed infection*	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
200	160	80	40	20	36	17.5	25	12.5	46	23	11	5.5	21	10.5	60	30	41	20.5

Table 20: Distribution of mixed infection in neonatal calves diarrhea.

No. of mixed infection	*E-Coli & Cpto*	*Salmonella & crypto*	*Salmonella & Clostridia*	*E-Coli & Salmonella*	*E-Coli & Giost*	*Rota & Corona & E-coli*	*Rota & Corona & Crypto*	*Rota & Corona & other viruses*
41	13	2	2	1	13	5	2	3

20% of calves excreted *Rotavirus* at the same time as *Cryptosporidium*. This is in agreement with results published in most surveys all over the world. Garacia[80] found that detection rate of other enteropathogens considered in calves with *Rota*virus were 20.4% for *Corona virus*, 85.2% for *cryptosporidia,* 16.7% for F5+ *E-coli* and 1.8% for *Salmonella*.

Conclusion

The study indicated the effects of age and location (management) on neonatal diarrhoeic infection in cattle in three randomly selected farms in Egypt. The data confirms the principal causal agent in neonatal calf diarrhoea in Egypt as *Escherichia coli, Rota*virus, *Corona* virus, *Clostridium perfringens, Salmonella* spp and *Cryptosporidium* spp as a single or mixed infection by the isolated agents. In addition, diet, management and sanitation are also significant contributory factors in the aetiology of neonatal calf diarrhoea in Egypt.

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SOME STUDIES ON NEONATAL CALF DIARRHOEA IN EGYPT

PART 2: Control

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ETUDES SUR LA DIARRHEE DU VEAU NEONATAL EN EGYPTE

2ème Partie : Contrôle

Résumé

Deux vaccins commerciaux disponibles ont été utilisés dans un essai pour le contrôle de la diarrhée du veau néonatal. Trente vaches gravides au dernier trimestre de gestation ont été réparties en trois groupes égaux. Dans le premier groupe, on a administré aux animaux le vaccin Entero-3 (5ml/animal, 2 doses, à deux semaines d'intervalle) ; dans le deuxième groupe, le vaccin Scour guard 3 « K » (2ml/animal, 2 doses, à deux semaines d'intervalle) et dans le dernier groupe, les animaux n'étaient pas vaccinés (groupe-témoin).

Des prélèvements de sérums ont été recueillis des animaux vaccinés et des sujets témoins au jour 0, 7, 14, 21, 28, 35 après la vaccination et le 1er, 4è, 8è, 14è, 21è, 28è jour après la mise bas des veaux.

La réponse immunitaire des animaux vaccinés a montré un accroissement linéaire des titres d'anticorps neutralisant dans le sérum après la deuxième dose d'injection avec le niveau maximum à la mise bas comparé aux animaux non-vaccinés du groupe-témoin. L'immunité passive chez les veaux après l'ingestion de colostrum a indiqué que le titre d'anticorps neutralisant dans le sérum a montré un titre maximum le premier jour, puis a baissé peu à peu et a atteint un niveau minimum de protection le 35è jour comparé aux veaux du groupe non-vacciné.

On peut conclure qu'il faudrait faire le test de sensibilité pour choisir le meilleur antibiotique à utiliser le plus tôt possible avec d'autres produits thérapeutiques pour le traitement des animaux. La vaccination des vaches gravides avec des vaccins commerciaux disponibles confère une bonne protection contre la diarrhée du veau néonatal.

Mots-clés : Diarrhée du veau néonatal, veaux, test de sensibilité, vaccin, vaccination, immunité active, immunité passive.

Summary

Two commercially available vaccines were used in a trial to control neonatal diarrhea. Thirty pregnant cows at the last trimester of gestation were allocated into three equal groups. In the first group, animals were administered Entero -3 vaccine (5ml/animal, 2

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doses, at two weeks interval); in the second, Scour guard 3 "K" vaccine (2 ml/animal, 2 doses, 2 weeks interval) and the last group was the no-vaccinated control.

Sera samples were collected from the vaccinated and control animals at day 0, 7, 14, 21, 28, 35 post vaccination and 1st, 4th, 8th , 14th , 21st , 28th day after birth for the calves.

The immune response of animals vaccinated showed a linear increase in serum neutralizing antibody titers after 2nd dose of injection with the maximum level at time of parturition compared with non vaccinated control animal. The passive immunity in calves after colostrum intakes revealed that serum neutralizing antibody titers showed maximum titer in first day, then decreased gradually and reached the minimum protective level at 35 days compared to calves in non-vaccinated group.

It can be concluded that the Sensitivity test should be done to determine the best bet antibiotic to be used in treatment as early as possible with other therapeutics. Vaccination of pregnant dams with available commercial vaccines give good result for protection against neonatal calf diarrhoea.

Key word: Neonatal calf diarrhoea, calves, sensitivity test, vaccine, vaccination, active immunity, passive immunity.

Introduction

Neonatal calf diarrhea is a multifactorial disease which despite decades of research on the topics remains the most common cause of deaths in neonatal calves.

Studies have shown that antibiotics[1,2], probiotics[3], maternal vaccines[4,5], are efficient in controlling diarrheal diseases. However, with many of these products there are concerns about antimicrobial resistance, product availability, producer acceptance, and cost.

Passive transfer of immunoglobulin in colostrum is the important source of immunoglobulin protection available to neonatal calves, inadequate intake and absorption of maternal antibody has been associated with risk of diarrhoea and deaths of neonates[6] .

Trial of vaccination of pregnant cows in the last quarter of pregnancy was done by several researcher based on using mono, bi or trivalent vaccine in Egypt and overseas[5,7,8,9,10,11,12,13], they reported that protection of the calves against neonatal diarrhea has been achieved by vaccination of the dams with combined inactivated vaccines containing rotavirus, *corona* viruses and *E-coli* K_{99} on two to three months before the anticipated calving date.

The objectives of this study were to assess the sensitivity of antimicrobial test to previously isolated bacteria and the protection of calves through the vaccination of the pregnant dams with inactivated killed trivalent vaccines.

Material and Methods

Animal for Vaccination trails:

Apparently healthy 20 pregnant cows at the last trimester of pregnancy were selected from private farm at Dakahalia governorate, Egypt for vaccination with either Entero-3 or Scour guard vaccine; in addition 10 pregnant cattle were kept as non-vaccinated control.

Serum samples

Blood samples were collected from both vaccinated and non vaccinated control pregnant cows and their calves for monitoring immunity without anticoagulant for serum separation. The separated serum were kept at -20^{0}C until required for analyses.

Vaccines:

A. Entero-3 vaccine, the vaccine is registered and produced by Veterinary Serum and Vaccine Research Institute, Department of Rinderpest like diseases. It is a combined inactivated vaccine used to protect cattle and buffalo calves against *Rotavirus, Corona* viruses and *E-coli* K_{99} infections. This vaccine was kindly supplied by Rinderpest Like Diseases Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

B. Scour Guard 3"K" vaccine is registered and produced by Pfizer, animal health, Exton, PA 19341, USA. It is used to protect cattle and buffalo calves against *Rotavirus, Corona viruses* and *E-coli* K_{99} infections.

Viruses and bacterial antigens

A. *Rota* virus antigen, Local strain of *Bovine Rota* Virus (BRV) was used for serological test. The virus was isolated from calves suffered from diarrhea[14].

B. Corona virus antigen, Nebraska strain of *Bovine Corona* Virus (BCV) was used for serological test.

C. E-coli K_{99} antigen, Enterotoxigenic *E-coli* K_{99} strain was kindly supplied from Animal Reproduction Research, Institute, Giza, Egypt.

Reference antisera:

Specific polyclonal antisera for BCV, BRV, and *E-coli* K_{99} were kindly supplied from the Serum and Vaccine Research Institute, Abbasia, Cairo.

Experimental vaccination by Entero-3 and Scour Guard 3K vaccines:

The first group of pregnant cows (10) was administered Entero-3 vaccine, in a dose of 5ml/animal, 2 doses, at 2 weeks interval by intramuscular injection (i/m). the second one (10) administered *Scour guard 3 "K"* vaccine, in a dose of 2ml/animal, 2 doses, at 2 weeks interval by i/m and the last 10 were kept as non-vaccinated control. All pregnant cattle were kept under the same environmental conditions. Dams and their calves were kept under clinical observation for 1 month post parturition. Sera samples were collected at day of 1st injection (0 day),7, 14, 21, 28 and 35 days post vaccination for dams while sera samples from offspring were collected at the end of 1st, 4th, 8th, 14th, 21st and 28th days after birth to monitor the active immunity.

Antimicrobial sensitivity test:

The agar disc diffusion technique was used by standard method[15]. The test was carried out on all isolate of *salmonella* and *E-coli spp.* The antibiotic kits and their potency are illustrated in Table 1.

Serum Neutralization Test (SNT)

The test was conducted for titrations of specific developed antibodies in vaccinated cattle dams as well as in their offspring. The test was performed according to the method described by Cerberey and Lee[16] using flat bottom sterile micro-plates 96 well as follows.

- 50 µl of MEM was added in all wells in the plate.
- 50 µL of each serum sample was

transferred to wells of first line using micro titer pipette to obtain dilution 1:2 then serial two fold dilutions were conducted.

- 50µL of MEM containing 100 TCID50 / ml of virus were added to all wells of the plate.
- before inoculation of virus on cell culture; the virus was treated with 0.1 ml of trypsine for 30 minutes at 37°C[17]
- The plates were incubated at 37°C for one hour.
- 100µL of the cell suspension of MDBK cell was added to the microplate. Then incubated at 37°C and was observed daily for five days for the presence of CPE.
- The reciprocal of the highest dilution of serum inhibiting CPE was expressed as the neutralizing antibody titer.

Microplate agglutination test:

This test was done by the method described by Collins *et al*[18] for determination of *E-coli* K_{99} antibody titer.

Results and Discussion

Trials for treatment and vaccination

a. Treatment

Protocol of the treatment was applied in the examined farms by using oxytetracyclin, sulfaquinoxaline, tannic acid, pectine, koaline, neomycin, scour pan and amoxicillin, chloromphenicol, enrofloxacine and fluid therapy. Some cases responded well to treatment and others did not respond and were discarded or died. Table (2) show Antimicrobial sensitivity test to determine the antibiotic of choice used with other therapy in cases of neonatal calf diarrhoea caused by isolated bacteria. Ciprofloxacin had the highest sensitivity 91.3%, 90.90% followed by Enrofaxacin 86.96, 72.72 for *E-coli* isolate and *Salmonella* isolate respectively followed by Gentamicin 82.61% for *E-coli* isolate and by chloromphenicol 63.63% for *Salmonella* isolates.

Table 1: The different antimicrobial agents used in the agar diffusion method and interpretation of their sensitivity.

NO.	Antimicrobial agent	Manufacture	Disk potency	Diameter of inhibition zone (mm)	
				Resistant	Sensitive
1	Enrofloxacin	Oxiod	10µg	≤ 15	≥ 21
2	Ciprofloxacin	Oxiod	5µg	≤ 15	≥ 21
3	Chloramphenicol	Oxiod	30µg	≤ 12	≥ 18
4	Colistin sulphate	Oxiod	10µg	≤ 8	≥ 11
5	Flumequine	Oxiod	30µg	≤ 16	≥ 16
6	Gentamicin	Oxiod	10µg	≤ 12	≥ 15
7	Oxytetracyclin	Oxiod	30µg	≤ 14	≥ 19
8	Amoxicillin	Oxiod	10µg	≤ 13	≥ 18
9	Neomycin	Oxiod	30µg	≤ 12	≥ 17
10	Tylosine	Oxiod	15µg	≤ 13	≥ 18
11	Trimethoprim/ sulphamethazole	Oxiod	1.25 + 23.75µg	≤ 10	≥ 16

Table 2: Antimicrobial sensitivity test for *E-coli Spp.* and *Salmonella Spp.* isolated from clinically diseased calves.

Antimicrobial agent	*E-coli spp.* (46 isolate)			*Salmonella* spp. (11 isolate)		
	No.	%	Zone	No.	%	Zone
Enrofloxacin	40	86.96	≥ 21	8	72.72	≥ 21
Ciprofloxacin	42	91.30	≥ 21	10	90.90	≥ 21
Chloramphenicol	20	43.48	≥ 18	7	63.63	≥ 18
Colistin sulphate	5	10.87	≥ 11	2	18.18	≥ 11
Flumequine	6	13.04	≥ 16	0	0	≤ 16
Gentamicin	38	82.61	≥ 15	0	0	≤ 12
Oxytetracyclin	2	4.35	≥ 19	1	9.09	≥ 19
Amoxicillin	4	8.70	≥ 18	1	9.09	≥ 18
Neomycin	5	10.87	≥ 17	0	0	≤ 12
Tylosine	0	0	≤ 13	0	0	≤ 13
Sulphatrimethoprim	0	0	≤ 10	0	0	≤ 10

b. Vaccination Trials

Microagglutinating titers obtained by cows received Entero-3 or scour guard vaccine presented in Table (5) Fig (5) revealed that the mean agglutinating titers increased after injection of second dose of vaccine and reached maximum titer at time of parturition. While in the calves received colostrum, Table (6), Fig (6) the titers was higher in first day then gradually decreased till minimum protective level at 28 days of age was reached.

The results obtained in farms where the clinical cases were examined are similar to those of Ismail *et al*[19], who concluded that norofloxacin had the highest sensitivity among all antimicrobial agents they tested followed by chloromphenicol and gentamycin, and high resistance to cephalexine and neomycin in bacteria isolated from dairrhoeic calves. On the other hand Sharma *et al*[20] studied the association of *Escherichia coli* in neonatal calf diarrhoea (NCD) and their serotype and antibiotic sensitivity pattern; 39 serotypes from 138 strains of *E. coli* recovered at the C.R.I. Kasauli (H.P.). The serotypes O123, O28, O23, O2, O8, O25, O55, O60, O4 and O38 were predominant in Bikaner, India. Maximum number of strains was sensitive

Table 3: Immune response of Immunocompetant pregnant cows post vaccination with (Entero-3) vaccine or Scour Guard vaccine

Groups	Viruses	Mean Log_{10} serum neutralizing antibody titers for *Rota and Corona* viruses					
		0 day*	7 day	14 day**	21 day	28 day	35 day
Vaccinated With Entero-3	*Rota*	0.48	0.84	1.38	2.28	2.58	2.7
	Corona	0.48	0.96	1.32	1.98	2.4	2.46
Vaccinated Scour Guard	*Rota*	0.42	0.78	1.44	2.1	2.58	2.7
	Corona	0.42	0.84	1.38	1.86	2.28	2.4
Non-vaccinated	*Rota*	0.38	0.34	0.32	0.25	0.16	0.28
	Corona	0.42	0.43	0.44	0.41	0.35	0.38

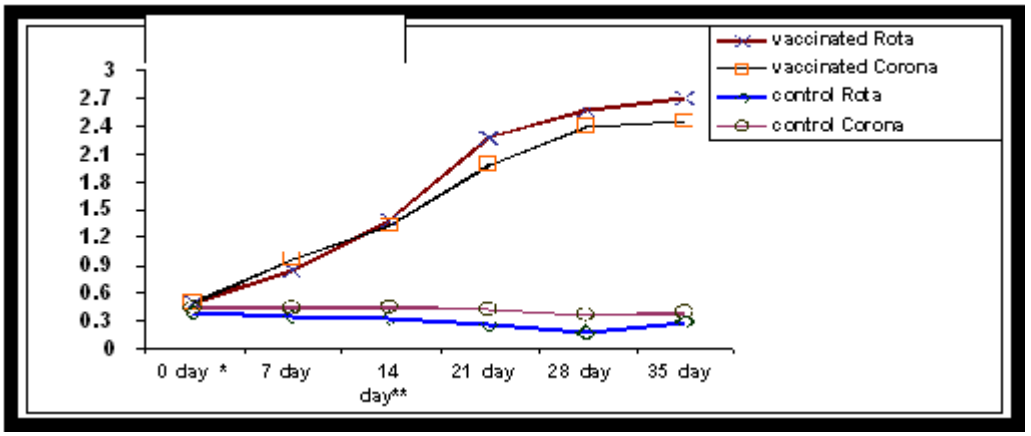
* zero day 1st dose 5ml i/m ** 2nd dose 5ml i/m

Table 4: *Rota* and *Corona* mean $\log_{10}$ serum neutralizing antibody titers in (Entero-3) or (Scour Guard) vaccinated pregnant cows at delivery time and in their offspring until one month of age

Viruses	Mean $\log_{10}$ serum neutralizing antibody titers for *Rota and Corona* V.							
	Dam		Offsprings					
	Delivery time*	Titer	1 day	4 day	8 day	14 day	21 day	28
Rota	27 day	2.7	2.58	2.4	2.22	1.86	1.56	1.
Corona		2.46	2.28	2.04	1.74	1.5	1.26	0
Rota	31 day	2.7	2.46	2.46	2.16	1.92	1.30	1.
Corona		2.4	1.98	2.04	1.8	1.44	1.08	0.
Rota	25 day	0.30	0.35	0.32	0.35	0.35	0.35	0
Corona		0.26	0.25	0.26	0.22	0.20	0.20	0.

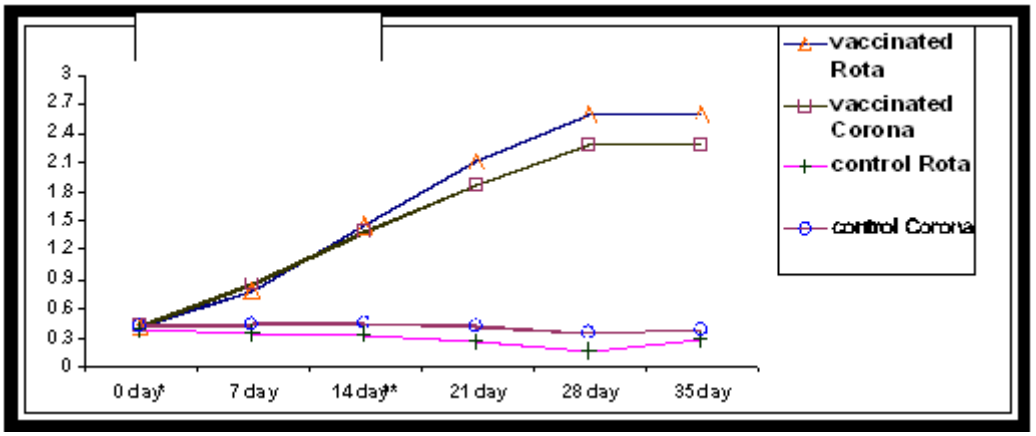
* Delivery time from second dose injection

Fig 1: Immune response of Immunocompetant pregnant cattle vaccinated with (Entero-3) vaccine and non-vaccinated group.



* zero day 1st dose 5ml i/m ** 2nd dose 5ml i/m

Fig 2: Immune response of Immunocompetant pregnant cattle vaccinated with (Scour Guard 3 K) vaccine and non-vaccinated group.



* zero day 1st dose 5ml i/m ** 2nd dose 5ml i/m

Fig 3: *Rota* and *Corona* mean log10 serum neutralizing antibody titers in (Entero-3) vaccinated pregnant cows at delivery time and in their offspring until one month of age

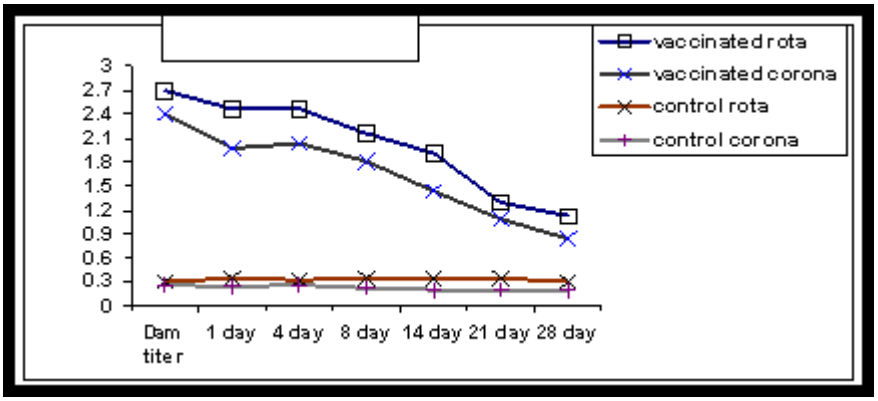


Fig 4: *Rota* and *Corona* mean log10 serum neutralizing antibody titers in (Scour Guard 3K) vaccinated pregnant cows at delivery time and in their offspring until one month of age.

