

Animal Health Research Institute
EL-Minia Lab

STUDIES ON MICROBIAL INFECTION ASSOCIATED REPEAT BREEDER IN BUFFALOES AND COWS AND ITS SENSITIVITY TO DIFFERENT ANTIBIOTICS IN VITRO

(With 7 Tables)

By

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دراسات على العدوى الميكروبية المصاحبة لحالات الشيع المتكرر في الأبقار
والجاموس وحساسية هذه الميكروبات لبعض المضادات الحيوية

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استهدفت الدراسة إلقاء الضوء على العدوى الميكروبية المختلطة (البكتيرية والفطرية) المصاحبة للشيع المتكرر في الأبقار والجاموس في محافظتي المنيا وأسيوط. اشتملت الدراسة على ١٢٠ حيوان (٥٠ جاموسة و٧٠ بقرة) تعاني من شيوع متكرر ٢٥ سليلة ظاهريا و٩٥ تعاني من التهابات رحمة مختلفة. أظهر الفحص الميكروبيولوجي إلى عزل ٢٠٠ عترة منها ٣٠ بنسبة (١٥%) من الحالات السليمة ظاهريا و ١٧٠ من حالات الالتهابات الرحمية بنسبة (٨٥%)، وقد شملت العترات المعزولة على ١٦٠ عترة بكتيرية بنسبة (٨٠%) و ٢٥ عترة فطرية بنسبة (١٢,٥%) و١٥ عدوى مختلطة (بكتيرية وفطرية) بنسبة (٧,٥%)، وكانت أهم العترات البكتيرية المعزولة هي أيشيريشيا كولاي بنسبة (١١,٩%) والكوريني بكتريم بيوجين بنسبة (٩,٤%) والميكروب العنقودي الذهبي بنسبة (٩,٤%) والميكروب السبحي فيجالز بنسبة (٨,١%) والميكروب السبحي بوفيز بنسبة (٧,٥%) والكوريني بكتريم بوفيز بنسبة (٦,٩%) والميكروب السبحي بيوجين بنسبة (٦,٩%) ومن الميكروبات اللاهوائية ميكروب الكلوستريديم بيرفريجنز بنسبة (٣,٧%) وميكروب ايروكتريم لننيم بنسبة (٣,١%) وقد شملت العزلات البكتيرية على عزل ٤٠ عدوى بكتيرية مشتركة بنسبة ٢٠% من اجمالي العترات المعزولة. وبالنسبة للعدوى الفطرية تم عزل فطر الاسبرجيلس والكنديدا بنسبة (٢٤%) لكل منهما وفطر البنسلليم والفيوزاريم والخمائر بنسبة (١٢%) وفطر الميكور بنسبة (٨%) وكل من الابيددا والروبيز بنسبة (٤%) من اجمالي الفطريات المعزولة. وبالنسبة للعدوى المختلطة (بكتريا وفطريات) تم عزل كل من أيشيريشيا كولاي مع الاسبرجيلس والميكروب العنقودي مع الاسبرجيلس وأيشيريشيا كولاي مع الفيوزاريم والميكروب السبحي مع كل من الخمائر والكنديدا والبروتيس مع الفيوزاريم بنسب مختلفة. وتم عمل اختبار الحساسية للعترات المعزولة باستخدام المضادات الحيوية المختلفة وقد وجد أن الأترروفلوكساسين والأوكس تتراسيكلين والجنتاميسين هم أكثر

المضادات الحيوية حساسية بينما كانت مقاومة لكل من نيومايسين وإيرثرومايسين و أمبسلين. ومن خلال هذه الدراسة ينصح في حالات علاج الشيوخ المتكرر أنه يجب الأخذ في الاعتبار العدوى المختلطة (البكتيرية والفطرية) لان استخدام المضادات الحيوية فقط يؤدي ألي نشاط الفطريات وعلى هذا ينصح باستخدام أدوية لها تأثير مزدوج على كل من البكتريا والفطريات بعد عمل اختبار الحساسية.

SUMMARY

The present study aimed to throw light on the microbial infection and special mixed infection (bacterial and fungus) associated repeat breeder in buffaloes and cows. This work was carried out on 120 cervico-vaginal and uterine swabs collected from (50 buffaloes and 70 cows) suffering from repeat breeding (25 subclinically and 95 clinically infected cows from dairy farms at EL-Minia and Assiut Provinces. Bacteriological examination revealed that, 200 different microbial causative agents (30 isolates with incidence of 15% for subclinically and 170 isolates with incidence of 85% for clinically infected animals). The total microbial infection represented by 120 (60%) single bacterial isolates, 40 (20%) mixed bacterial isolates, 25 (12.5%) fungi isolates and 15 (7.5%) mixed infection (bacterial and fungi). The most common aerobic microorganisms isolates were *E.coli* 19 (11.9%) followed by *Croynebacterium pyogenes* 15 (9.4%). The most common mixed bacterial isolates were *E.coli* + *Croynebacterium pyogenes* + *Proteus* spp with incidence of 22% and *Staphylococcus aureus* + *Croynebacterium bovis* with incidence of 20%. 25 fungi isolates (12.5%) from total microbial isolates were found. The most important fungi isolates were *Aspergillus* spp (24%) and *Candida* (24%). Fifteen cases out of 120 repeat breeder cases (12.5%) proved to have mixed infection (bacterial and fungal). The most common mixed infection caused by *E.coli* + *Aspergillus* spp (33.3%). After Sensitivity test, the most active antibiotics were Enerofluxacin, Oxytetracycline, Gentamycin and Nalidixic acid. Most bacterial isolates were resistant to Neomycin, Erythromycin and Ampicillin. Sensitivity test revealed that most bacterial isolates, in this study, were highly sensitive to Enerofluxacin, Oxytetracycline, Gentamycin and Nalidixic acid and resistant to Neomycin, Erythromycin and Ampicillin.

Key words: *Bacteria – fungus – repeat breeder – Buffaloes – cows*

INTRODUCTION

Repeat breeding in animals has great economic importance as it causes increased calving interval, less number of offspring, decreased milk production and wastage of time and money on treatment. The repeat breeder has long been a problem world wide with an overall incidence rate of 10-25% (Bartlet *et al.*, 1986). In Egypt, the incidence of repeat breeder syndrome ranged from 64.44% to 71.5% from the other infertility problems in buffaloes (Atalla, 1984 and Osman, 1984). Cervicitis and endometritis may be responsible for early embryonic death or repeat breeding problems which are mostly caused by increase in the number of the bacteria and /or in their virulence (Blanch, *et al.*, 1992). Many bacteria present in genital tract as saprophytes but under unfavorable conditions might become pathogenic and causes clinical or sub-clinical signs of endometritis. Yousef (1984) isolated seven types of microorganisms from 10 normal cows and 10 (29.4%) different types of microorganisms from 34 cases of repeat breeder cows. Deka *et al.* (1985) concluded that 75% of cows with abnormal parturition were positive for uterine microflora, 50% were considered pathogenic and 50% as non-pathogenic. Dawson (1963) and Shouman *et al.* (1977) reported that *Micrococcus citrus*, *Bacillus subtilis*, anthracoid, *Streptococcus faecalis*, *