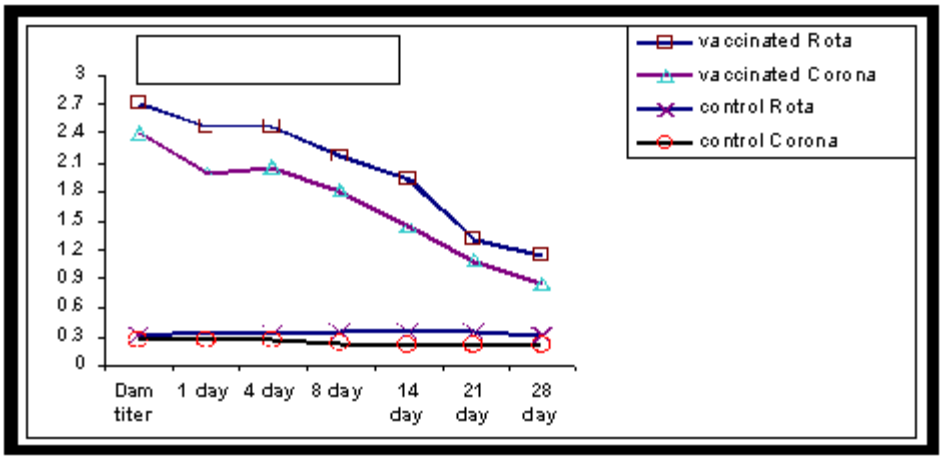


Table 5: *E-coli* K_{99} Microagglutination titer in late pregnant cows post vaccination with either Entero-3 or Scour guard-3K vaccine.

Vaccine used	Microagglutination titer at weeks post vaccination				
	Zero*	2**	3	4	5
Entero-3	0	768	1024	1536	1536
Scour guard	0	512	1024	2048	2048
control	12	12	32	16	32

* Time of first dose ** Time of second dose

Table 6: *E-coli* K_{99} Microagglutination titer in offsprings of cows post vaccination with either Entero-3 or Scour guard-3K vaccine.

Groups	Dams vaccination	Titer of dams at parturition	Microagglutination titer at age of					
			1st day	4th day	8th days	14th days	21st days	28th days
Vaccinated	Entero-3	1036	2048	2048	2048	1024	512	512
	Scour guard	2048	2048	2048	1280	1280	486.4	512
Control		32	32	64	32	128	32	16

Fig 5: *E-coli* K_{99} Microagglutination titer in late pregnant cows post Vaccination with either Entero-3 or Scour guard-3K vaccine.

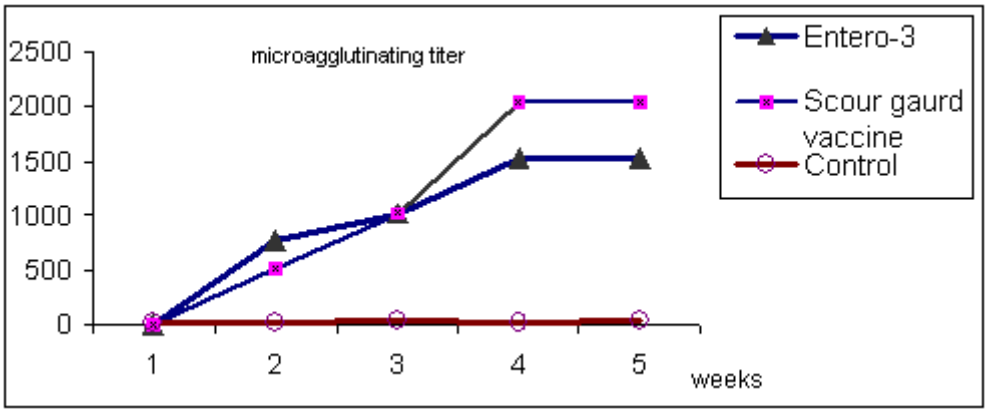
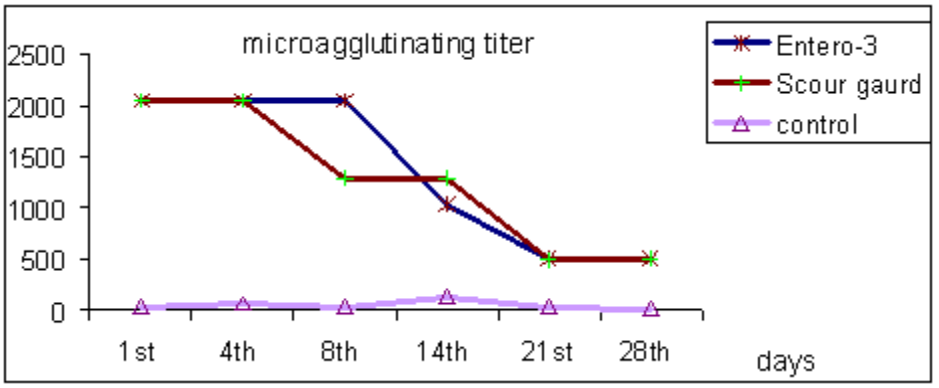


Fig 6: *E-coli* K_{99} Microagglutination titer in offspring is of cows post Vaccination with either Entero-3 or Scour guard-3K vaccine



to norofloxacin (97.28%), kanamycin (93.24%), ciprofloxacin and Chloramphenicol (92.6% each), gentamicin (85.62%) and contrinazine (71%).

Antibiotics alone have never been the answer to calf scours. Fluids have always been the most important, regardless of the causative agent. The calf dies from dehydration, so we must keep fluids going in the front end faster than they come out the back end. Scours are more effectively prevented than treated[21].our efforts to minimize neonatal calf diarrhoea are focused on its prevention, while the early appearance of diarrhea makes the use of calf vaccines impractical and inefficient, the disease preempts prevention, the active immune response of the calves is not yet developed, while there are still many theories to when the calf becomes immuno-competent, thus prevention is best achieved through maternal vaccination or establishing passive

immunity. Our present experimental field study describes the successful application of trivalent killed adjuvanated vaccines which included *Rota* and *Corona* viruses and *E-coli* K_{99} bacterin, the two commercially available vaccines in Egypt were used one locally produced (Entero-3) and the other is imported produced by Pfizer (Scour guard). The vaccination approach considered the importance of vaccinating pregnant cattle by two doses, the first one was 8-10 weeks and the second booster dose was 2–4 weeks before parturition to ensure the availability of high protecting antibody titers against *Rotavirus, Corona virus* and *E-coli* in the sera and colostrums of these animals.

The goal of a vaccination program is to prevent the occurrence and severity of an infectious disease. The payoff, however, comes in the huge dividends that result in reducing the incidence of lost pregnancies, poor-performing calves and calf mortalities. Vaccination does not completely prevent infection; it only stimulates the development of immunity.

In these trials, the vaccinated and control non vaccinated pregnant cattle as well as their offsprings were kept under observation till the end of the experiment when calves reached one month of age, it was noticed that calves born from vaccinated cows remained in a good health condition all through the experimented period, except that one calf in each vaccinated group that suffered mild diarrhoea, compared to the non-vaccinated control group in which 6 manifested varying degrees of diarrhoea. Saleh *et al*[22] reported that morbidity rates from neonatal calf diarrhoea was 4% in calves feed on colostrums of cows immunized with trivalent vaccine containing *Rota* & *Corona* viruses and *E-coli,* while it was 24.7% in calves feed on colostrum from non-vaccinated cows.

The serum neutralizing antibody titers to *Rota* and *Corona* viruses in vaccinated cows and non-vaccinated represent in (Table 3 and Figs 1 & 2) is evident that there is a remarkably increase in the mean neutralizing antibody titers at the time of the 2nd dose, reaching to $\log_{10}$ 1.38 and 1.32 to *Rota* and *Corona* viruses respectively in groups vaccinated with Entero-3 vaccine and log10 1.44 and 1.38 to *Rota* and *Corona* viruses respectively in groups vaccinated with Scour guard vaccine compared with 0.32 and 0.44 in non vaccinated groups. At calving the mean serum neutralizing antibody titers for *Rota* and *Corona* viruses in vaccinated cows were sharply increased till reached $\log_{10}$ 2.7 and 2.46 for *Rota* and *Corona viruses* respectively in groups vaccinated with Entero-3 vaccine and $\log_{10}$ 2.7 and 2.4 for *Rota* and *Corona* viruses respectively in groups vaccinated with Scour guard vaccine. Doaud *et al*[12] stated that the application of trivalent vaccine (entero-3) in breeding farms, gave satisfactory results when pregnant cows were vaccinated at late stage of pregnancy, elevated level of serum and colostral antibodies against BRV, BCV and *E-coli* colostral antibodies passively protected the offspring during the critical period, and remained with a high titer to the end of sampling time of 30 days post parturition

The results demonstrated that calves born from vaccinated dams their neutralizing antibody titers were sharply increased after ingestion of colostrums, the mean *Rota* and *Corona* neutralizing antibodies titers were $\log_{10}$ 2.7 and 2.46 for *Rota* and *Corona viruses* respectively in groups vaccinated with Entero-3 vaccine and $\log_{10}$ 2.7 and 2.4 for *Rota* and *Corona* viruses respectively in groups vaccinated with Scour guard vaccine,

the antibody titer remained high all over the experiment. On the contrary, calves born from non-vaccinated cows after ingestion of colostrums, their mean serum neutralizing antibiosis titer began very low. The results are represented in (Table 4 and Figs 3&4). Omyama[9] stated that calves born to cows vaccinated with trivalent killed adjuvant *bovine Rota,Corona viruses* and *E-coli* bacterin had higher antibodies titers in their serum against *Rota, Corona* and *E-coli* than sera of calves' born to non-vaccinated cows. Nawal *et al*[8] estimated antibodies response in pregnant dams vaccinated with killed *bovine Rota, Corona viruses* and *E-coli* bacterin; they concluded that calves delivered from vaccinated dams gave high titers if compared to a titer given by calves delivered from non-vaccinated dams.

The passive immunity that vaccine provided through maternal colostrum ingested by their calves, born in a breeding herd on a farm with customary management, but with antecedents of a high rate of calf neonatal diarrhea, demonstrated that the strategy of vaccinating pregnant mothers between 60 and 30 days prior to calving and with an interval between doses of approximately 20days, was very satisfactory[23].

Micro-agglutinating titers obtained by cows received Entero-3 or Scour Guard vaccine are presented in Tables (5,6), Figs (5,6) revealed that the mean agglutinating titers increased after injection of the second dose of vaccine and reached maximum titer at time of parturition. While in the calves received colostrums from vaccinated dams, the titers was higher in first day then gradually decreased till reach minimum protective level at 28 days of age. This result go in harmony with that obtained by other authors[11, 12] who showed that, a single inoculation of pregnant cows with the combined *Rota*virus, *Corona* virus, *E-coli* F_5 (99) vaccine significantly improved the level of passive immunity in their calves for at least 28 days and added that overall herd immunity could be improved by bringing the titers of antibodies in colostrums and milk up to a similar level which should help to control these three significant causes of neonatal diarrhea in cattle when it is used as a part of good farm management program. The results obtained indicated that their is no significant difference between antibody titers gained by using Entero-3 or Scour Guard 3K vaccine in sera of vaccinated pregnant cattle and in their offsprings.

It can therefore be concluded from the present results that Ciprofloxacin is the antibiotic of choice can used in cases of neonatal calf diarrhea with *Salmonella* and *E-coli* infection with other therapy. Improving hygienic measures in the farm and calf management are necessary with vaccination of pregnant cows at last trimester of pregnancy with Entero- 3 or Scour Guard 3K vaccine to control neonatal calf diarrhea.

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A CROSS-SECTIONAL SURVEY OF MANGE MITE INFESTATIONS IN GOATS IN TURKANA DISTRICT, NORTH-WESTERN KENYA

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UNE ENQUÊTE TRANSVERSALE SUR L'INFESTATION PAR L'ACARUS DE LA GALE CHEZ LES CHEVRES DANS LE DISTRICT DE TURKANA, AU NORD-OUEST DU KENYA

Résumé

Une enquête transversale sur l'infestation par l'acarus de la gale a été menée dans le district de Turkana, au nord-ouest du Kenya. Seize *adakars* (composés de groupes d'éleveurs qui émigrent avec leurs animaux), avec 10 – 15 ménages par *adakar*, ont été sélectionnés pour conduire l'étude. Sept ménages dans chaque *adakar* étaient choisis au hasard pour l'enquête, parmi lesquels cinq ont fait l'objet d'une enquête par questionnaire. Pendant l'enquête, 560 animaux étaient cliniquement examinés pour la gale, 208 (37%) avaient des lésions cutanées, ce qui indique la présence de la gale. Il y avait plus de cas chroniques que de cas aigus, 94,7% (197/208) et 5,2% (11/208) respectivement. Les animaux avec des lésions cutanées avaient un prurit accentué et des taches cutanées érythémateuses sous forme hypersensible ; tandis que dans la forme chronique, les lésions étaient caractérisées par l'hypertrophie de l'épiderme, qui était corné et croûteux sur tout le corps. La forme chronique était beaucoup plus fréquente (P<0,05) que la forme hypersensible. Les deux sexes et tous les groupes d'âge étaient affectés, et la répartition était très forte sur les oreilles. Dans l'étude, 109 (52%) échantillons d'éraflements cutanés étaient positifs pour l'acarus de la gale. *Sarcoptes var scabiei* était le principal agent causal.

Summary

A cross-sectional survey of mange mite infestation in goats was carried out in Turkana District, North-western Kenya. Sixteen adakars (Animal camps, comprised of groups of pastoralists who migrate together with their animals) each containing 10 – 15 households were conveniently selected for the study. Seven households in each adakar were randomly selected for sampling. Five of them were subjected to a questionnaire interview. In the survey 560 animals were clinically examined for mange, 208 (37%) had skin lesions indicative of mange. There were more chronic cases observed than acute cases, 94.7% (197/208) and 5.2% (11/208) respectively. Those with skin lesions showed marked pruritis and erythematous skin patches in the hypersensitive forms, while in the chronic form, lesions were characterized by hypertrophy of the epidermis, which was horny and scabby all over the body. The chronic form was significantly ($p<0.05$) more common than the hypersensitive form. Both sexes and all age groups were affected and the distribution was highest on the ears. In the study, 109 (52%) samples of the skin scrapping samples were positive for mite. The main causative agent was *Sarcoptes var. scabiei.*

Introduction

In drought prone areas of Africa, small ruminants, particularly goats, are the dominant livestock species. Although small ruminants are known to adapt to harsh environments, the cumulative effects of overcrowding, poor nutrition and diseases can result in serious production losses[1, 2]. Existing data on the impact of diseases on indigenous small ruminants is scanty; consequently disease control policies relating to these class of livestock are inadequate[3]. While parasitic disease of cattle in Africa attract a lot of attention[4] the contribution of small ruminants to human nutrition should not be forgotten. Turkana district is the largest in Kenya, covering a vast 77,000km^2. The current population of goats is estimated at 2,021,000 compared to cattle 197,000, 1 Million sheep, 172,400 camels and 35,160 donkeys[5]. Goats are kept for meat and milk production, as source of cash income and for their socio-economic and cultural reasons. Skins and manure are valuable by products of goat production. In addition they are considered as insurance or investment against crop failure[1]. There are scanty roads, telecommunication, veterinary services and infrastructure development in Turkana district. Livestock disease information, according to the District Veterinary Officer (DVO) reports is mainly relayed through verbal communication. The main disease constraints reported in the area include caprine pleuropneumonia, mange and helminthiasis. The incidence of mange has reportedly increased during the last three years especially in South Turkana. Mange not only causes direct loss to the farmer through animal mortality, poor growth and reproduction, the skin of mange infested animals is often down graded or rejected at the tannery. This leads to economic losses to the tanning industry and ultimately the country through reduced foreign earnings.

The increasing severity and periodic spread of the disease necessitated a survey to characterise the true status of disease and the institution of possible control strategies. Mange mite infection caused by *Sarcoptes scabiei, Psoroptes spp., Chorioptes spp. Demodex canis var. caprae* are among the most commonly encountered caprine dermatological problems[1,6]. Tropical environment is particularly advantageous for the spread and development of mites[6].

Materials and Methods

Study Area

Turkana District lies on the extreme north-western edge of Kenya, between longitude 34º and 36º40' East and latitude 10º30' and 5º30' north. Turkana District is divided into 17 administrative divisions. The district comprises both arid and semi-arid areas and is characterised by a warm to hot climate ranging in temperatures between 24ºC and 38ºC. The rainfall pattern in the district is erratic and generally unreliable. The topography comprise low lying open plains with altitude of between 300m and 900m above sea level and a few mountain ranges that rise up to 1800m above sea level. The vegetation is mainly composed of dwarf shrubs and grasslands, suitable for goat production.

Selection of study sites

A cross-sectional study was carried out in the Northern and Southern parts of Turkana district. The areas were conveniently selected on the basis of logistics, availability of animals, accessibility and security. Seven divisions were visited, namely Lokichogio,

Oropoi, Kakuma, Kaaleng, Lokitaung, Kibish and Katilu. Using the Global Positioning System (GPS) hand-held receiver, locations for each adakar latitude and longitude were recorded. Each adakar had 10-15 flocks from which 7 flocks were randomly selected for sampling. A total of 35 goats were sampled per adakar .The study was conducted during the wet months of May and September 2005.

Study design

In each adakar five goat owners were subjected to a questionnaire interview. The type of production system and the diseases prevalent in their herds was recorded for ranking in the order of importance. The local disease names indicated by the respondents were given their scientific equivalents based on clinical signs described, personal observation of clinical cases and by the assistance of animal health personnel working in the area. Respondents who reported disease indicative of skin conditions in their locality were asked to describe them in greater details in terms of clinical signs.

Parasitological examination

A total of 560 goats were physically examined for skin lesions indicative of mange. Those with clinical infestation were classified as either having the hypersensitive or chronic form of diseases. Lesions observed were categorised into 5 body sites namely, Ear/ pinna, Face, Back/neck, Flanks /abdomen and Legs. The age, sex and breed of the animals were recorded. A total of 208 samples of skin scrapings were collected.

Parasitological techniques

The skin scrapings were examined for presence of mites under a microscope after digestion in 10% potassium hydroxide. The identification of mites was according to the method described by Urguhart[7] and Soulsby[8].

Data processing

Cross- sectional study questionnaire record sheets were entered into Microsoft access ® (Microsoft Co-operation, Redmond, WA, USA) and analysed. The 95% confidence limits for binomial proportions (Snedecor and Cochran, 1989) were estimated using the Epitable facility in EPI-INFO program (Version 6.02 Centre for disease, Atlanta). A chi-square analysis was used to test for association between age or sex in prevalence's and distribution of skin lesions and the prevalence of mite species[9]. The level of agreement between mange diagnosis in the field by clinical signs and laboratory diagnosis using microscopy was judged by the Kappa statistic[9].

Results

In the questionnaire study, 78.3% (73/ 92) of the respondents' practised pastoralism, 20.7% (19/92) practised semi-sedentary pastoralism and 1.1% practised sedentary system. The sedentary system was practised by people living around trading centres. Semi-sedentary pastoralism was practised mainly in the South. The average distance covered by the animals during grazing was 10 km for the adakars and 2 km for the sedentary and semi sedentary system.

The major goat diseases/agents named by the farmers and ranked by them according to their economic importance include: Pneumonia, Mange, Ticks, Diarrhoea, and Orf. (Table 1). 27.2 % of the combined North and South respondents identified mange as the most important disease of their goats.

46.7% reported that it occurred in both seasons.

The respondents indicated that they took the following measures after an outbreak of mange: 53% had their animals treated with ivermectin® and 13% treated with antibiotics both of which were administered by the community animal health workers, 26% administered traditional treatment, while 8% did not treat their animals. 67 % of the respondent indicated that adults were the most affected, with more females affected than males.

Clinical mange survey

Of the 560 animals examined, 208 (37%) had lesions indicative of mange. These were observed in seven (7) out of the sixteen (44%) adakars visited. The cases were distributed as follows, Lokapel 73, and Kaneudion 19 cases both in the South. In the North, 11 cases were in Kakuma, 3 in Lokore, 2 in Lokichogio and 1 in Kalobeyei. The herd incidence in the seven adakars is shown in Figure 1. The highest incidence was observed in Lokapel followed by Kaneudion, both in the southern block, with percentage incidences of 81.1 % and 79.1% respectively, and the lowest was in Kalobeyei (10%). Skin lesions consisted of a thin hair coat, alopecia, scabs on the skin and in the ears or thickened skin and wounds. 69% (144/208) of the cases had the generalised form of mange. All the other cases had more or less localised lesions. The distribution of the lesions is given in Table 2. The highest distribution was found on the ears (205/208) 98.5% while legs had the least distribution.

The hypersensitive and chronic forms of mange were observed in 5.2% (11/208) and 94.7% (197/208) of the cases respectively. There was marked prurit and erythematous skin patches in the hypersensitive forms while in the chronic form, lesions were characterised by hypertrophy of the epidermis, which was horny and scabby all over the body in some goats, giving them an unpleasant appearance. The chronic form was

Table 1. Major diseases/agents affecting goats in Turkana district as mentioned by the farmers

		Frequency	Percent	Cumulative percentage
Valid	Pneumonia	55	59.8	59.8
	Mange	25	27.2	87.0
	Ticks	2	2.2	89.2
	Diarrhoea	2	2.2	91.4
	Orf	2	2.2	93.6
	Others	6	6.4	100.0
	Total	92	100.0	

Table 2. Type and distribution of mange lesions observed clinically on the various parts of the skin surface in goats and the number positive on microscopy from Turkana District.

Part	Number of animals	Positive on microscopy	Negative on microscopy	Hypersensitive form	Chronic form
Ear	205	109	96	11	194
Face	84	75	9	3	81
Back	105	90	15	7	98
Flanks	107	90	17	7	100
Legs	103	88	15	5	98

significantly ($p<0.05$) more common than the hypersensitive form. Northern Turkana had a prevalence of (17/94) 20.2% while Southern Turkana had a prevalence of 80.7%. The prevalence between the two blocks was significantly different $P> 0.05$.

Age and sex Influence

Two hundred and eight (208) samples were grouped into two categories namely young goats under (18 months) and the adults, over 18 months (Table 3). The grouping was done on the basis that those below 18 months do not go out to graze, but are left behind within the adakars. The youngest age sampled for mange was two months. The prevalence in adults was 62.85% and 43.4% in the young group. There was no significant difference ($p>0.05$) between the two age groups. The prevalence of the sexes was 44.4% for males and 56.7% for females. This was not statistically different ($p>0.05$). Comparisons were done for age and sex and there were no significant differences.

Parasitological Results

Of the 208 skin scrapping samples examined 109 (52%) were positive for mange. In all these cases *Sarcoptes var. scabiei* (figure 4) was seen. There was significant difference between prevalence based on the number of clinical cases observed and number of samples in which mites were observed by microscopy ($p>0.05$). Of the 197 cases of chronic mange, mites were observed in 98 cases, while in the 11 hypersensitive cases 8 were positive for Sarcoptes scabiei mites. There was also significant difference in the rate of mite observation between the two clinical mange conditions.

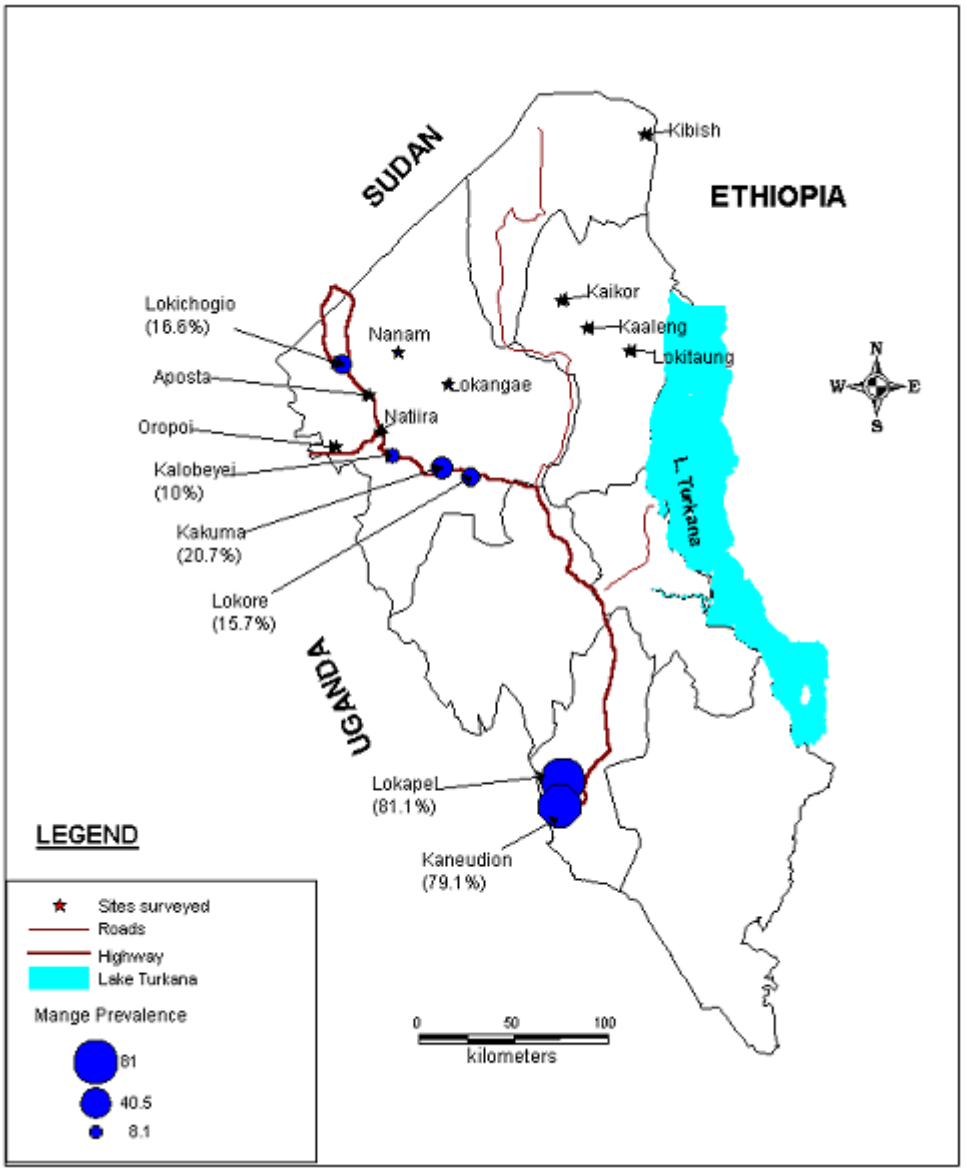
Table 3 a: The number of positive mange cases in goats in Turkana District according to age

Age	+ve	-ve	*Total*
Young	33	43	76
Adult	76	56	132
Total	109	99	208

Table 3 b: The number of positive mange cases in goats in Turkana District according to sex

Sex	+ve	-ve	*Total*
Male	32	77	109
Female	40	59	99
Total	72	136	208

Figure 1. Map showing Prevalence of Mange in goats in fourteen Adakars in Turkana District



Potential risk factors for mange

Potential risk factors in the surveyed area were mainly herd size and management which are associated with geographic location and climate. Traditional type of husbandry is practiced in the area, where animals come in contact with other herds during grazing and at watering points. Owing to the large herds' sizes, there is close contact of animals thus enhancing mite transmission. The survival of mites off the host is also likely to be high in a humid tropical environment.

Discussion

From the results of the present study, it has been established that mange infestation in goats is widely distributed in Turkana District. In the questionnaire survey, 92 pastoralists were interviewed and 95% indicated that mange had occurred in their herds at one time, 27.2% indicated that it was the most important disease in their herds ranked second to pneumonia's. The source of infection as indicated by 53% of the respondents was from newly introduced animals. In this area, new introductions are commonly through livestock rustling and exchange of animals as bride price/gifts. Although the majority of the respondents indicated that the disease occurs in both dry and wet season (46.7%), reports elsewhere indicate that the disease mainly occurs during the wet and the cold season[10]. In the present study 41.3% of the respondents were in agreement with the findings. In the field survey, the percentage of goat herds found affected by mange was 44% (7/16). In total 208/560 cases were observed. The average number of animals affected per herd was 30 (208/7) animals. The field survey therefore confirms the results of questionnaire interviews that mange is an important disease in this area. This was further confirmed by the parasitological examination of the samples of skin scrapping. Of the 208 samples 109 (52%) were found positive for *Sarcoptes scabiei.* This high prevalence in Turkana could be attributed to the traditional type of husbandry where animals come in contact with other herds during grazing and at watering points. The survival of mites off the host is also likely to be high in a humid tropical enviroment[11].

The current results indicate that there was a significant difference between clinical signs and mite detection with the rate of detection in clinical specimens being much lower than that reported elsewhere in the region but much higher than findings reported outside the region. A prevalence of 87% was in clinical samples from goats in Ethiopia[1] compared to a prevalence of 21.6% in Malaysia[12] and 33% in Fiji[7]. The difference between clinical signs and mite detection has been attributed to reduced mite multiplication by the host's hypersensitivity response[13]. This is further complicated by the fact that *S. scabiei* is difficult to find in skin scrapings[14]. This is an indication that clinical signs may not be sufficiently reliable for mange diagnosis where laboratory diagnosis is unavailable.

In all the samples that were positive for mites, the mite species observed was *Sarcoptes scabiei* which seems to be the common mite in this region. In Ethiopia it was found that 87% of samples from goats were positive for *Sarcoptes scabiei*[1]. Out side the region, *Sarcoptes scabiei* was reported to be responsible for generalised mange in goats in Malaysia[7]. Other genera of mites observed in goats are *Psoroptes spp., Chorioptes spp. Demodex canis, Demodex caprae* and *Psoroptes*

canuculi,[7,15].

Clinically the highest numbers of lesions were recorded on the ears. This was in agreement with Dorny et al., (1994) who observed that sarcoptic mange lesions usually start on the ears and nose and then spread over the entire body within 2 to 3 weeks[7].

There was no significant difference of mange infestation in goats within the age and between the sex. This could be attributed to the fact that the animals came from the same herds and environment. However the prevalence was higher in the adults (62.8%) compared to young (43.4%), though there was no significant difference. This is contrarily to what is known that young animals are more susceptible than adults[10]. The apparent higher prevalence in the adults can be attributed to the fact that adults acquire the infection at an early age and do not clear the infection thus suffering cumulative effects of mange over the years. Similar observations were made indicating that old debilitated goats had extensive or severe lesions as compared to the young[15]. A high prevalence of mange was found within the adakars. In two of the adakars (Lokapel and Kaneudion) the prevalences were 81.1% and 79.1% respectively, while in the rest of the adakars it averaged 30%. This was indicative of efficient transmission of mites between animals[7]. Transmission of mites between different species of animals has also been found possible[14]. In view of the fact that goats in the study area are grazed together with other species (e.g. sheep) these can serve as a source of infection for the goats and vice versa.

The clinical implication of sarcoptic mange infestation in animals is a major cause of emaciation and death[7] more often chronic cases are characterized by a remarkable decrease in immune response[16] severe and generalized skin lesions and extreme weakness, which leads to death. Most animals observed in Turkana with severe lesions of mange could have been in the fourth phase of the disease. In the southern part of the district, majority of the respondents reported loss of animals due to mange.

The uncontrolled livestock movement in the district coupled with lack of awareness of modern drugs, the poor economic background of the population, lack of awareness of the infectious nature of the disease, and the relative difficulty in accessing professional veterinary services may have led to uncontrolled spread of mange.

Conclusion

There is need for more education and information to the pastoral farmers and animal health workers to increase the level of awareness in disease control strategies with reference to Mange. Further research is needed to establish the relationship between clinical mange signs and presence of mites as well as seasonal influence on mange occurrence so as to implement appropriate control strategies.

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LICE INFESTATION ON CATTLE IN ENDEGAGN DISTRICT, SOUTHERN ETHIOPIA: SPECIES COMPOSITION, PREVALENCE AND SEASONAL PATTERN

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INFESTATION PAR LES POUX DES BOVINS DANS LE DISTRICT D'ENDEGAGN AU SUD-OUEST DE L'ETHIOPIE : COMPOSITION, PREVALENCE ET TYPE SAISONNIER DES ESPECES

Résumé

Une étude a été conduite pour déterminer la composition, la prévalence et le type saisonnier des poux de bovins dans le district d'Endegagn au sud-ouest de l'Ethiopie. Au total, 500 bovins ont systématiquement été examinés selon les pratiques d'usage pendant la période allant de septembre 2005 à juin 2006. L'étude a révélé que sur un total de 500 bovins examinés, 157 (31,4%) étaient infestés par une espèce de pou ou plus. On a pu observer quatre espèces de pou sur les bovins dans le district couvert par l'étude avec un taux de prévalence globale de 23,6% (pour *Linognathus vituli*) ; 5,4% (*Damalina bovis*) ; 2,6% (*Solonopotes capillatus*) et 1,8% (*Haematopinus eurysternus*). La prévalence mensuelle et la prévalence globale de l'infestation par les poux étaient beaucoup plus élevés (P<0,05) chez les bovins de moins d'un an que chez les bovins de 1 – 3 ans et de plus de 3 ans. L'étude a indiqué que la prévalence globale de l'infestation par les poux des bovins dans le district était très forte pendant les mois de février (6,4%) ; mars (5%) et janvier (4,8%), et très faible durant les mois de septembre (1,2%) ; juin (1,4%) et octobre (2%). L'étude a aussi montré qu'une plus grande proportion de bovins a subi une infestation faible à modérée, tandis qu'une faible proportion a souffert d'une forte infestation. De même, une plus grande proportion de bovins infestée par les poux avait un mauvais état corporel, alors qu'une faible proportion de bovins infestés avait un état corporel assez bon à bon. Les jeunes bovins âgés de moins d'un an constituaient la plus grande proportion (P<0,05) de ceux qui étaient fortement infestés et avaient un mauvais état corporel par rapport aux autres groupes d'âge. Dans la présente étude, on n'a jamais aperçu de *Haematopinus quadripertusus* sur les bovins examinés dans le district. Il est recommandé de mener une étude plus approfondie et de mettre en œuvre les mesures de prévention et de lutte appropriées.

Mots-clés : Bovins, district d'Endegagn, poux, prévalence, type saisonnier, sud de l'Ethiopie

Summary

A study was conducted to determine the species composition, prevalence and seasonal pattern of lice of cattle in Endegagn district in southern Ethiopia. For this purpose a total of 500 cattle were examined systematically according to standard procedures during

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the period from September 2005 to June 2006. The study revealed that out of a total of 500 cattle examined for lice 31.4 %(157) were found infested with one or more species of lice. Four species of lice were encountered on cattle of the study district with an overall prevalence rate of 23.6% *Linognathus vituli,* 5.4%*Damalina bovis,* 2.6% *Solonopotes capillatus* and 1.8% *Haematopinus eurysternus.* Both monthly and overall prevalence of lice infestation was significantly (P<0.05) higher on cattle of less than one year of age than cattle of 1-3 years and cattle of more than 3 years of age. The study indicated that the overall prevalence of lice infestation on cattle of the district was highest during the months of (6.4%)February, (5%)March and (4.8%)January whereas was lowest in months of (1.2%)September, (1.4%June) and (2%)October. The study revealed that greater proportions of cattle infested with lice were with light to moderate degree of lice infestations whereas only small proportions were with heavy degree of lice infestations. Likewise greater proportions of cattle infested with lice were having poor body condition whereas only minor proportions of infested cattle were with fair to good body condition score. Young cattle of less than one year of age constituted significantly (P<0.05) greater proportions of both the heavily infested and those with poor body condition score cattle than the other age groups. In the present study *Haematopinus quadripertusus* was never encountered on examined cattle of the district. Further detailed study and implementation of appropriate control and prevention of lice of cattle is recommended.

Key Words: Cattle, Endegagn district, lice, prevalence, seasonal pattern, South Ethiopia.

Introduction

Ethiopia possessing 43.4 million cattle, 24.6 million sheep, 24.3 million goats, 9 million equines, 2.3 million camels and 50 million poultry[1] ranks first in Africa and 9th in the world in livestock population. However, the productivity of this huge number of cattle is very low due to a multitude of constraining factors such as poor genetic potential, widely distributed parasitic and infectious diseases, poor nutrition and inadequate management.

The mixed farming and extensive grazing systems in Ethiopia, other tropical and subtropical countries is an ideal environment for both ecto and endo parasites of cattle. Parasitic diseases have direct consequences on lowered market value, reduced body weight gain, reduced milk and wool yield, deteriorate hide and skin quality, reduce draught power, reduce dung output for fertilizer and fuel, reduce efficiency of feed conversion, reduce productive lifespan, cause immunospression and increase susceptibility to other diseases[2].

Ectoparasites have worldwide distribution and are especially important in developing countries due to limited resource for their control, prevention and eradication[3]. In Ethiopia ectoparasites are responsible for major impediments to the productivity and well being of livestock particularly their effect on skins and hides quality for export. Skins, hides and leather are the second leading export items next to coffee for Ethiopia. Of the total value of export items skin and hide account for 12-16% for Ethiopia[4]. Skin and hides utilization in Ethiopia is currently estimated at 48% for cattle, 75% for goats and 97% for sheep[5]. However about 30% of all skins and hides processed in tanneries are unsuitable for export due to various defect of which 65% are associated with

ectoparasites[6]. Lice, ticks, mange mites, keds, flies and fleas are the most important ectoparasites responsible for major pre-slaughter defects, downgrading and rejection of hides and skins hence result in serious economic loss to small holder farmers, the tanneries and the country as a whole.

Lice are one of the most common and economically important ectoparasites of cattle worldwide. Pediculosis (dermatitis due to lice) is more common in cattle than any other species of domestic animals[7]. Lice are permanent ectoparasites of cattle and can't survive more than few days off their host animal and are highly host specific. Lice infestation reduces 25-30 kg of body weight and 15-25% in milk production per animal per year[8]. In addition lice infestation in affected cattle is usually associated with health and economic problems due to reduced productivity, anemia, loss of hair due to scratching, biting and rubbing, disruption in feeding, hide damage resulting in leather defect called flecks and light spots, secondary skin infections and damage, reduced calf birth weights or abortion in pregnant animals and damage to fences, equipment and buildings due to excessive rubbing and scratching. Yeruhan *et al*[9] in a study conducted on heifers in Israel reported that severe infestation of *Haematopinus quadripertusus* is associated with *keratoconjunctivitis* and *periorbital papillomatosis.*

The species of lice so far described to infest cattle are one species of chewing lice *(Bovicola (Damalina) bovis)* and three species of sucking lice, viz, *Linognathus vituli* (the long-nosed cattle louse), *Solenopotes capillatus* (the little blue cattle louse) and *Haematopinus eurysternus* (the short-nosed cattle louse) all of which are considered as cosmopolitan lice of cattle with worldwide distribution. The other fifth species, *Haematopinus quadripertusus* (the cattle tail louse), a sucking type of lice with limited geographical distribution and mainly occurs in tropical and subtropical countries of the world. The buffalo louse, *Haematopinus tuberculatus,* is reported to infest cattle in countries where buffalo is domesticated and kept in close association with cattle like Egypt, the Philippines, Australia, Madagascar, China and Myanmar[7, 10, 11].

Except *H. quadripertusus* all the other species of lice populations of cattle show distinct seasonal pattern by building during the months of autumn and reaching a peak in months of winter, declining in spring and remaining low throughout the summer months[11,12]. Nutrition, sunlight, temperature, humidity, crowding, host skin reaction, hair coat condition, hair length, grooming and host resistance affect louse number [11, 12, 13].

The actual prevalence of lice in cattle is grossly underestimated due to the fact that lice aren't readily apparent on animals until their numbers become high and the infestation leads to secondary health problems. Many studies indicated that lice of cattle are common in poorly nourished stocks and young age classes of cattle[7,11,13.]

Specific studies on lice of cattle in Ethiopia are not available and almost all studies are virtually limited to the surveys conducted during studies of other ectoparasites especially ticks and mange mites and their effects on hides and skins. Hence detailed study on lice of cattle and other domestic animals in Ethiopia is needed to design appropriate control strategy. Endegagn district is one of the parts of Ethiopia that is highly affected by

ectoparasites lice that lower cattle production, productivity and lower hide quality. Therefore, in the present study, we tried to determine the prevalence, species composition, intensity and seasonal variation of lice infestation on cattle in Endegagn district in southern Ethiopia and also to draw the attention of cattle owners and veterinary professionals the importance of controlling lice of cattle.

Materials and Methods

Study Area

Endegagn district, where the present study was conducted, is located at 200kms to south west of Addis Ababa. The district has a total area of 136 km^2 and altitude ranging from 1700-2500m above sea level. During the study period Endegagn district received an average annual rainfall of 1200mm and temperature ranging from 15-20^0c. Farming in the district is mixed crop-livestock production type and cattle are owned by smallholdings kept under extensive management system.

Study Animals and Design

The study was conducted on indigenous zebu cattle kept by small-scale owners in Endegagn district from September 2005 to June 2006. All the examined cattle were randomly selected and grouped into three age classes: as group 1 cattle of < 1 year of age, group 2 from 1-3 years of age and group 3 cattle of > 3 years of age. The body condition score of each examined cattle were determined based on the standard scoring method indicated by Matthwman[14]. "Poor body condition" was given to extremely thin cattle with wasted thigh muscle of concave appearance, "fair" to moderate condition with straight thigh muscle and "good" to cattle having bulge thigh muscle with convex appearance and very fat cattle. During the study period 50 cattle per month from the 5 randomly selected peasant associations (PA) were thoroughly examined for presence of lice according to classical procedures. Degree of lice infestation on each positive cattle was determined by recording the number of lice over 6.5cm^2 of skin and was ranked into: as light degree when < 4 lice per 6.5cm^2, moderate degree when 4-10 lice per 6.5cm^2 and heavy degree of infestation when >10 live lice were counted per 6.5cm^2 of skin in examined infested cattle by hair parting technique so as to expose the skin over the entire area within examination region as described by Watson *et al*.[13] Colwell *et al.*[15], Milnes *et al.*[16] and Holdsworth *et al.*[17] All the examined cattle did not received any treatment against ectoparasites prior to the study period.

Lice collection and Identification

Cattle were restrained and the body surface of the animal was visually examined for presence of lice. During examination great attention was paid to the already determined predilection sites of both chewing and sucking cattle lice, namely dewlap (5 X 15cm), rump (5 X 15cm), tail (5X15cm), top line (5X15cm), withers (5X15cm), around each eye (10X15cm each), right and left cheek (5X15cm), muzzle (5X25cm) and across the poll area (10X25cm)[13,15,16,17]. Cattle were considered positive when a single louse of any species was noted at any of these examination locations. Lice were removed manually using forceps or by hands carefully to avoid any damage to the body of lice and placed in vials containing 70% ethanol alcohol for subsequent identification. Lice were identified in species under stereomicroscope using the key

Table 1. Overall prevalence of lice infestation in different age groups of cattle of Endegagn district.

Age group of cattle	No examined	No infested	Prevalence
< 1 year	166	79	15.8%*
1-3 years	168	42	8.4%
> 3 years	166	36	7.7%
Total	500	157	31.4%

* Indicates P<0.05

morphological characteristics presented by Urquhart[7], Soulsby[10].

Data Analysis

Microsoft excel soft-ware was used to store the data and analysis of ordinary statistics. SPSS 12.5 for windows soft ware program was used for data analysis. Overall and monthly prevalence of lice and degree of infestation on cattle of different age groups, peasant association and body condition score of cattle were all compared as in ANOVA. Significance was set at P<0.05. Mean, confidence interval, percentage values and standard deviation error were all used when appropriate to compare and described lice infestation on cattle of the study area.

Results

In the current study examination of a total of 500 cattle for the presence of lice infestation revealed the presence of 4 species of lice belonging to 3 genera with an overall prevalence rate of 31.4% (157). Cattle were infested with one or more species of lice (Table 1). The four *species* of lice identified on the cattle of the Endegagn district were 23.6%(118) *L. vituli*, 5.4% (27) *D. bovis,* 2.6%(13) *S. capillatus* and 1.8% (9) *H. eurysternus* (Table 1). *L. vituli* was identified as the most predominant species throughout all months of the study period followed by *D. bovis* as the second prevalent species of lice on cattle of the study area. The prevalence of *S. capillatus* and *H. eurysternus* was consistently at low level and significantly (P<0.05) lower than the prevalence of *L.vituli* and *D. bovis* throughout the study period (Table 2). Out of the total positive cattle 93.6%(147) were with single lice *species* infestation whereas 6.4%(10) were infested with two or more *species*. Lice infestation was significantly (P < 0.05) higher in young cattle of <1 year of age 15.8%(79) compared to cattle of 1-3years of age 8.4%(42) and > 3 years of age 7.2%(36) as indicated in (Table 1). There was statistically insignificant (P>0.05) difference in the prevalence of lice infestation between cattle of different sexes.

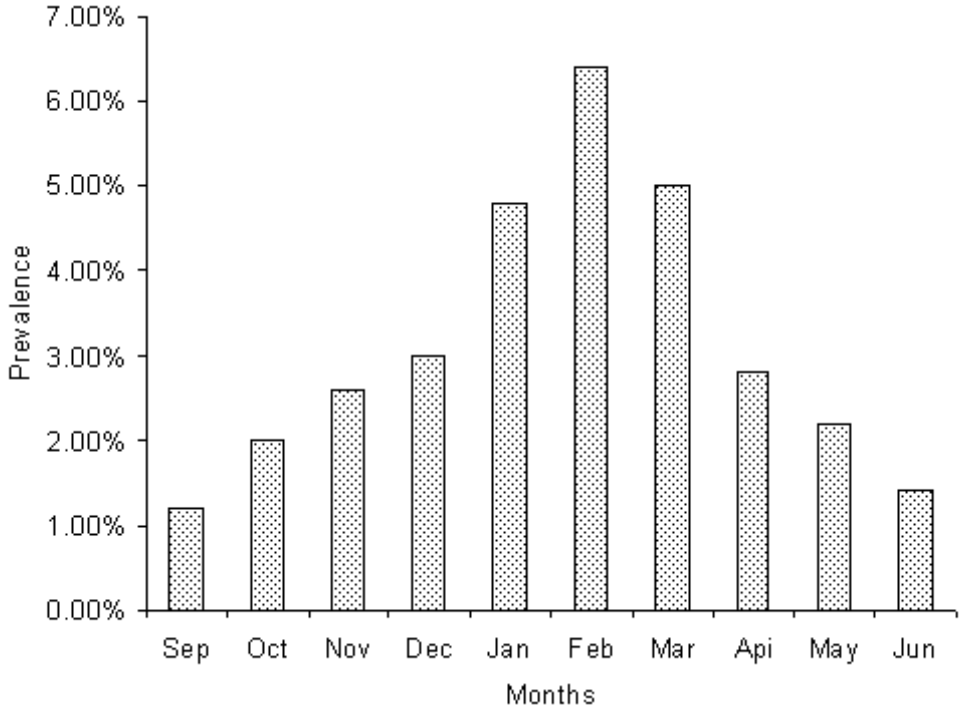
In the present study the overall monthly prevalence of lice infestation on cattle was highest in February (6.4%), March (5%) and January (4.8%) whereas was lowest in months of September (1.2%), June (1.4%), October (2%) and May (2.2%) as presented in Figure 1.

Table 2. Monthly Prevalence of species of lice infestation on cattle in Endegagn district.

Month	*L. vituli*	*D. bovis*	*S. capillatus*	*H. eurysternus*
Sep2005	0.8%(4)	0.2%(1)	0.2%(1)	0%(0)
Oct2005	1.8%(9)	0.4%(2)	0.2%(1)	0.2%(1)
Nov2005	2%(10)	0.6%(3)	0.2%(1)	0.2%(1)
Dec2005	2.2%(11)	0.4%(2)	0.4%(2)	0.2%(1)
Jan2006	3.4%(17)*	1%(5)	0.4%(2)	0.4%(2)
Feb2006	4.6%(23)*	1.2%(6)*	0.4%(2)	0.4%(2)
Mar2006	4%(20)*	0.6%(3)	0.2%(1)	0.2%(1)
Api2006	2.4%(12)	0.4%(2)	0.2%(1)	0%(0)
May2006	1.6%(8)	0.4%(2)	0.2%(1)	0%(0)
Jun2006	0.8%(4)	0.2%(1)	0.2%(1)	0.2%(1)
Total	23.6%(118)*	5.4%(27)*	2.6%(13)	1.8%(9)

() Number in brackets represents number of cattle infested with lice.
*Indicates P< 0.05

Figure 1. Monthly overall prevalence of lice infestation on cattle of Endegagn district.



Both the overall and monthly prevalence of lice infestation on cattle in the five Peasant Association of the district did not attain statistical significance insignificant (P>0.05) variation during the study period (Table 3). Likewise, the prevalence of the 4 species of lice of cattle in the studied PAs of the district failed to reveal any statistically difference during the study period (P>0.05) (Table 3).

Greater proportions of the positive cattle were with poor (66.9%) body condition, whereas small proportions of the infested cattle were with fair (21%) and good (12%) body condition score (Table 4). Among the positive cattle, 65% had light infestation, 23% moderate and 12% heavy degree (Table 4). Of the total positive cattle greater proportions 8.9% of young cattle of less than 1 year of age were with heavy degree of infestations whereas small proportions 3.2% of cattle of 1-3 years age and minor proportions 0.7% of cattle of greater than 3 years of age were with heavy degree of lice infestations as shown in table 4. The study also indicated that out of the total positive cattle higher proportions (36.9%) were cattle of less than 1 year of age were with poor body condition score whereas small proportions (15.9%) of cattle of 1-3 years of age and (14 %) of cattle of greater than 3

Table 3. Prevalence of species of lice of cattle in Endegagn district by PA

PA	No of cattle infested	Specie of lice			
		L. vituli	*D. bovis*	*S. capillatus*	*H. eurysternus*
Araticho	5.6%(28)	4.2%(21)	1.2%(6)	0.6%(3)	0.4%(2)
Becha	6.4%(32)	4.8%(24)	1%(5)	0.4%(2)	0.4%(2)
Bucha	6.6%(33)	4.8%(24)	1.2%(6)	0.6%(3)	0.4%(2)
Gomira	7%(35)	5%(25)	1%(5)	0.6%(3)	0.4%(2)
Shawura	5.8%(29)	4.8%(24)	1%(5)	0.4%(2)	0.2%(1)
Total	31.4%(157)	23.6%(118)	5.4%(27)	2.6%(13)	1.8%(9)

Table 4. Degree of lice infestation in relation to body condition score and age of infested cattle

Age of cattle	No infested	Body condition score			Degree of Lice infestation		
		Poor	Fair	Good	Light	Moderate	Heavy
< 1 year	50.3%(79)	36.9%(58)*	8.9%(14)	4.4%(7)	27.4%(43)	14%(22)	8.9%(14
1-3 years	26.7%(42)	15.9%(25)	5.1%(9)	5.1%(8)	17.8%(28)	5.7%(9)	3.2%(5)*
> 3 years	22.9%(36)	14%(22)	6.4%(10)	2.5%(4)	19.7%(31)	2.5%(4)	0.7%(1)*
Total	157(100%)	66.9%(105)*	21%(33)	12%(19)	65%(102)*	22.3%(35)	12.7%(2

*indicates significant difference

years of age were with poor body condition score as indicated in table 4.

Discussion

In the current study, a high overall prevalence (31.4%) of lice infestation on cattle of the district was recoded. This was most probably attributed by factors like inadequate plane of nutrition, poor management, poor sanitation, inadequate veterinary services and widely distributed diseases of cattle such as gastrointestinal parasites, trypanosomosis, and infectious diseases in the study area all of which favor great lice infestation on the cattle of the district. It also indicates that little attention has been given to the importance of lice infestation on cattle of Endegagn district.

This observation is in line with previous studies of Watson *et al.*[13], Hussain *et al.*[18] and Colwell *et al.*[15]. On the other hand the overall prevalence of lice infestation reported herein is higher than the previous works of Hagos and Markos[19] who reported 8.2% overall prevalence of lice infestation on cattle in a study conducted in central zone of Tigray region, Regassa[20] report of 13% overall prevalence of lice on cattle of Nekemptie, Faris[21] report of 15.5% overall lice prevalence on young cattle at Holeta and its surrounding and Amanuel[22] report of 17.9% overall prevalence of lice of cattle in eastern Showa. Differences in agro-ecology, weather, environment and age of the study cattle are the most likely explanation for the observed variations.

This study identified *L. vituli* as the most predominant species of lice of cattle in Endegagn district followed by *D. bovis* as the second prevalent spp during all months of the study period. *S. capillatus* and *H. eurysternus* were encountered at a very low prevalence throughout the study period. This observation is in agreement with earlier works of Colwell *et al.*[15] and Scharff[23] and George *et al*[24] all of which reported the predominance of *L. vituli* infestation on cattle. On the other hand this finding is in contrast to the work of Milnes and Green[25], Nafstad and Gronstol[26], Colebrook and Wall[27] and El-Metenaway *et al.*[28] all of which reported the predominance of *D. bovis* with overall prevalence of 75-90%. This is most probably attributed to the differences in seasonal, ecological, management, and breed of cattle of study that could govern the predominance of species of lice of cattle of an area.

The result of the present study indicated that both the monthly and overall prevalence of lice infestation decreased with age of cattle as was significantly ($P<0.05$) higher on young cattle than adult cattle. This finding may suggests that young cattle are more susceptible to lice infestation due to underdeveloped immune status that favors great lice infestation unlike in adult cattle in which age related responsiveness and development of immunity causes lower fecundity of lice or susceptibility to new infection as described by Colwell and Himsl-Rayner[29]. This observation is in accord with many other previous works like Watson *et al.*[13], Hussain *et al.*[18], Milnes and Green[25], Nafstad and Gronstol[26], Colebrook and Wall[27] and El-Metenaway *et al.*[28] all of which reported similar observations.

The present study revealed that monthly prevalence of lice infestation on cattle of the study area is significantly higher during Feb (5.4%), March (4.8%) and Jan (3.6%) than the other months of the study period. This observation supports the works of many other

previous studies such as kettle[12], Watson[13], who reported seasonality of lice infestation on cattle as populations of lice begins increasing in months of winter reaching highest level in months of spring but lowest in months of summer and autumn. This observation was due to cool skin temperature, denser coat, low nutritional status, presence of many stress factors and relative cooler weather condition during months of winter and spring all of which favor female lice fecundity leading to increase in lice population on cattle. On the other hand exposure to sun light, improvement in nutrition from new growing grass and shedding of coat during the months of summer and autumn all cause decline in lice infestation of cattle.

The observation of absence of any statistically significant (P>0.05) variation both in monthly and overall prevalence of lice and also spp prevalence of lice of cattle in different PAs of Endegagn district in the current study suggest the similarities in agro-ecology, management, breed and environment of cattle in different PAs of the district.

The study revealed that significantly (P<0.05) greater proportions of positive cattle infested with lice were those with poor body condition score than cattle of fair and good body condition. Similarly, cattle of less than 1 year of age accounted for significantly (P<0.05) greater proportions(36.9%) of cattle of poor body condition score than the other age groups as shown in table 4. This finding supports the well-documented fact that lice infestation is common in malnourished, stressed and diseased cattle than well fed, healthy and good body condition cattle and is in agreement with previous work of Nelson[30] and Gibney *et al.*[31].

The study indicated that greater proportions of positive cattle carrying lice were with light to moderate degree of lice infestation whereas smaller proportions of the infested cattle were with heavy degree of infestation. The study also indicated that significantly(P<0.05) higher proportions of positive cattle of less than 1 year of age accounted in carrying higher intensity of lice infestation than the other age groups as shown in table 4. This observation suggests that lice infestation on cattle usually causes chronic disease and remained unnoticed until much of the economic loss has already occurred as described by Campbell *et al.*[32] However, subclinical infestations could cause retarded growth, reduced productivity and increase susceptibility to other diseases and chronically infested animals also act as a potential source of infestation for other animals.

In conclusion this study showed that productivity of cattle of the study area was constrained by significant lice infestation. Cattle owners and veterinary professionals should implement appropriate control and preventive strategies at early stages to minimize impediments due to lice of cattle of the area. Further annual epidemiological studies in different agro-ecological regions are recommended to elucidate the economic significance and losses associated with lice of cattle and other domestic animals in the area.

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BOVINE PULMONARY TUBERCULOSIS AT BAHIR DAR MUNICIPALITY ABATTOIR, ETHIOPIA

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TUBERCULOSE PULMONAIRE BOVINE A L'ABATTOIR DE LA MUNICIPALITE DE BAHIR DAR EN ETHIOPIE

Résumé

Une étude transversale a été menée de décembre 2005 à juin 2006 pour déterminer la prévalence de la tuberculose bovine chez 1441 bovins abattus et aussi pour valider la qualité de diagnostic de l'inspection de routine à l'abattoir de la municipalité de Bahir Dar, dans le nord-ouest de l'Ethiopie. On a aussi comparé la nécropsie complète et la méthode d'inspection de routine de la viande. La prévalence de la tuberculose pulmonaire bovine était de 0,78% selon la nécropsie complète et seulement 0,069% avec la méthode d'inspection de routine. La probabilité de ne pas pouvoir détecter un animal ayant des lésions avec l'inspection de routine de la viande était d'environ 83,4%. A peu près 44,4% des lésions tuberculeuses macroscopiques ont été observées dans le ganglion lymphatique trachéo-bronchique et 33,4% dans le ganglion lymphatique médiastinal. L'analyse des facteurs de risque a révélé que les bovins croisés étaient plus affectés que le Zébu local (test χ^2, P = 0,003). Le sexe avait aussi un effet sur la prévalence de la maladie ; effet qui était statistiquement important chez les femelles (test χ^2, P<0,05). Les résultats obtenus dans la présente étude montrent clairement l'importance d'une intervention appropriée en plus des méthodes d'inspection de routine de la viande, pour déterminer l'ampleur de la tuberculose pulmonaire bovine.

Mots-clés : Bovin, tuberculose, prévalence, lésion postmortem, abattoir, Bahir Dar.

Summary

A cross sectional study was conducted from December 2005 to June 2006 to determine the prevalence of bovine tuberculosis on 1441 slaughtered cattle and validate the diagnostic quality of routine abattoir inspection at Bahir Dar municipality abattoir, North Western Ethiopia. Comparison between the detailed postmortem examinations and the routine meat inspection procedures was also made. The prevalence of bovine pulmonary tuberculosis was 0.78% based on detailed postmortem examination and only 0.069% with on the routine meat inspection procedure. The estimated probability of missing an animal with lesions using routine meat inspection was therefore 83.4%. About 44.4 % of gross tuberculous lesions were found in the tracheo-bronchial lymph node while 33.4% in the mediastinal lymph node. Analysis of risk factors revealed that cross breed cattle were more likely to have high proportion of reactors than the local Zebu cattle (χ^2 test, P = 0.003). Sex was also found to have effect on the prevalence of the disease and this effect was statistically significant (χ^2 test, P< 0.05) in females. The results obtained in this study clearly demonstrate the importance of proper and detailed intervention in order to determine the right magnitude of pulmonary bovine tuberculosis besides to the routine meat inspection procedures.

Key words: Bovine, Tuberculosis, Prevalence, Postmortem lesion, Abattoir, Bahir Dar.

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Introduction

Tuberculosis (TB) is an infectious disease of world wide distribution, infecting both man and animals, affecting all age groups, sparing no organ of the body and responsible for more deaths throughout the world than any other bacterial diseases[1]. Bovine tuberculosis is a major problem in Ethiopia especially in dairy calltle. Testing for infection in cattle is mainly based on an intra-dermal tuberculin test. However, the method lacks specificity due to antigenic cross-reaction of *Mycobacterium bovis* with other atypical mycobacteria like *M. phlei, M. avium* and other mycobacteria to which animals are often exposed[2]. In Ethiopia bovine TB causes significant financial losses due to condemnation of infected organs and also causes loss of production due to loss of condition[3].

The primary cause of human tuberculosis (HTB) is *M. tuberculosis* although it is sometimes caused by *Mycobacterium bovis (M. bovis)*. However, *M. bovis* is responsible for increasing animal and human health problems in several developing countries[4]. Infection in human occurs largely through consumption of infected milk but also spread can occur by inhalation[5] of infective spores. The practice of drinking raw cattle milk has led to high prevalence of infection by the disease in Ethiopia agrarian communities. In Ethiopia most surveys carried out on bovine tuberculosis were based on abattoir reports[6,7] and also tuberculin testing of animals in designated localities[8,9]. In Ethiopia, tuberculin test and postmortem findings are used for the diagnosis of BTB in live animals and in slaughtered animals respectively.

Bahir Dar is known for its high livestock population. The town has one municipality abattoir with 59 registered butchers that effect slaughter and food supply chain and inspected meat. Therefore the objectives of this paper were to determine the prevalence of BTB in animals slaughtered at Bahir Dar Municipality Abattoir and to compare and evaluate the efficiency of routine and detailed meat inspection to detect tuberculosis cases.

Materials and methods

Study subject

The study was conducted on cattle slaughtered in Bahir Dar municipality abattoir. A total of 1441 heads of cattle and their carcasses were included in the study. This number includes 1392 adult males and 49 adult females.

Study design and sampling

Since specific identification number was given to animals and carcasses for administrative purpose in the abattoir, stratified random sampling was used to select carcasses for detailed laboratory examination. Accordingly, out of 1441cattle slaughtered during the study period, 638 cattle were subjected to extensive postmortem examination. This figure includes 589 adult males and 49 adult females. Of the 638 cattle, 122 adult males and 29 adult females were crosses of Zebu with Holstein-Friesian and 467 males and 20 females were Zebu. Lymphnodes (Mediastinal, tracheo-bronchial) and the lungs were visualized, palpated, and incised for the presence of tuberculous lesions. During sampling, both abnormal and the apparently normal tissue of the organs in

question were included. Samples from suspicious lesions were collected in 10% formal buffered saline and suspicious examinations were confirmed to be tuberculosis after direct microscopic and histopathological examinations.

Postmortem Examination

The routine postmortem examination in Bahir Dar Abattoir involves palpation and incision of the liver, visual examination of the lungs and kidneys, palpation and incision of lymph node like pre-scapular, pre-femoral and poplitial lymph nodes. The lymph nodes and other tissue specimens were subjected to a detailed examination; for the presence of abscesses, cheesy masses and tubercles. Detailed postmortem examination involved also visual inspection, palpation and incision of the lungs and tracheo-bronical and mediastinal lymph nodes for the detection of tuberculosis lesions. Organs or the whole carcass with milliary TB and carcasses with involvement of multiple lymph nodes were condemned during both routine and detailed postmortem examination.

Detailed Laboratory Examination

The lung tissue and tracheo-bronchial and mediastinal lymph nodes were collected and examined with a bright light source. They were cut into slices of 2mm using a sharp surgical blade, then the cut surface were examined for abscesses, cheesy mass and calcified masses. Tissues with suspected lesions were subjected to microscopic and histopathological examinations.

Microscopic Examination

An impression smear was prepared from the suspected lesions dried and fixed by passing through a Bunsen burner flame, 3 times a filter paper was placed over the slide and flooded with carbol-fuchsin; heated until steam rises then acid-alcohol, decolorizer, was added and left to act for 2 min, rinsed, drained and washed followed by addition of methylene blue for 30 sec. Finally, washed and air dried for microscopic examination, under oil immersion microscope for the presence of acid- fast *bacilli* [10].

Histopathological Examination

Tissue samples from the lungs and tracheo-bronchial and mediastinal lymph nodes showing frank macroscopic lesions were collected in 10% formal buffered saline after which they were dehydrated in serials of alcohol, cleared by xylene, embedded in paraffin, sectioned at 4 micrometers, deparaffinized in the 60^0C incubator, again cleared by xylene and hydrated to water. The processed sample was stained with hematyoxylene-eosin and finally examined under the microscope.

Data Collection and Analysis

The individual animal identification number, breed and sex were recorded. The range and frequency of anatomical sites where tuberculosis lesion were detected were also recorded for cattle subjected to detailed laboratory examination. Moreover, the result of routine abattoir inspection, detailed laboratory examination, microscopic examination and histopatholgical examination were also recorded. The data were systematically arranged and stored in MS-excel spread sheet and imported to be analyzed by intercooled stata 7.0 and win epi 2.0 soft wares. Descriptive statistics was used to determine the percentages of positive and negative values. Chi-square was used to

Table 1: Number of animals examined by routine abattoir inspection and detailed laboratory examination to detect animals with tuberclous lesion

Types of examination	N° of lungs examined	Positive	Negative	Percentage (%)
Routine abattoir inspection	1441	1	1440	0.07
Detailed laboratory examination	638*	6**	633	0.9

* Selected from 1441 negative carcasses during routine abattoir inspection

** A positive carcass from routine inspection was also included

Table 2: Summery of tuberculous cases diagnosed by different methods

	N° of Lungs examined by		N° (%) of TB cases			
Types of Carcasses Examined	Routine abattoir inspection	Detailed laboratory examination	Routine *	Detailed *	Microsocpic *	Histo pathology
Adult male	1392	589	0	4(67)	1(17)	1(17)
Adult female	49	49	1(17)	2(33)	0	1(17)
Total	1441	638	1(17)	6(100)	1(17)	2(34)

* (%) is based on detailed laboratory examination

determine the associations between the factors, and the strength of associations among the results and assumed risk factors was analyzed by multivariate logistic regression. Also win-epi 2.0 was used to determine diagnostic test evaluation, prevalence and confidence interval. In all the analysis, the confidence level was held at 95% and $P<0.05$ was set for significance.

Results

The prevalence of pulmonary BTB in cattle slaughtered at Bahir Dar municipality abattoir during the study period on the basis of detailed postmortem examination was 0.78% (5 out of 638 inspected samples). A total of 1441 cattle were screened for the presence of tuberculous lesions. Of these, 1 (0.07%) and 5 (0.78%) were regarded as TB infected respectively by the routine abattoir inspection and detailed laboratory examination (Table 1). Lungs showed severe degree of gross lesions featured by the presence of multiple nodules of varying sizes distributed through out the parenchyma. These nodules when cut showed a cavity in the centre filled by thick cheesy pus, with occasional calcification in the centre. Smear made out from the pus material and tubercles of the lungs and mediastinal lymph nodes showed presence of acid fast bacilli, when stained by Zeihl Neelsen's method. A direct smear from specimens of carcasses with tuberculous lesion revealed acid-fast bacilli, in 1 of the 6 infected lungs. On the other

Table 3: Comparison of detailed necropsy procedure and routine meat inspection

		Detailed Necropsy		
		Positive	Negative	Total
Routine meat inspection	Positive	1	0	1
	Negative	5	633	638
Total		6	633	639

Sensitivity = 16.6%

hand, histopathological examination using hematoxylene-eosin staining detected two additional tuberculous lesions showing typical microscopic appearance of a tubercle, lymphocyte infiltration, area of necrosis and multi-nucleated langhan's giant cells sometime found in ring forms and surrounded by fibroblasts(Table 2).

The sensitivity of routine abattoir inspection was therefore only 16.6% when compared to detailed laboratory examination (Table 3).

Higher prevalence rate (4.1%) was recorded among female than in male animals (0.68%) (Table 4) and the difference in prevalence between the two sexes was statistically significant (P<0.05). Similarly, higher prevalence rate (2%) was recorded in cross breeds than the local cattle/zebu (0.62%). These rates also revealed statistically significant difference (P<0.05) (Table 4).

Discussion

In this study, 0.78% (n=638) of the lungs subjected to detailed laboratory examination contained tuberculous lesions. This finding is in agreement with previous reports from North East Nigeria[11] and Rabat-Morocco[12]. However, the current finding is lower as compared to previous reports. This

Table 4: Tuberculous infection by sex and breed

Variable	Category	N° of Examined	N° (%) of positive	95% CI	χ^2	P value
Sex	Male	589	4(0.68)	0.4-0.7		
	Female	49	2(4)	2.1-8.0	24	0
Breed	Local	487	3(0.62)	0.3-2.6		
	Cross	151	3(2)	1.3-3.1	9	0.003

Table 5: Distribution of tuberculous lesions in tissues of tuberculous lungs

Tissues	N° of lesions	Percent (%)
Tracheo-bronchial lymph node	4	44.4
Mediastional lymph node	3	33.33
Lungs	2	22.22
Total	9	100

is because the prevalence of BTB is lower in beef cattle than in intensive dairy cattle[5,13].

The routine abattoir inspection procedure detected only 1 (16.6%) of the 6 cattle with pulmonary tuberculosis lesion. That means abattoir necropsy procedure failed to detect an estimated 83.4% of animals with visible pulmonary lesions. This low level of sensitivity (16.6%) indicates a high risk of public and veterinary health challenges following method of inspection used, otherwise, careful examination of the lungs and associated lymph nodes can result in the detection of 96% of cattle with macroscopic pulmonary lesions[14].

Microscopic examination of impression smears from tuberculous lesions is always considered the least sensitive of all the diagnostic methods[15]. Similarly in this study less sensitivity 1(16.6%) of acid fast bacilli was detected. This may be due to small number of bacteria present in the lesions or from absence of viable Mycobacteria in calcified lesion of tuberculosis[10] .

Nodular lesions of different sizes were found in the lungs and associated lymph nodes which upon palpation, some were soft and others were hard. Upon incision, the contents of the lesions were purulent, thinly fluid, sticky and cheesy or calcified and harder, these lesions were collected for histopahtological examination, and some times two or more samples were taken from a single lesion. The results of haematoxylien-eosin staining showed the presence of necrosis and calcification, aggregated epitholoid cells, lymphocytes and langhan's giant cells, surrounded by fibroblasts. However, histopathological examination revealed lower number 2(33.3%) of lungs with tuberculous lesions, compare to detailed laboratory examination. This might result from classifying non tuberculous lesions as tuberculous by detailed laboratory examination[10,16].

In this study, higher rate of BTB results were recorded in female animals (4.1%) than in male animals (0.68%); though the number of the two sexes was not proportional[7] and also reported higher prevalence of BTB among females than male animals[9]. This is not purely the effect of sex but, the high proportion of adult females infected is due to old age and management in a closed spaces, it is most probable for them to be infected during their long productive life and due to many other stress factors associated with female animals such as pregnancy, parturition and lactation[5].

A prevalence of 2% and 0.61% was recorded in cross and local breeds, respectively. The prevalence was found to be lower in Zebu cattle but higher in cross breed cattle. This finding is in agreement with the study[9] who reported a prevalence of 14.4% and 8.6% in cross and local breeds. The probable reason could be due to the fact that genetically improved cattle may suffer more severely from deficient housing and malnutrition; subsequently, become more prone to infection than local breeds.

Table 5 displays the frequency of anatomical sites with tuberculous lesions. The mean number of lesions in the lungs with TB lesions is (1.5) in the 6 tuberculous lungs, tuberculous lesions were found in 9 anatomical sites. Out of these 4(44.4%) the lesion were found in trachio-broncial lymph node, 3(33.3%) in the mediastinal lymph node and 2(22.2%) in the lungs. The

distribution of tuberculous lesions in the present study is similar to the reports[17,18].

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ECTOPROTOZOAN AND MYXOSPOREAN INFECTIONS IN SOME FRESHWATER FISH OF FAKO DIVISION, SOUTH WEST PROVINCE OF CAMEROON

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INFECTIONS PAR LES ECTOPROTOZOAIRES ET LES MYXOSPOREANS DE CERTAINS POISSONS D'EAU DOUCE DANS LE DISTRICT DE FAKO DANS LA PROVINCE DU SUD-OUEST DU CAMEROUN

Résumé

Une étude a été menée dans le district de Fako dans la province du sud-ouest du Cameroun en vue de recueillir des données sur la prévalence et la répartition des parasites des poissons d'eau douce, et d'évaluer les facteurs physiques et chimiques de l'eau susceptibles d'influencer la santé des poissons et les infections parasitaires. Au total, 146 échantillons de *Clarias gariepinus, Oreochromis noliticus, Barbus camptacanthus* et *B. occidentalis* provenant de quatre différents habitats ont été examinés. Deux ciliés ectoparasitaires (*Ichthyophthirius multifiliis* et *Trichodina spp*) et trois myxosporeans (*Myxobolus njinei,* un *Myxobolus* non identifie et *Henneguya* sp) ont été identifiés sur les poissons. L'état physique et chimique de l'eau a permis de connaître le niveau de pollution des divers habitats. Les poissons qui étaient dans les sites pollués étaient plus exposés à l'infection que ceux des sites non pollués. Le ruisseau de Ndongo et les étangs de Great Soppo qui avaient le taux d'infection le plus élevé (69,2% et 100% respectivement) avaient aussi le taux le plus faible d'oxygène dissous (3,3mg/L et 4mg/L) et de saturation d'oxygène (26,8% et 37,2%), et les températures les plus élevées (25,2^{0}C et 25,1^{0}C respectivement).

Mots-clés : Poissons, parasites, ciliés, myxosporeans, infection, pollution, habitats.

Summary

A study was carried out in Fako Division of South West Province, Cameroon to provide baseline data on the prevalence and distribution of freshwater fish parasites and assess the physicochemical factors of water that may affect fish health and parasite infection. A total of 146 fish samples of *Clarias gariepinus, Oreochromis noliticus, Barbus camptacanthus* and *B. occidentalis* from different habitats were examined. Two ectoparasitic ciliates (*Ichthyophthirius multifiliis* and *Trichodina sp*) and three myxosporeans (*Myxobolus njinei,* an unidentified *Myxobolus sp* and *Henneguya sp*) were identified from the fish. The level of pollution in the different habitats was indicated by the physicochemical parameters of the water. Fish in polluted sites were more exposed to infection than those in non-polluted sites. Ndongo stream and Great Soppo fish tanks which had the highest prevalence of infection (69.2% and 100% respectively) were also the sites with the lowest dissolved oxygen content (3.3mg/L and 4.0mg/L respectively) and oxygen saturation (26.8% and 37.2% respectively), and highest temperature (25.2^{0}C and 25.1^{0}C respectively).

Key words: fish, parasites, ciliates, myxosporeans, infection, pollution, habitats.

Introduction

The fishery sector in Cameroon constitutes the most important source of animal protein in the diet of the lower income population. Parasitic infections and diseases are some of the factors hindering high productivity and growth in fish farms. The fish body harbours a great number of ecological niches that may be colonized concurrently or separately by a great variety of parasites[1]. Protozoan parasites are some of the largest group of pathogenic organisms in warm water fish ponds[2]. Ecological conditions, like organic loads in pond water due to feeding, form the basis for increased repetition of life cycle stages and intensive spread of many external parasites[3]. These invade the fish, bringing about rapid death and often spreading the new infections throughout the water[4]. The correct diagnosis, treatment and control of disease problems commonly affecting fish will help in achieving an efficient and profitable aquaculture[2].

This study was aimed at providing baseline data on the prevalence and distribution of freshwater fish parasites of Fako Division in South West Province of Cameroon, and assessing the physicochemical factors of water that may affect fish health and parasitic infection.

Materials and Methods

Study site

Fish used for the study were collected from two streams along the Mutengene-Buea road (Ndongo stream, where water pollution is mainly from domestic waste and organic pollutants from washing of vehicles upstream, and Ekande stream, where the water pollutants are mainly organic from washing of vehicles) and two ponds (Ewongo fish pond, formed by embankment of the Ewongo stream located along the Limbe-Mutengene road, and Great Soppo fish tanks in Buea town, which is an artificial pond consisting of six concrete tanks).

Fish sampling

Fish samples were collected from the four sites monthly from February to June 2006. Fish from the streams were caught using a catch net of about 2cm mesh size, while fish from the ponds were caught using a bowl after first emptying the water. The fish were transported alive to the laboratory at the University of Buea and kept in aquarium. They were examined within two days for parasitic protozoans.

A total of 146 fish samples of *Clarias gariepinus, Oreochromis noliticus, Barbus camptacanthus* and *B. occidentalis* were examined. Eleven of them were examined for both *ectoparasitic protozoans* and *myxosporeans,* 21 were examined for myxosporeans only and 114 were examined for *ectoparasitic protozoans* only.

Laboratory investigations

Each fish specimen was carefully examined for symptoms indicative of parasitic infection. Thereafter, skin biopsy was done by gently scraping a small area on the surface of the fish with the edge of a microscope cover slip in the cranial to caudal direction[5]. The mucus from the skin scraping was then transferred immediately to a drop of aquarium water on a glass microscope slide and examined under the microscope for free swimming stages, attached or encysted protozoans. If movements in the wet mount revealed the presence of any parasite, Giemsa stained slide was prepared

for confirmatory identification of parasite species observed in the wet mount.

Fins that showed any abnormality were cut and examined under the microscope for the presence of protozoan parasites. The gill cover was opened and a small amount of tissue aspirated from the gills using a Pasteur pipette and placed in a drop of water on a glass microscope slide for microscopic examination.

The skin, fins and eyes were examined for ectoparasitic myxosporeans using a stereomicroscope. The internal organs were removed and inspected with a stereomicroscope for endoparasitic myxosporeans. The position of the cyst, organs infected and the size of the cyst were noted. Spores were observed in squashed preparations of the infected organ or by removing a cyst from the infected organ, breaking it on a slide and examining it at 200 or 400X magnification.

Water samples from the study sites were collected and the physicochemical parameters (temperature, conductivity, pH, dissolved oxygen, percentage oxygen saturation, salinity and biological oxygen demand) measured using multiparameter analyzer which permitted rapid analysis and measurements to be done at the study sites[6].

Results

Parasitic infection

Fifteen of the 32 fish samples examined for myxosporeans were infected (infection rate of 46.9%). Similarly, 50 of 125 fish samples (40.0%) examined for ectoparasitic protozoans were infected. The ectoparasitic protozoans (all ciliates) identified were *Ichthyophthirius multifiliis* and *Trichodina sp,* while the myxosporeans were *Myxobolus njinei,* an unidentified species of *Myxobolus* and *Henneguya sp.* The unidentified species of *Myxobolus* had spherical spores with length greater or equal to the width; the intercapsular process was present but

Table 1: Prevalence of ciliates in four fish species from some freshwater habitats of Fako Division

Fish species	Parasites identified	Site of infection	Number of fish examined	Number of fish infected	Infection rate (%)
Clarias gariepinus	*Ichthyophthyrius multifiliis*	Skin Fins Gills	25	19	76.0
Oreochromis noliticus	*Ichthyophthyrius multifiliis*	Skin Gills Fins	14	2	14.2
Barbus camptacanthus	*Ichthyophthyrius multifiliis*	Skin Gills	68	11	16.2
	Trichodina sp	Skin Gills	68	8	11.8
Barbus occidentalis	*Ichthyophthyrius multifiliis*	Skin Gills Fins	18	5	27.8
	Trichodina sp	Gills	18	5	27.8
Overall			125	50	40.0

Table 2: Prevalence of myxosporeans in four fish species from some freshwater habitats of Fako Division.

Fish species	Parasites identified	Site of infection	Number of fish examined	Number of fish infected	Infection rate (%)
Clarias gariepinus	*Henneguya sp*	Gills Skin	8	1	12.5
Oreochromis nolticus	*Myxobolus njinei*	Fins	8	1	12.5
Barbus campacanthus	-	-	3	0	0
Barbus occidentalis	*Myxobolus njinei*	Fins Gills Liver Skin Intestine Gonads Heart Kidney	13	8	61.5
	Myxobolus sp	Fins Gills Liver Skin kidney	13	10	76.9
Overall			**32**	**15**	**46.9**

than half the length of the spore and the spores were coelomic.

Infection rate of ciliates

The infection rate of ciliates and the organs infected are shown in Table 1. The infection rates in the different fish species ranged from 11.8% in *B. camptacanthus* to 76% in *C. gariepinus. B camptacanthus* and *B. occidentalis* were both infected with *Trichodina sp* and *I. multifiliis,* but no mixed species infection was observed. The overall infection rate of ciliates was 40.0%. *I. multifiliis* was the most prevalent ciliate infection, as the parasite was found in all the four fish species, and *C. gariepinus* was the most succeptible to it. On the other hand, *Trichodina* infection was found only in *B.camptacanthus* and *B. occidentalis,* with a higher but insignificant infection rate in *B. occidentalis.*

Infection rate of myxosporeans

The infection rate of myxosporeans is shown in Table 2. All 13 *B. occidentalis* specimens were infected. Eight (61.5%) of them were infected with *Myxobolus njinei,* while 10 (76.9%) were infected with an unidentified species of *Myxobolus.* Five (38.5%) of the 13 infected fish had mixed

infection of *M. njinei* and the unidentified species of *Myxobolus. M. njinei* also infected *O. noliticus* (12.5%). Out of 8 specimens of *C. gariepinus* examined, only one (12.5%) was infected with *Henneguya sp.* The overall infection rate of *myxosporeans* was 46.9%. The infection rate was higher ($p<0.05$) in *B. occidentalis* than in either *C. gariepinus* or *O. noliticus.*

The unidentified species of *Myxobolus* was the most prevalent *myxosporean* infection in the freshwater fish species studied. *C. gariepinus* was infected only by *Henneguya sp* whereas *B. occidentalis* was susceptible to both *M. njinei* and the unidentified species of *Myxobolus*. The unidentified *Myxobolus sp* infected only *B. occidentalis* while *M. njinei* was found in both *B. occidentalis* and *O .noliticus.*

The 11 fish specimens examined for both ectoprotozoans and myxosporeans did not reveal any mixed infections of the 2 parasite types. There was no significant difference between the infection rates of ciliates (40%) and myxosporeans (46.9%).

Habitat effect on infection

I. multifiliis was the most prevalent ciliate in all the study sites while *Trichodina sp* was found only in fish from Ndongo and Ekande streams (Table 3). The highest infection rate (100%) was observed in the Great Soppo fish tanks with *I. multifiliis,* while the lowest infection rate (5%) was observed in Ekande stream with *Trichodina sp.* Ndongo and Ekande streams harboured both parasites.

Myxosporeans were more extensively studied only in fish from Ndongo stream, where all three *myxosporean* species identified were observed. The 6 fish specimens from Ewongo fish ponds were uninfected.

Physicochemical factors of water

The values of some physicochemical factors of water in relation to infection rate in the four study sites are shown in Table 4. The pH values of water in all the habitats were within the normal range. There was variation in temperature at the various sites, with the highest temperature (25.2^0C) recorded in Great Soppo fish tanks and the lowest (22.7^0C) in the Ewongo fish pond. Dissolved Oxygen levels (DO) in Ndongo stream (4.0mg/L) and Great Soppo fish tanks (3.3mg/L) were low. The area with the highest oxygen content was Ekande stream (4.6mg/L) but the area with the highest percentage oxygen saturation was Ewongo fish pond (51.5%).

The sites with the highest prevalence of infection were Great Soppo fish tanks (100%) and the Ndongo stream (69.2%), and these were also the sites with the lowest dissolved oxygen content (3.3mg/L and 4.0mg/L respectively) and percentage

Table 3: Prevalence of parasitic infection in fish from some selected freshwater habitats in Fako Division

Site	No. of fish examined	No. of fish infected	Infection rate (%)
Ewongo pond	25	4	16.0
Ekande stream	40	6	15.0
Ndongo stream	65	45	69.2
Great Soppo	15	15	100.0
Overall	146	70	47.9

Table 4: Infection rate of protozoans in relation to the physicochemical factors of water

Site	pH	T°C	DO(mg/L)	% O_2 (mg/L)	Cond. (µs)	Sal. (mg/L)	BOD (mg/L)	Infection rate (%)
Ewongo pond	7.7	22.7	4.5	51.5	0.2	0.2	2.5	16.0
Ekande stream	8.1	23.4	4.6	43.2	0.2	0.3	2.4	15.0
Ndongo stream	7.7	25.1	4.0	37.2	0.04	0.3	1.9	69.2
Great Soppo	**7.9**	**25.2**	**3.3**	**26.8**	**0.3**	**0.4**	**3.3**	**100.0**

NB: pH = Hydrogen ion concentration; T?C = Temperature in degrees Celsius;
DO = Dissolved Oxygen; Cond. = Conductivity, % O2= percentage oxygen saturation; Sal. = Salinity; BOD = Biological oxygen demand

oxygen saturation (26.8% and 37.2% respectively), and highest temperatures (25.2^{0}C and 25.1^{0}C respectively). On the other hand, the sites with the lowest prevalence of infection were Ewongo fish pond (16%) and Ekande stream (5%), and these were also the sites with the highest dissolved oxygen content (4.5mg/L and 4.6mg/L respectively) and percentage oxygen saturation (51.5% and 43.2% respectively), as well as lowest temperature (22.7^{0}C and 23.4^{0}C). Biological oxygen demand also varied between the sites, with the highest values at Great Soppo fish tanks (3.3mg/L) and the lowest at Ndongo stream (1.9mg/L).

Discussion

All four species of fish examined for ectoparasitic protozoan infection were susceptible to *I. multifiliis.* Similar results were obtained by Lom and Dykova[7] who observed that most species of freshwater fish were susceptible to *I. multifiliis,* though some were more susceptible than others. Price and Clayton[8] also reported that *I. multifiliis* was the most prevalent and most pathogenic ciliate infection of freshwater fishes. *C. gariepinus* was the most susceptible fish species, probably because the absence of protective scales on its body enhances penetration of trophozoites into the body of the fish. *I. multifiliis* is fatal to fish of all sizes and chronic infection will cause serious damage to the skin, fins and gills. The skin and gills were sites of heavy parasite infection in our study, with ulcers and lesions observed on the skin of highly infected fish. These lesions may be caused by the feeding action of trophonts (trophozoites) and the detachment of the mature tomites which are usually embedded in the epithelial cells[1].

Our study revealed that freshwater fishes were generally resistant to *Trichodina sp.* However, it would appear that the high content of organic pollutants in the water may predispose fishes to *Trichodina* infections as observed in Ndongo and Ekande streams.

The spores of the unidentified species of *Myxobolus* resembled those of *M. agolus*, *M. israelensis* and *M. exiguus* by being

spherical with the length greater or equal to the width, and the intercapsular process being present but indistinct[9]. The spores, however, differed from those of *M. agolus* and *M. israelensis* in that their polar capsules occupied more than half the length of the spore.

The *Henneguya sp* observed in this study resembles *H. malapteruri* and *H. bopeletic*[10] with the following characteristics:- spore body was ovoid with total length of spore >40µ, and the caudal appendages were separated from the base. However, it differed from *H. bopeletic* in that the polar capsules of *H. bopeletic* were longer. It also differed from *H. malapteruri* in that the caudal processes were shorter.

Longshaw *et al*.[11] reported that myxosporean infection is most common in compromised and polluted water habitats. This assertion seems to explain their occurrence in Ndongo stream which was the most polluted of the four sites. The physico-chemical factors of water were found to affect disease prevalence in freshwater. The highest temperature was recorded in the Great Soppo fish tanks. This was probably because the tanks were made of cement which absorbed heat from the sun and transmitted it to the water, thus heating it up and affecting other parameters like dissolved oxygen. This led to compromised water conditions which were suitable for parasite growth. Folack[12] reported that when there are many bacteria in a water body or when the water becomes too warm, dissolved oxygen levels might be reduced. Thus, dissolved oxygen levels in Ndongo stream might have been low for these reasons. Water bodies with high microorganism content have a low biological oxygen demand, which ties with the results from Ndongo stream. The high value of biological oxygen demand of the water in the Great Soppo fish tanks showed that though the water conditions were compromised, the tanks were not polluted.

Conclusion

It may be concluded from this study that the *Clarias gariepinus* was the most susceptible fish species to ectoparasitc protozoan infection while *Barbus occidentalis* was the most susceptible fish species to myxosporean infection. *Ichthyophthirius multifiliis* was the most prevalent and pathogenic ectoparasitic protozoan while the unidentified species of *Myxobolus* was the most prevalent myxosporean. The effects of temperature and dissolved oxygen on fish health were greater than those of other physicochemical parameters.

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FACTORS INFLUENCING SMALLHOLDER DAIRY PRODUCTION AND MARKETING PERFORMANCE IN RUNGWE DISTRICT, TANZANIA

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FACTEURS INFLUENÇANT LA PETITE PRODUCTION LAITIERE ET LA PERFORMANCE DE COMMERCIALISATION DANS LE DISTRICT DE RUNGWE EN TANZANIE

Résumé

Une étude sur la performance d'un échantillon randomisé de 120 petits exploitants de bétail laitier a été conduite dans le district de Rungwe en Tanzanie. Les résultats ont montré que le bétail laitier contribuait environ à hauteur de 34% au revenu du ménage. La production laitière était de 6,8 – 9,8 litres/vache/jour, soit une moyenne de 8,1 litres/ jour. Le lait vendu par ménage était en moyenne de 11,5 ± 0,9 litres/jour. Les analyses économiques effectuées sur la ferme ont montré que la marge bénéficiaire brute mensuelle des exploitants variait entre 2.603 et 32.270 Tshs (2,2 – 26,9 $ EU) par mois. Les analyses de régression ont révélé que le revenu net tiré de la production laitière et l'accès au crédit avaient un effet positif important sur l'utilisation des intrants (P<0,01). L'accès au marché et la participation active avaient un impact positif important (P<0,05) sur la quantité d'aliments commerciaux servis à une vache/jour, et un effet très important (P<0,01) sur la quantité de lait produite par vache/jour. Les obstacles majeurs à l'amélioration de la production étaient le coût élevé des intrants, le faible coût du lait et le manque de débouchés pour écouler l'excédent de lait. D'après les conclusions, le renforcement des capacités des petits exploitants à accéder aux marchés et à y participer activement est important pour une adoption accrue des technologies laitières améliorées.

Mots-clés : Petite production laitière, production, commercialisation, Tanzanie.

Summary

A study on the performance of a random sample of 120 smallholder dairy farmers was conducted in Rungwe district, Tanzania. Results showed that dairy cattle were contributing an average of 34% of the household income. Milk production ranged from 6.8 – 9.8 litres per cow per day (8.1 litres/day). Milk sold per household was an average of 11.5±0.9 litres per day. Economic analyses of the farm enterprises showed that farmers' monthly gross margins ranged from Tshs. 2,603 – 32,270 (US $ 2.2 – 26.9) per month. Regression analyses showed that net income from dairying and access to credit had significant positive influence on input use (P<0.01). Access to market and active participation had significant (P<0.05) positive impact on amount of commercial feed given to a cow/day and highly significant (P<0.01) influence on amount of milk produced by a cow/day. Major constraints to improved production were high cost of inputs, low milk prices and lack of market for surplus milk. The findings suggest that enhancing ability of smallholder farmers to reach input/output markets and active participation in them are key to increasing adoption of improved dairy technologies.

Key words: Smallholder dairy production, marketing, Tanzania

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Introduction

In order to attain self sufficiency in milk production, the Tanzania government has since mid 1970 implemented various cross-breeding programmes aimed at enhancing the milk production potential of the local cattle population. The southern highlands dairy development programme (SHDDP) which was implemented between 1978/79 to 2002 in Iringa and Mbeya regions of the southern highlands of Tanzania is one of such initiatives. SHDDP was supported by the Swiss government[1]. As a result of this support, Rungwe district had by 2002 about 30,493 dairy cattle[2]. With a population of about 306,000 people[3], it is one of the districts with the highest concentration of dairy cattle per capita in the country.

In spite of the favourable climate, the milk production of dairy cattle in Rungwe district has been reported to be about 1800 litres against a potential lactation yield of 2,400 – 4,000 litres per cow[4,5] . The reasons for the low level of milk production are not well known. However, accessibility to markets is important to ensure continuous flow of income to producers for livelihood and re-investment[6]. Following the closure of the only milk processing plant in Mbeya in 1996, milk outlets have become limited and could be constraining efforts by smallholder farmers in adopting technologies that could improve the productivity of the dairy industry in the region. Factors constraining the biological and economic performance of the industry need to be well understood before sustainable development startegies can be implemented. While several studies[7, 8, 9] have looked at the economics and marketing aspects of smallholder production in the Southern Highlands , there is lack of information on factors influencing the use of inputs and services required to sustain higher milk production and marketing performance of smallholder dairy systems in sub-Saharan Africa. The objective of this work was therefore to study the factors that influence performance of smallholder dairy farming in rural communities such as those of Rungwe district so that proper strategies to address them can be formulated and implemented.

Material and Methods

Study area

Rungwe district is located in Mbeya region in the Southern Highlands and has an area of 2,211 km^2. It is lies between longitudes 330 20' E and 340 00'E and latitudes 8050'S and 9020'S. Its altitude varies from 1,000m to 2,958m. Annual precipitation of 1,700mm to 2,400mm is distributed almost throughout the year except September and October. Temperature range is between -6^{0}C and 25^{0}C (Planning Commission and Region Commissioner's Office Mbeya, 1997). The area is volcanic and mountainous plateau with numerous steep valleys and hills. The study covered four divisions of the district: Tukuyu, Ukukwe, Pakati and Busokelo. Ukukwe, Pakati and Busokelo are located 5, 10 and 30 km from Tukuyu town which is the main commercial and administrative centre located along the Mbeya – Kyela highway.

Data collection

Multi-stage and simple random sampling techniques were used in selecting 120 respondents. Primary data were collected using a pre-coded structured questionnaire, which captured information on both qualitative and quantitative data from sample households. Socio-economic data of the respondents included: household size, age, gender, education level, experience in dairying, source of labour and capital used

in establishing and running the dairy enterprise. Secondary data was collected to provide information that supplemented primary data. This was extracted from district Headquarter reports and other documentary materials that were of relevance to the study.

Briefly, market access was defined in terms of frequency of milk sales which ranged from twice per day (high market access) to less than three times per week (low market access). A second criterion for defining market access was time taken to reach milk market outlets, i.e. less than one hour, one to three hours and more than three hours travel time categorised respectively as high, medium and low market access[10,11].

Data analysis

The collected information was analysed using Statistical Package for Social Science[12]. Descriptive and quantitative assessments were employed. For descriptive analysis percentages, means, ranges, cross tabulation and other statistical measures were employed to assess variability in socio-economic characteristics of respondents, market access and dairy performance among the smallholder dairy farmers.

Profit margins

In order to justify farmers' decision to engage and invest, economic viability of smallholder dairy enterprise is an important aspect that needs to be examined. The monthly gross margins estimates were used in evaluating smallholder dairy enterprise viability. Gross margins were estimated as follows:

$PM_{ij} = \Sigma(Pij\ Vij - VCij)$

Where: PM_{ij} = Profit margin earned by i^{th} farmer in j^{th} market, P_{ij} = Unit price sold by i^{th} farmer in j^{th} market, V_{ij} = Milk volume sold by i^{th} farmer in j^{th} market, VCij = Variable cost incurred by i^{th} farmer in j^{th} market and Σ = Summation sign

Influence of market access on dairy production and marketing

Influence of market access and socio-economic factors on dairy performance was examined using partial Analysis of Variance (ANOVA) by studying their relationship on individual selected variables such as pasture plot size, quantity of minerals and concentrates given to cow per day, age at first calving, calving interval and total milk produced per cow/day.

Results

Socio economic characteristics of respondents

A total of 120 households keeping dairy cows, thirty in each of the Rungwe district's divisions, were interviewed. . Briefly, 76% of respondents were male, 88% had formal education (primary to secondary school), 49.1% were of 30 – 50 years of age. The household size was an average of six persons and owned an average of three heads of cattle. About 56.7% depended on family labour. About 80.8% of respondents had established their dairy enterprises using own funds. A high proportion (79.1%), had been in the dairy farming business for over 5 years.

Dairy herd size, production and reproductive performance

Table 1 presents the average herd size and composition per sample households. All surveyed households kept improved dairy cattle. The herds were found to be composed of lactating cows, dry cows, heifers, bulls and calves. On average the number of dairy cows were 1.6, 1.6, 1.1 and

1.4 in Ukukwe, Busokelo, Pakati and Tukuyu respectively and there was a significantly ($P<0.05$) lower in Pakati division.

Dairy cattle production parameters in the four divisions showed that the highest average milk yield of 9.8 litres /cow/day was recorded in Tukuyu while the lowest milk yield of 6.8 litres/cow/day was observed in Pakati division. However, there were no clear differences in terms of age at first calving across the locations. Although not statistically significant ($P>0.05$), the number of services per conception and calving interval were lowest in Busokelo division.

Farmers' perception towards dairy performance trend

Respondents were asked to indicate if the performance of their cows has been increasing or decreasing over the years. Results in Table 2 show that 56.3% of the respondents indicated that the performance of their cows has been decreasing over the years. Most of the reasons given by the respondents (Table 2) to account for the decrease were: low use of inputs due to high cost of inputs (28.6%), lack of market for surplus milk (21.0%) and low milk prices (6.7%).

Milk production and marketing

The average amount of milk produced per respondent household was 11.5 litres with a range of 7.9 litres in Pakati to 14.5 litres in Busokelo division.(Table 2). Average milk sold was 5.7±0.7 litres per household per day. . Pakati division had the lowest amount of milk sold per day per respondent (2.5 litres). The results reveal that the amount of milk sold depended on total milk produced and varied from 36.5 % in Pakati division to 70.5% of milk produced per household in Tukuyu division.

Table1: Average dairy herd size, production and reproductive performance of dairy cows in four divisions of Rungwe district

Category	Ukukwe	Busokelo	Pakati	Tukuyu	Overall
Milking cow	1.6^{a} (0.2)	1.6^{a} (0.2)	1.1^{b} (0.0)	1.4^{a} (0.1)	1.4 (0.0)
Dry cow	0.1 (0.0)	0.3 (0.1)	0.2 (0.0)	0.2 (0.1)	0.2 (0.0)
Heifer	0.2 (0.0)	0.3 (0.0)	0.1 (0.0)	0.4 (0.1)	0.3 (0.0)
Calves	1.3 (0.1)	0.9 (0.1)	1.0 (0.0)	1.0 (0.1)	1.0 (0.0)
Bulls	-	-	0.1 (0.0)	0.1 (0.0)	-
Total	3.3^{a} (1.3)	3.1^{a} (0.3)	2.6^{b} (0.1)	3.1^{a} (1.3)	3.0 (0.1)
Production and reproductive performance					
AFC (months)	38.2(0.8)	39.6 (1.5)	38.7 (2.6)	35.5 (0.8)	37.7 (0.8)
CI (days)	546.6 (28.7)	498.4 (22.8)	590.7 (37.5)	555.6 (39.5)	547.5 (16.2)
Service per conception	2.7 (0.4)	1.8 (0.3)	1.9 (0.6)	2.4 (0.9)	2.2 (0.2)
Milk yield per cow/day	7.4^{a} (0.8)	8.4^{a} (0.7)	6.8^{b} (0.6)	9.8^{a} (0.9)	8.1 (0.4)

Figures in brackets show standard error (SE) and figures with the same superscripts within rows are not significantly different.

Milk prices and sales in different market outlets

Results of 94 respondents across four divisions of Rungwe district (Table 3) revealed that the majority (95.6%) of respondents either sold their milk at home to vendors and neighbours or carried it to customers on foot thus no transport was required and only 4.4% used bicycles to transport their milk to the market. The reliance of "on-foot" transport may be due to lack of access to other means of transport.

The average milk price in the district was 167.3 T.sh/l. Moreover, prices differed according to different type of buyers. Vendors who mostly operated in the remote rural areas of Busokelo division paid the lowest price to the farmers, an average of 143.6 T.sh/l and followed by private and co-operative group shops (163.6 and 164.2 Tsh/l respectively. Vendors collected milk from farmers to sell either in Kyela or Mbeya Urban centres. The lowest price paid by vendors may be attributed to transport cost because they used public transport, which was 50 shs per litre of milk transported to Mbeya or Kyela. The same applied to private and group cooperative shops where surplus milk had to be transported to Mbeya or Kyela. Individual consumers paid the highest price , an average of 172.3Tsh/l. However, most of the milk was sold to cooperative group shop because farmers choose milk outlet based on the quantity it absorbed and mode of payment.

Most dairy farmers (41%) did not make any contractual arrangements with buyers. Verbal contracts were the most predominant. Cooperative and self help groups had some binding agreement stipulated in their record books which included mode of payment, quantity to be supplied, quality control, time of supplying and some conditions for one (supplier) to qualify to be a member of a group such as entry fee and honesty.

Table 2: Farmers' perception (%) and reasons for dairy performance trends (%) on smallholder dairy farms in Rungwe district (n =119)

	Parameter	Ukukwe (n=30)	Busokelo (n=30)	Pakati (n=30)	Tukuyu (n=29)	Overall (n=119)
	Milk production /Hh/day (mean±S.E) (litres)	11.0±1.8	14.5±2.0	7.9±1.0	12.9±2.1	11.5±0.9
	Milk sold /Hh (mean±S.E) (litres)	6.0±1.1	5.3±0.9	2.5±1.2	9.1±2.1	5.7±0.7
	(%)	(54.5)	(36.6)	(31.6)	(70.5)	(49.6)
Trend in milk yield						
Increasing	%	10.0	10.0	3.3	17.2	10.1
Unchanged	%	43.3	33.3	33.3	24.1	33.6
Decreasing	%	46.7	56.7	63.3	58.6	56.3
	Reasons for Trend:					
Increasing	Proper use of input	6.7	6.7	3.3	13.8	7.6
	Good milk market	3.3	3.3	0.0	3.4	2.5
Unchanged	No improvement in dairy practices	43.3	33.3	33.3	24.1	33.6
Decreasing	High input cost	26.7	26.7	30.0	31.0	28.6
	Low milk price	3.3	0	16.7	6.9	6.7
	Lack of milk market	16.7	30.0	16.7	20.7	21.0

All figures are percentage of respondents.

Table3: Amount of milk sold, price, distance mode of transport and payments received in different market outlets by smallholder farmers in Kyela .

Customer type	n	Amount sold/day	Price/litre (Tsh)	Range (prices)	Distance in km
Group shop	26	10.6^{a}(1.8)	164.2^{b}(2.9)	140-180	1.1
Individual consumer	42	1.3^{b}(0.8)	172.3^{a}(5.3)	150-300	0.6
Vendors	14	4.8^{a}(1.1)	143.6^{c}(2.3)	130-160	-
Kiosk/retail shop	15	5.3^{a}(0.8)	163.6^{b}(4.4)	100-200	2.5
Overall Total(mean)	97	6.5(0.7)	167.3(3.0)	100-300	1.1
Mode of transport	94	On foot	Bicycle		
		95.7%	4.3%		
Mode of Payment	97	Daily cash	Weekly	Bi-weekly	Monthly
		36.1%	9.3%	36.1%	18.6%
Nature marketing contract		No Contract	Informal (Verbal) agreement	Informal (written agreement)	
	105	41.0%	24.8%	34.3%	

Figures in brackets show standard error (SE) and figures with the same superscripts within columns are not significant different

Average monthly income from dairying

The results on income and costs per household per month in Table 4 indicate that milk is the major source of income in Rungwe district accounting for an average of 34% of the respondents' income with a range of 15.6% in Busokelo Division and 50.2% for Pakati division.

Regarding cost structure, feed costs accounted for 66% of total cash expenditure per month Table 4). Farmers in Tukuyu division spent more money on dairying (Tsh 22,685/ month) followed by Ukukwe Pakati and Busokelo divisions.

Despite high costs of production incurred by the farmers in Tukuyu, their monthly gross margins (T.shs 32,271 / month) was almost double that of Ukukwe and Busokelo and 10 times higher than of Pakati division..

Influence of market access and factors limiting dairy performance

The results (Table 5) shows that improved market access has significant impact on land size allocated for pasture, amount of concentrates given to a cow per day and total milk produced per cow/day. Age at first calving and calving interval increase as market access becomes more difficult although this is not statistically significant (P>0.05).

Disease control is important for good performance of dairy cows. In the study area, tick borne disease is the most problematic. Dipping or spraying of animals is a necessary measure to ensure good health and performance of cattle. The results of ANOVA analysis reveal that though not statistically significant (P>0.05), increase in market access tends to have a positive

impact on frequency of spraying.

Problems limiting increase in dairy herd performance

The study also investigated the constraints encountered by smallholder dairy farmers in their day-to-day activities. The results (Fig 1) shown earlier of descriptive statistics suggested that dairy production in the study area faces impediments that lower the performance of the industry. Among the mentioned problems high input cost (85.8%), low milk price (62.5%) and lack of market for surplus milk (50% were found to be the most limiting factors. The other problems mentioned by farmers in order of importance were animal diseases, availability of feeds during the dry season, access to credit facilities, low conception rates, distance to milk market (mentioned by 33.3% of dairy in farmers of Pakati division), lack of own capital, land availability, poor breeds, availability of extension agents and lack of labourers.

Table 4: Average monthly income from dairying

	Divisions				
	Ukukwe	Busokelo	Pakati	Tukuyu	Overall
Monthly income from					
Sale of milk	29 500.0	21 033.5	12 743.3	41 963.0	26 310.0
Sale of manure	224.5	-	372.7	451.4	262.2
Sale of animals	2 480.5	4 942.0	3 319.4	8 791.7	4 883.3
Hiring bulls	-	-	-	3 750.0	937.5
Total monthly income	**32 205.0**	**25 975.5**	**16 435.4**	**54 956.1**	**32 393.0**
% of total HH income	30.1	50.2	15.6	38.8	34
Variable costs					
Pasture production	519.4	-	135.6	422.2	269.3
Purchase of minerals	775.0	1 025.0	469.2	1 184.2	863.3
Cost of Concentrates	11 356.6	6 630.0	7 733.3	14 078.3	9 977.5
Animal treatment	365.0	347.5	202.8	495.0	352.6
Spraying	511.4	473.7	258.3	761.1	501.1
Labour	4 072.2	3 902.8	4 750.0	5113.9	4 459.7
Breeding	725.0	302.8	283.3	630.6	485.4
Total variable costs	**18 324.6**	**12681.8**	**13 832.5**	**22685.3**	**16 908.9**
Monthly Gross margins	**13880.4^{b}**	**13293.7^{b}**	**2602.9^{c}**	**32270.8^{a}**	**15484.1**

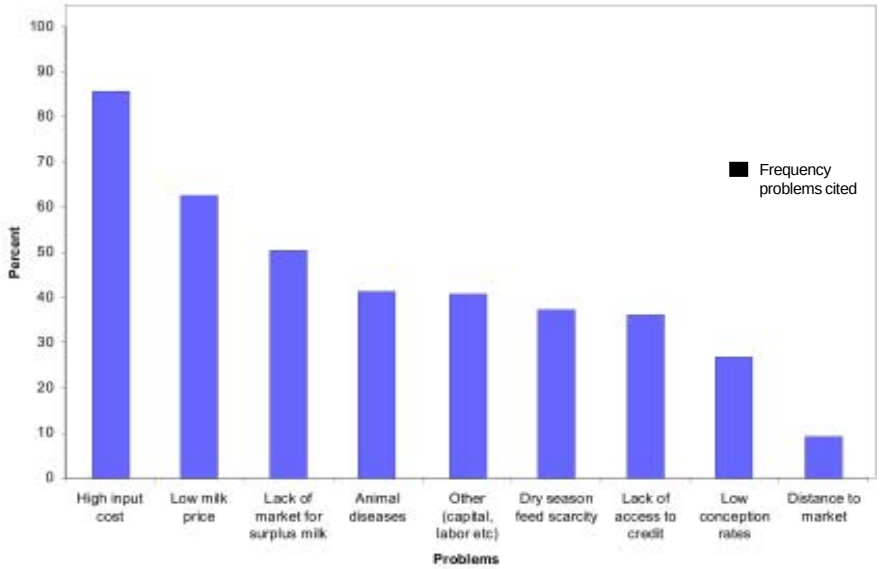
Figures within the row with the same superscripts are not significant different Tshs 1,200 = 1 Us $

Table 5: The impact of market access on input use and dairy performance by smallholder dairy farmers in Rungwe district

		Market access			
Parameter	Indicator	High	Medium	Lowaccess	F-value
Frequency of milk sale[a)]	Pasture plot size (n=97)	1.0(25)	0.59(44)	0.49(28)	**4.251***
	Minerals in g (n=68)	33.3(27)	24.1(29)	20.8(12)	2.236
	Concentrate kg (n=102)	4.5(28)	3.5(46)	3.5(28)	**3.527***
	AFC in months (n=64)	35.5(14)	37.5(32)	40.1(18)	2.161
	CI in days (n=92)	539.9(27)	529.7(38)	580.0(27)	0.868
	Milk produced in litres (n=119)	10.4(29)	7.4(54)	6.436)	**9.214****
	Spraying/month (n=115)	2.5(26)	2.3(54)	1.8(35)	2.153
Time spent to travel to market[b)]	Pasture plot size (n=97)	0.9(60)	0.3(19)	0.4(18)	**5.837****
	Minerals in g (n=68)	28.7(56)	20.0(7)	22.0(5)	0.719
	Concentrates - kg (n=102)	4.2(65)	2.3(19)	2.9(18)	**6.171****
	AFC in months (n=64)	36.5(42)	40.0(13)	40.3(9)	2.534
	CI in days (n=92)	524.5(57)	568.1(19)	604.8(16)	1.920
	Milk produced/cow/day in litre (n=119)	9.2(73)	4.9(25)	6.4(21)	**13.236****
	Spraying/month (n=115)	3.6(59)	2.2(15)	3.8(9)	2.719

** Significant at 1%level and * Significant at 5% level
a) x2/day = High; x1/day = Medium; < 3days/week = low market access
b)< 1 hrs = High; 2-3 hrs = Medium; > 3hrs= Low market access

Fig. Problems limiting increase in dairy performance in Rungwe district



Discussion

Socio economic characteristics of respondents and the dairy herd size in the study area were similar to those found in rural based, smallholder farming communities in other areas of Tanzania[12,13]. The proportion of cows in the herd is of paramount importance in dairying since it contributes directly to revenue generation of the herd . About 47% of the herd were milking cows which reflects the long calving intervals and high number of services per conception both of which need improvement.

The milk yields of 8.1 litres per cow per day were slightly higher than those reported in Tanga[13] probably because of the relatively more favourable climate for dairying. It is noteworthy that Pakati division which has the poorest access to markets for fresh milk, had significantly ($P<0.05$) lower average milk production of 6.8 litres per cow per day than Tukuyu division (9.8 litres/cow/day). The higher dairy performance in terms of milk yield in Tukuyu may be attributed to proximity to both input and output markets with associated high milk price and higher use of inputs such as concentrates.

Across the four divisions, differences in reproductive performance as measured by age at first calving, calving interval and services per conception were not statistically significant ($p>0.05$).This may be attributed to the fact that most farmers depended on natural services from bulls kept by fellow farmers within a village. However, more than 56% of the farmers were of the view that the performance of their cows has been decreasing over the years due to the fact that farmers have low purchasing power such that they fail to afford required inputs, which were highly priced. Furthermore, the low prices offered for milk and lack of milk market outlets also discourage farmers from using dairy inputs to improve their cows' performance. For example, in Ndubi village it was reported that the price of milk has remained at Tsh 150/litre since 1995 but the price of inputs was increasing day after day. This has made some farmers to stop spraying their animals with acaricides. Farmers who reported improvement in performance of their dairy cows attributed this to good milk market access and proper use of inputs according to the advice given by the extension agents. On the other hand, farmers who have not adopted improved dairy practices reported the performance of their cows to have remained unchanged over the years[7].

On average amount of milk produced per respondent household was 11.5 litres of which about 50% (range 29.5 - 68.4%) was consumed at home and the rest sold for cash within the neighbourhood or transported on foot. Similar findings have been reported[9, 13]. The average milk price in the district was 167.3 T.sh/l. This was similar to that reported earlier[10] in the district which support the observation by farmers that milk prices have stagnated for a long time The marketing pattern which show that farmers market most of their milk on foot within the neighbourhood or sell it to vendors who pay the lowest price (143.6 T.shs/litre) is a result of limited milk market infrastructure (roads, milk collection and cooling equipment and transportation). While individual consumers paid the highest price on cash basis, selling through co-operatives and self help groups, though paying a relatively lower price was a more reliable form of marketing milk in terms of risk sharing, contractual price stability and regularity of market outlet[14].

In terms of gross margins from dairying enterprises (Table 4) dairying accounted for about 34% of the household income in Rungwe District while feeds made up to 66%

of total cash expenditure on the dairy enterprise. The level of gross margins are similar to other findings on smallholder dairy[13,15] These results are in agreement with an earlier report that three quarters of all expenses go into animal feeding especially minerals and concentrates, which were considered to be expensive[9]. However, these results for Rungwe district are higher than those reported by Kisusu[15] and Msangi[13] who found that labour costs accounted for about 55% and 38% respectively of the total dairy expenditure.

The results in this study (Table 5) shows that improved market access has significant impact on land size allocated for pasture, amount of concentrates given to a cow per day and total milk produced per cow/day. This implies that the shorter time spent on traveling to the market and the higher the frequency the milk is sent to the market the bigger the land that is allocated for pasture and the same applies to concentrate use and milk yield. This confirms the empirical evidence[16,17,18,19] that good market access leads farmers to produce high value commodity for which they have comparative cost advantages. It can be inferred that access to output market increases demand for inputs. It is notable that market access did not have significant influence ($p>0.05$) on reproductive indices (age at first calving and calving interval) or on frequency of spraying of animals against ticks. This is probably because these parameters are influenced more by farmer's skills and knowledge that would enable them detect heat and apply other relevant husbandry practices such as disease control to achieve better performance rather than output market access *per se*.

Among the problems that limit dairy performance (poor reproductive and production indices) by smallholder dairy farmers, high input cost of (feeds, veterinary drugs) relative to price of milk, lack of market for surplus milk and associated poor marketing infrastructure (both institutional and structural) and animal diseases appear to be the most significant. Improving market access appears to be key to improving the willingness of farmers to allocate more resources (land, labour) to dairying and hence enhancing dairy performance.

Conclusions

The study showed that smallholder dairy production in Rungwe district is an important economic activity to the majority of rural households contributing up to 50% of total household income. The level of performance is however low as indicated by low production and reproductive indices. as well as poor access to fresh milk markets. Major constraints to improved production and marketing include high input costs, low milk price, lack of markets for surplus milk and animal diseases.

Improving market access to fresh milk markets had significant impact on use of inputs (land allocated to pasture, feed concentrates) that lead to higher milk production. Addressing milk marketing constraints will be key in uplifting the performance and contribution of dairying to household incomes and poverty reduction of rural households.

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PERFORMANCE OF FINISHER BROILER CHICKENS AS AFFECTED BY GRADED LEVELS OF COOKED COWPEAS *(VIGNA UNGUICULATA)* IN THE GROWER-FINISHER DIET

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LES EFFETS DU TAUX D'INCORPORATION DE NIEBE CUIT *(VIGNA UNGUICULATA)* SUR LA PERFORMANCE DES POULETS DE CHAIR EN FINITION

Résumé

Cent soixante (160) poulets de chair mâles âgés de 21 jours et pesant en moyenne 416,32 g ont été répartis au hasard dans 40 unités expérimentales de 4 poulets chacune. Chacune des cinq rations expérimentales contenant respectivement 0% de niébé (F0, ration témoin) ; 15% (F1) ; 20% (F2) ; 25% (F3) et 30% (F4) de niébé cuit a été servie à 8 unités expérimentales pendant 28 jours selon un dispositif expérimental complètement randomisé pour évaluer la performance de production des poulets de chair en finition. Il n'y a pas eu de différence significative (P>0,05) entre les différents lots pour la consommation alimentaire totale. Le poids vif, le gain de poids et l'indice de consommation enregistrés pendant toute la période de l'essai dans les lots F3 (contenant 25% de niébé cuit) et F4 (contenant 30% de niébé cuit) ont été statistiquement faibles (P<0,01) par rapport aux autres groupes, et les coûts de production / kg de poids vif étaient très élevés (P<0,05) pour les rations F3 et F4. Les poulets servis de F0, F1 et F2 ont été comparables pour ces paramètres. Le rendement en carcasse, le pourcentage des différents organes analysés par rapport au poids vif ainsi que le taux de créatinine dans le sérum des poulets ont été statistiquement comparables (P>0,05) pour tous les traitements. Il a été conclu que l'on peut servir une ration contenant jusqu'à 20% de niébé cuit sans affecter négativement la performance des poulets de chair.

Summary

One hundred and sixty (160) male broiler chicks, 21days-old and weighing 416.32g on average, were randomly distributed into 40 experimental units of four birds each. Five experimental diets containing respectively 0% (F0, control), 15% (F1), 20% (F2), 25% (F3) and 30% (F4) cooked cowpeas were each fed to 8 experimental units during 28 days in a completely randomised design and broilers' performances measured. Parameters measured were, weight gain, feed conversion ratio (FCR), feed cost per kg weight gain, carcass evaluation and presence of antinutritional factors (ANFs). There was no significant difference (P>0.05) between treatment groups for total feed consumption. Live weight, weight gain and feed conversion ratio were statistically poorer and cost of production highest (P<0.01) for the F3 and F4 birds as compared with the other groups, which were not different (P>0.05) for these parameters. All the treatment groups were not statistically different (P>0.05) for carcass yield, proportions of organs and serum creatinine level. It was concluded that up to 20% of cooked cowpeas could be used in the finisher diet without negatively affecting the performance of broilers.

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Introduction

Soybean meal, with 44-48% crude protein is the major source of plant protein in poultry diets[1,2]. According to Robinson and Singh[3], the price of soybean meal is forecast to increase higher on the international market due to the high demands of China and the emergent countries of Asia. As a consequence, there is the risk that this traditional source of protein for poultry would become too expensive and scarce in the years to come, particularly in low-income African countries south of the Sahara. It is, therefore, necessary to search for good substitutes using readily available local feedstuffs. Grain legumes could be good substitute for soybean meal, as they are known to have a similar amino acid profile[4]. However, recent studies[5,6,7,8] agreed that the utilization of raw cowpeas *(Vigna unguiculata)* was limited by the presence of antinutritional factors (ANFs), which negatively affect broilers' feed consumption and growth, thus confirming previous reports[2,9,10,11,12,13] on the necessity to detoxify grain legumes before they can be included in monogastric animals diets. Different methods have been developed to de-activate the ANFs including heat treatment.

The objective of this study was, therefore, to evaluate the effects of graded levels of cooked cowpeas on the production performance of finisher male broilers.

Materials and Methods

Animals and Diets

This study was conducted at the Experimental Farm of the University of Dschang. A total of 160 male Hubbard broiler chicks, 21 days old and with an average live weight of 416.32g were used. The chicks were started in deep-littered open side type house, with a conventional diet to contain crude protein (CP) (21.89%), metabolizable energy (ME) (2990.79Kcal/kg), calcium (0.96%) and phosphorous (0.1%). They were immunised against Newcastle disease and infectious bronchitis at 7 and 23 days of age and infectious bursal disease (Gumboro) at 10 days of age. Anti-stress (Aliseryl W.S®: 1g/5l of water) was given during the first three days, before and after each vaccination, during the transfer of birds from the brooding to the finishing housing, and during transition of starter to finisher diet. Coccidistatics Vetacox®, and an anti-infection drug Furaltadone® were administrated in drinking water for 3 consecutives days from 10th day of age and every week thereafter until 6 weeks of age. An antibiotic, Oxytetracycline, was used to treat a respiratory disease at 3 weeks of age. Feed and water were provided *ad libitum*.

Cowpea grains bought from the local market were cooked for 3 minutes in a pressure cooker at the temperature of about 115^{0}C under a pressure of 155Pa. Cooked grains were sun-dried for one week to a humidity level of about 11%. Five experimental diets containing 15% (F1), 20% (F2), 25% (F3) or 30% (F4) cowpea and one control (F0) were formulated as shown in Table 1. Samples of cowpea and feeds were analysed according to AOAC[14].

Experimental Design and analysis

The 160 birds were caged in pairs. Each of the five experimental diets (treatments) F0, F1, F2, F3 and F4 was allocated to 8 experimental units in a completely randomised design. The experiment lasted 28 days and parameters measured were

Table 1. Ingredients and nutrient composition (%) and cost (FCFA) of experimental diets.

Feedstuffs	Experimental diets				
	F0 (Control)	F_1	F_2	F_3	F_4
Maize	62	53	50	47.5	44.5
Wheat middling	8	10	10.5	9	9
Soya bean meal	8	11	10	10	7.75
Cottonseed meal	5.5	3.5	3	2.5	2.25
CMAV 10% [1]	10	0	0	0	0
Cooked cowpea	0	15	20	25	30
Fish meal	4	5	4	3.5	4
Bone meal	1.25	1.25	1.25	1.25	1.25
NaCl	0.5	0.5	0.5	0.5	0.5
Synthetic D-L Methionine	0.25	0.25	0.25	0.25	0.25
Synthetic Lysine	0.5	0.5	0.5	0.5	0.5
Total %	100	100	100	100	100
Calculated composition (%)					
M.E., Kcal/Kg	3072	3037	3067	3087	3125
Crude protein	18.75	18.55	18.71	18.89	19.09
Crude Fibre	3.9	3.8	3.5	3.2	2.9
Lysine	1.3	1.3	1.2	1.2	1.1
Methionine	0.53	0.52	0.58	0.50	0.50
Total phosphorous,	0.7	0.53	0.52	0.48	0.49
Calcium	0.93	0.9	0.9	0.9	0.9
Cost of production of kg of ration (FCFA/kg)[2]	264.10	255.35	267.35	268.22	279.6

1(CMAV 10%) composition (g kg –1): protein (20), fat (40), fibre (20), Ca (90), P (37.5), lysine (28), methionine (23), Methionine +Cystine (28), M.E. (2300 Kcal Kg –1), vitamin (100kg): A (15x106 IU), D3 (3x106 IU), E (53X104 mg), vitamin (mg kg–1): K3 (26), B1 (25), B2 (60), B6 (25), B12 (0,3),, folic acid (20), trace minerals (g kg–1): Fe (1650mg kg–1), Cu (200mg kg–1), Zn (1300mg kg–1), Mg (850mg kg–1), Se (3mg kg–1))

[2] 1 Euro = 655.95 FCFA

? Metabolizable Energy calculated according to Sibbald quoted by Wiseman34

feed consumption, weight gain, feed conversion ratio (FCR), feed cost per kg weight gain, carcass evaluation and presence of antinutritional factors (ANFs). At the beginning of the trial, initial live weights of the birds were determined by weighing them in groups at day-old and by pair, by cage each week. Weighing of birds took place in the morning hours (6.30-7.30 am). Weight gain was determined by difference of the live weight of two consecutive weeks. Daily feed consumption was determined by subtracting the leftover from the quantity offered each day. The weighing of birds and feed was done using a top loading (20 kg capacity. Goat Brand®) weighing scale.

Table 2. Chemical composition (%DM) of raw and cooked cowpea

Nutriments	Raw cowpea(why this information)	Cooked cowpea
M.E. (Kcal kg^{-1}DM) *	4460.89 etc.	4506.58 etc.
Crude protein	26,51	25,47
Organic matter	94,89	95,52
Dry matter	88,22	89,31
Ash	5,11	4,98
Ether extract	2,20	2,13
Crude Fibre	5,28	5,27

* See Table 1

DM: dry matter

Feed conversion ratio was determined as feed intake divided by weight gain. Cost per weight gain was calculated as FCR x cost per kg feed. At the end of the trial, 8 birds per treatment were used for carcass evaluation[15]. Blood samples collected from the jugular vein of sacrificed birds were analysed for creatinine according to Jaffe's reaction, using a commercial kit by Boehringer Mannheim GmbH Firm (Mannheim, Germany).

All the data collected were subjected to analysis of variance. The Duncan's Multiple Range test was used to determine the extent of dispersion between the means[16]

Results

The chemical composition of raw and cooked cowpeas is presented in Table 2. Cooking affected the nutritive value of cowpeas. Cooked cowpeas had higher ME and organic and dried matter contents than raw cowpeas. However, CP, ash, fat and CF contents decreased after cooking.

In general, the feeding of cooked cowpea diets significantly (P<0.05) affected all the performance parameters of broilers, except feed consumption and percent mortality (Table 3). Weight gain was significantly (P?0.05) lowest for birds fed on the diet containing 30% cooked cowpeas and highest for those on F2 with 20% cowpeas. However, there was no significant difference between the F2, F1 and the control birds for weight gain. The F3 (25%) and F4 (30%) diets induced the highest (P?0.05) feed conversion ratio and cost per kg of weight gain. There were no significant differences between the birds fed control, F1 (15% cooked cowpea) and F2 (20% cooked cowpea) diets for these parameters. Data on carcass characteristics and creatinine level in the fowl's serum are summarised in Table 3.

There was no significant difference between all the treatment groups for carcass yield, the percentage body weight of gizzard, liver and abdominal fat. The birds under the diet with 30% cooked cowpeas recorded the highest leg and heart percent as compared to the control. There was no significant difference between the groups fed cooked cowpea between the control and all the

groups fed up to 25% cooked cowpea for leg and heart percent, in general. However, the birds fed 15% cooked cowpeas was significantly higher (P<0.05) than those of the control but did not significantly differ (P>0.05) from the birds fed 20 and 25% cooked cowpeas. The serum creatinine level was not significantly different (P>0.05) among treatment groups.

No treatment effect was recorded on mortality rate throughout the experiment (Table 3).

Discussion

The ME and CP levels obtained in the raw cowpeas were higher than values reported[17,18]. The difference among varieties of grains used could probably be the cause. The decrease in the CP level of the cooked beans could be related to the denaturation of protein by heat treatment, thus reducing the level of this nutrient together with the protease inhibitors in the grains during the cooking process[18].

In general, feed consumption was comparable in all groups of birds and the total feed consumed was in the 3000-3500g range generally considered as normal[20]. The results of the present study agreed with those of Nji Fru *et al*.[21] who reported that the incorporation level of autoclaved Bambara groundnuts in finishing broiler diets did not affect their feed consumption. However, the total feed consumption in all groups was lower as compared to that of 4787g suggested by Hubbard[22]. The lower

Table 3. Growth performances, carcass characteristics (% body weight) and serum creatinine (mg/dl) of finisher male broilers fed graded levels of cooked cowpea diets from 3 to 7 weeks of age

Parameters	Diets (% inclusion of cooked cowpea)					SE
	Control (0%)	F_1(15%)	F_2(20%)	F_3(25%)	F_4(30%)	
Feed consumption (g)	3286.12a	3348.43a	3313.75a	3366.40a	3236.26a	38.60
Weight gain (g)	1337.74a	1362.49a	1357.43a	1207,61b	1094.93c	33.30
Feed conversion ratio (g feedg^{-1} gain)	2.48c	2.48c	2.46c	2.79b	3.02a	0.07
Feed cost/kg weight gain (FCFA)*	509.45c	508.90c	518.85c	603,88b	747,92a	21.15
Mortality, %	0.31a	0.43a	0.43a	0.50a	0.56a	0.02
Carcass yield (% BW)	69.01a	73.17a	71.53a	71.17a	68.16a	1.75
Organs weight (% BW)						
Liver	3.16a	3.75a	3.26a	3.54a	3.38a	0.32
Gizzard	2.58a	3.63a	3.07a	3.31a	3.56a	0.33
Head	3.16a	3.50a	3.51a	3.72a	4.20a	0.28
Heart	0.62c	1.62ab	1.17abc	0.97bc	1.73a	0.22
Legs	3.16b	3.50ab	3.51ab	3.73ab	4.20a	0.33
Abdominal fat	1.94a	2.41a	2.71a	2.47a	1.69a	0.38
Creatinine level (mg/dl)	4.8a	4.8a	4.8a	5.2a	5.2a	0.46

a, b, c : Means in the same raw carrying the same letter are not significantly different (P>0,05).
SE: Standard Error
* See Table 1
BW: Body Weight

performances observed[7,8] respectively with 5% cooked and 6% of toasted cowpeas were probably due to the poor control of the temperature during the detoxification of grains.

In general, weight gain was lower and feed conversion ratio higher in all groups of birds including the controls as compared to the suggestions of Hubbard[22]. These poorer performances could be related to the bacterial infection that occurred in all treatment groups at 6 weeks of age. The inclusion of up to 20% cowpeas in the diet resulted in higher weight gain but not significantly different from the control group. However, as the inclusion level of cowpeas increased above 20%, there was a rapid linear drop in weight gain. This suggests, in agreement with earlier reports[7,8,9,23,24,25,26,27], the presence of residual quantities of antinutritional factors in the cooked cowpea. A longer cooking time could probably have been more efficient. The growth performances recorded in the present study with rations containing more than 25% cooked cowpeas were higher than those who used only 5% boiled and 6% toasted cowpeas respectively[7,8]. Under pressure-cooking, there is a better control of the temperature and this could have improved the nutritional value of the grains[28,29]. The results of this study are in agreement with those of Nji Fru *et al*.[21] who found a proportional decrease in total growth when above 19% autoclaved Bambara groundnuts were used in broiler finishing diets. The lowest body weight gains recorded in the birds fed on the diets containing 25 and 30% cooked cowpeas were associated with the poorest feed conversion ratio and the highest cost of production. Therefore, it would not be technically justified to include the tested feedstuff above the 20% level.

No significant difference was detected among treatment groups for carcass yield, proportion of liver, gizzard, head and abdominal fat, percent mortality and serum creatinine. This indicated that the incorporation of up to 30% cooked cowpeas in the finisher diet not affect the carcass characteristics of the broilers. Although the carcass yields recorded for the all groups of birds was in the range suggested[15,29], the percentage of liver, gizzard, head and heart were highest. Japou[6], Mbakop[7] and Chakam[8], recorded an increase in the liver and gizzard percents in birds fed raw, cooked and toasted cowpeas respectively.

The creatinine levels recorded in all the groups of chickens were higher than the normal range (0.5-1.5 mg/dl) suggested[31]. The increase in the level of serum creatinine could be associated with the use of Oxytetracycline® to treat all the birds against respiratory affections. Miller *et al*[32] indeed reported an azotemia or high concentration of serum creatinine associated with the consumption of drugs such as Gentamicine®, Oxytetracycline®, Amphotericine B®, Furosemide® and Trimethoprim-sulfadiazine®. These drugs are known to reduce the excretion capacity of kidneys thus, causing the accumulation of ammonia in the blood and the consequent increase of creatinine level in the serum.

Conclusion

The results of this study showed that up to 20% of cooked cowpeas could be used in the finisher diet without negatively affecting the production performance of broiler chickens.

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MORTALITIES IN GOATS AND SHEEP IN BOTSWANA

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Sheep and goats play an important socio-economic role in the rural areas of Botswana. Nevertheless, many Batswana farmers do not consider production of these animals a profitable and viable adventure[1] and hence pay little attention to problems that adversely affect the production of sheep and goats[2]. Goats and sheep populations were estimated to be 2.2 million and 0.4 million repectively[3]. It was quite evident from the study conducted by Panin[3] that small-stock production is both profitable and economically viable and accounts for about 15% of the total average rural household income of Botswana. It is advantageous to rear goats and sheep as they appear to thrive well in the harsh conditions of Botswana and hence the reason why over 80% are found in the hands of traditional farmers[4]. Goats and sheep provide milk, meat and skin to the rural communities. Panin[3] observed that goats are reared in larger numbers (90%) as opposed to sheep that stood at only 27%. There are various factors that may affect smallstock production performances. In Botswana, Sebunya and Diteko[2] observed that weather and climatic conditions greatly influence disease occurrence. Mucuthi and Munei[5] identified mortality through diseases and lack of water as being the most common limiting factor in smallstock production. Carmichael[1] identified helminthiasis as a serious generalized constraint in livestock production in Botswana.

The causes of mortalities in goats and sheep in Botswana have not been thoroughly investigated and determined. The aim of this study was to identify the major causes of mortalities in these animals with the ultimate goal of making recommendations on the most cost-effective strategic interventions that would minimize such mortalities and therefore boosting goat and sheep production in Botswana.

The data was derived from clinical cases submitted to the Botswana National Veterinary Laboratory (BNVL) for laboratory diagnosis from various districts of Botswana over a four year period (1999 – 2002). Mortality was defined as cases in which field officers recorded deaths that could be linked with the laboratory diagnosis. Causes of mortality were classified as follows: endoparasitosis, tick-borne diseases, non – infectious and other infectious causes. Seasonal occurrence of mortality in both species was also evaluated. Seasons are classified as summer (November – January), autumn (February – April), winter (May – July) and spring (August – October).

Percentages (%) and standard error (±SE) at 95% confidence interval were calculated according to Swinscow[6].

A total of 13 010 mortalities of which 86% were in goats and 14% sheep were recorded during the study period. Endoparasites accounted for 60.65% and 68.90% mortality rates in the goats and sheep, respectively (Table 1). This was followed by tick-borne disease conditions in particular heartwater that accounted for 20.43% and 17.47% for goats and sheep,

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Table 1: Mortality rate (%) by cause in goats and sheep in Botswana (1999-2002)

	% ±SE[1] mortality per animal species	
Cause	Goats	Sheep
Endoparasitosis[2]	60.66±0.91	68.90±2.13
Tick-borne diseases	20.44±0.75	17.47±1.74
Other infectious diseases	8.53±0.52	7.09±1.18
Non-infectious[3]	10.37±0.56	6.54±1.14

respectively. Other causes of mortality that included infectious diseases and non-infectious conditions which collectively accounted for 18.90% and 13.63% mortality rates in goats and sheep respectively were observed.

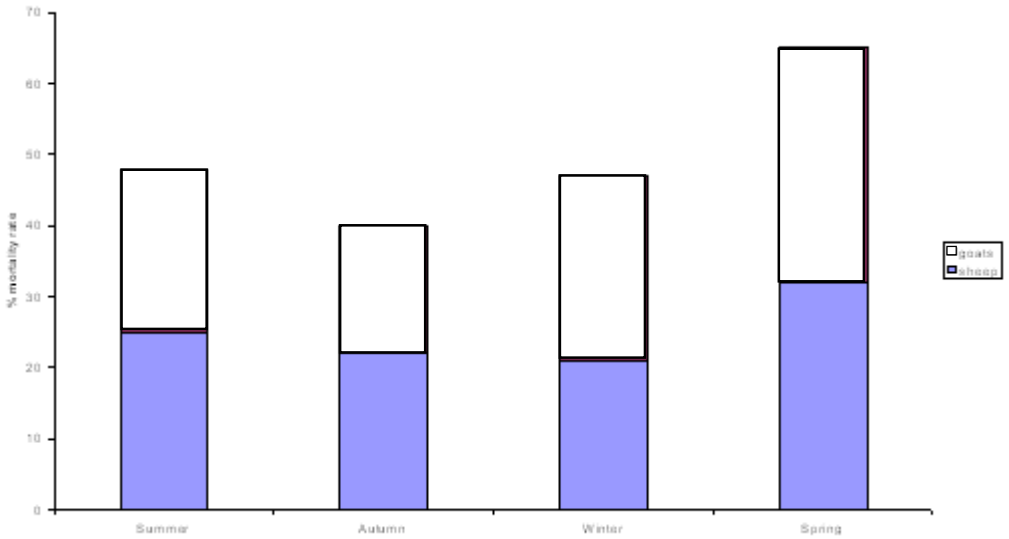
The mortality rate for both species was low in autumn and gradually increased in winter and reached peak levels in spring (Figure 1). A slightly higher mortality rate was experienced during summer months in both species (Figure 1).

Endoparasites (helminthes and coccidia) have since long been recognized as the major constraint limiting goat and sheep production in Botswana[1]. This study confirms that these parasites are by and large still the leading cause of death both in sheep and goats in this country. Heavy infestation with helminthes and/or coccidia causes loss in production through mortalities, loss in body weight and delay in sexual maturation.

Improvements in goat and sheep production can only be realized after effective control of helminthes and coccidia in the animal and on pastures. There are several drugs available in the markets whose effectiveness against these parasites has been verified by many workers[7, 8, 9, 10]. Antihelminthics and anti-coccidial drugs are commonly used to control endoparasites in livestock in Botswana and the government subsidizes heavily the cost of these and other veterinary drugs so that even the small-scale farmers can afford to buy them. Nevertheless, the problem of endoparasitism does not appear to be under control as evidenced by the high prevalence of the problem both in goats and sheep. This raises two possible hypotheses (i) many farmers probably do not treat their livestock for endoparasites or if they do treat, they are probably under-dosing; (ii) drug resistance might have developed, probably due to under-dosing. These are potential problems hampering effective control of internal parasites in livestock and therefore need to be critically evaluated and where possible addressed accordingly to improve the control of helminthosis and coccidiosis particularly in goats and sheep in this country.

The study indicated a high mortality rate in sheep compared to goats due to helminthosis. The differences could be

Fig. 1 Seasonal occurrence of mortality in goats and sheep in Botswana (1999-2002).



attributed to the feeding habits of both species. Sheep tend to graze close to the ground where larval numbers are much higher compared to goats that are predominantly browsers.

Amongst the tick-borne diseases, heartwater is identified as the major cause of mortality both in sheep and goats in Botswana. The disease appears to kill mostly exotic breeds of animals; an observation which has been noted in this country[12] and elsewhere in Africa[13,14]. Experiences on the control of heartwater by vaccination on large commercial and smallholder farms in Southern Africa have been documented elsewhere[15, 16]. These experiences which have been adopted for the control of the disease in commercial ranches in Botswana could with government subsidization be extended to the traditional livestock holdings in the rural communities in order to minimize the mortalities caused by the disease. Alongside vaccination, strategic tick control by dipping and/or spraying of animals with suitable potent accarides should be intensified in order to eliminate the main species of ticks responsible for the transmission of heartwater and the other tick-borne diseases in this country.

A seasonal mortality rate variation has been a feature in this study. Mortality rate reached peak levels during the spring period. This coincides with the breeding season when most animals are shedding over wintered larvae and hence increased pasture contamination[17]. During the same period, tick population begins to multiply and hence increased risk of transmission of heartwater. Mortality rate reached low peak levels during autumn when there is little tick and nematode activity. It is therefore recommended that animals be dewormed prior to the risk period which in this case is spring to reduce pasture contamination. The treatment should preferably be done with long-acting, broad-spectrum anthelminthics every month during the spring and summer

months so that all nematode larval stages including hypo-biotic larvae18 are killed.

The major diseases affecting the production of goats and sheep identified in this study require strategic control measures so that their negative impact on the productivity of these animals is minimized.

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ISOLATION OF *Haemophilus somnus* FROM PNEUMONIC LUNG IN SLAUGHTERED WHITE FULANI CATTLE IN JOS, NIGERIA

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Haemophilus somnus is a well documented cause of pneumonia and bronchopneumonia in cattle worldwide[1,2]. The organism spreads throughout cattle herd in a very elusive manner; usually a major problem has already developed before the disease is detected. The disease *"Histophilosis"* has caused a serious economic loss to cattle industry due to mortality and decreased productivity in the affected herds. The disease also affects feed lot cattle and calves[3]. *Haemophilus somnus* was reported as a bacterial cause of pneumonia in buffalo calves[4], and in bronchopneumonia of American bison[5], also in acute interstitial pneumonia in feedlot cattle[6].

Haemophilus somnus is gram negative and hemophilic which is difficult to identify owing to poor growth in routinely used biochemical test media, and isolation from a typical lesion is usually a primary determinant in the identification[7]. Primary isolation of *Haemophilus* species is important for the early diagnosis and treatment of a variety of infectious diseases[8].

There is dearth of published information on *Haemophilus somnus* – pneumonia in cattle in Nigeria. Therefore, this paper reports and documents the first isolation of *Haemophilus somnus* from pneumonic lung in slaughtered indigenous white Fulani cattle in Jos abattoir in North-central Nigeria.

A pneumonic right lung was identified during postmortem examination at Jos abattoir, macroscopic lesions observed on the affected lung include, fibrinous pleuritis and variable amounts of cranioventral pulmonary consolidation as described[9].The affected lung was aseptically collected in polythene bag and transported to the laboratory in Coleman box packed with ice. It was immediately processed on arrival at the laboratory.

A piece of lung tissue was sectioned; dorso-ventral surfaces were seared with a red hot spatula. Using a sterile swab, the sectioned surface was swabbed and inoculated onto plate containing Chocolate agar then streaked with wire loop, the plate was incubated for 24 hours at 37°C in microaerophillic atmosphere of 5% CO_2 [10].

Bacterial Identification and Biochemical test(s).

Grams' reaction and various sugar fermentation and biochemicals were tested according to standard method(s) as

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described by Carter and Chengappa[11].

Haemophilus somnus produces a wide range of septicemic conditions in bovine which include infectious thromboemboencephalitis (ITEME), Pneumonia, arthritis, genital infections and abortion. Panceira *et al*[12], noted three clinical manifestation of *Haemophilus somnus* infections, peracute (neurological), acute (respiratory) and chronic (arthritic), with frequent overlapping of the syndromes. The organism is also incriminated as etiologic agent of mastitis[13] in cattle.

Haemophilus somnus isolates from this study were tested for various sugar fermentation; the results were consistent with earlier reports. The isolates were positive for catalase, indole, Ornithine-decarboxylase, which is in conformity with previous findings[14, 15, 16, 17, 18, 19,]variable results were reported for indole and catalase[20]. The same isolates were however tested negative on Triple sugar iron and Urea which agrees with previous reports. Voges-Proskauer, Citrate and Nitrate were not tested even though the results of earlier workers were negative and positive respectively.

To assess growth on solid media, colonies were further streaked on Blood and Mac Conkey agar and incubated for 24 hours at 37°C; there was no growth on Mac Conkey agar which is a positive indication for *Haemophilus somnus*[19], and Blood agar yielded luxuriant growth of small less than 1mm, round, glistening, raised yellow-grey colonies with clear hemolysis, on Gram staining, the colonies were Gram negative *coccobacilli* as observed under the microscope, these are consistent with description of *Haemophilus somnus*[21].

Isolating *H. somnus* from pneumonic lung of slaughtered cattle further confirmed its involvement in causing pneumonia in cattle as previously documented[12].Thus; it should be considered an etiologic cause of respiratory disease in cattle amongst other bacterial cause of pneumonia such as, *Pasteurella multocida, Pasteurella haemolytica* and *Mycoplasma* species. Macroscopically the infected lung showed fibrinous pleuritis and variable amounts of cranioventral pulmonary consolidation suggestive of pneumonia; these coincide with the reports of Dungworth[22].

Because of the seeming dearth of published information and lack of research work on *Haemophilus somnus* pneumonia in cattle in Nigeria, it could be inferred that, these findings represents first isolation of *Haemophilus somnus* from pneumonic lung in indigenous cattle in Nigeria. Further investigations on *Haemophilus somnus* involvement in bovine broncho-pneumonia are imperative and on-going; these will safeguard future economic losses due to *"Histophilosis"* in cattle herds in Nigeria.

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SHORT COMMUNICATION

A SHORT REVIEW OF THE METHODS USED IN MUSEUM TECHNOLOGY

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Death is the irreversible loss of the properties of living matter, that is to say, death is the cessation of life and is invariably followed by the putrefactive forces of nature that destroy the tissue, unless measures are taken to arrest the latter process[1]. Tissue destruction occurs as spontaneous autolysis due to the action of intracellular enzymes and microbial decay due to enzymes from microorganisms. Putrefaction is the gradual disintegration of the body into gases, liquids and salts by both bacterial activity and enzymes from the body[1].

Museum techniques that deal with biological materials aim to protect the specimens from the forces of putrefaction, resulting from autolytic or microbial enzymes. Entire organisms, parts or organs may be preserved as wet mounts in glass or perspex jars[2]. Stuffed or mounted skins may be preserved as dry mounts as occurs in taxidermy[3], or the lumina of hollow structures are filled with solid non-perishable material such as polyester resins as occurs in methacrylate corrosion casting[4]. Mummification, the process of preservation by drying, was preponderant in the ancient times, particularly in Egypt[5, 6]. On the contrary embalming is used as a routine method for temporary preservation of cadavers in funeral homes, but as a more permanent procedure in anatomy dissection laboratories[7, 8]. Plastination is a relatively recent technique and involves replacement of tissue water and fat with a curable plastic polymer such as silicone by infiltration[9].

The term fixation refers to application of fixation fluids to tissues to prevent destruction by autolysis and decay. Fixation arrests autolysis, bacterial decomposition, coagulates tissue fluids and fortifies tissue The generally accepted method utilizes Kaiserling`s formula 1 (KI) solution, which consists of 40% Formalin (400 ml), Potassium acetate (60 g); Potassium nitrate (30g) and then constituted with water to make up to 2000 ml2. The specimen should be supported by fixative-soaked lint or cotton wool to avoid artificial flat surfaces and to allow all areas access. Many other modifications of formalin-based fixatives have since been made with ranges of 5-10% formalin[2].

Restoration of specimens

The natural colour of specimens disappears after fixation this is by Kaiserling's II (KII) method[2]. The specimen

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is removed from fixative washed in running water for 10-30 minutes and placed in 80% ethanol for 30 minutes to 12 hours. Normal colour develops during this time. The specimen is removed from ethanol and blotted dry. The specimen is then placed in preserving or mounting solution. If the specimen is left too long in ethanol, the colour fades irreversibly[2].

Preservation and presentation of specimens

The preservation solution is the final fluid in which the specimen is mounted for display. The recommended solution is known as Kaiserling III (KIII) whose components are sodium acetate (100g), glycerin (300ml), formalin 40% (5ml) constituted with water to make up to 1000 ml[2]. Presentation of the specimens is done either as dry mounts or wet mounts. Wet mounts are done in solutions, mainly KIII and are either put in glass jars or perspex jars. Many other formulae based on glycerin have been described[2].

Bone maceration and mounting

Maceration is the process by which raw bone is defleshed, leaving clean dry non-greasy bone[10]. The methods include boiling in plain water, or alternatively, a strong alkali may be added either a household detergent such as Ariel or even a proteolytic enzyme such pancreatin or papain and then heating the water to 95^0C hastens the process. Use of carnivorous hide beetles, *Dermestes maculatus,* which clear the flesh and not the bone[11], is practiced in some museums. Ultimately, the bones are bleached in hydrogen peroxide, degreased in carbon tetrachloride in a fume cupboard, articulated using wires and glue and mounted on a wooden base[2].

Taxidermy

Taxidermy is the art of preserving and mounting the skin together with the feathers, fur, hair and scales of animals[3]. The methods entail preparing the skin and treating it with the appropriate preservative solutions and then mounting the skin on an appropriately prepared artificial body to resemble the living animal in both appearance and mannerism.

Mummification

Mummification seems to have its origins in the late predynastic period, over 3000 ago. The process reproduces the desiccating effects of the hot dry sand on a body buried within it. Mummification is not a common procedure in the current museums and the technologies used then, remain largely obscure[12].

Embalming

Embalming entails treatment with balsams, herbs, antiseptics, resins, drugs and chemicals and is used for temporary preservation of human remains. To date, embalming has been perfected to include elegant mortuary procedures that result in well-preserved cadavers useful also for anatomical dissection and demonstration of surgical techniques[7, 8].

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Plastination

This is the process of preservation by infiltration of plastic polymers into the tissue. The water and fat are replaced the plastics, yielding specimens that can be touched, do not smell or decay, and even retain most microscopic properties of the original sample[9]. Plastinated organs and body slices are novel teaching aids for cross-sectional anatomy, are helpful for manifold scientific research activities and are a suitable diagnostic means in pathology.

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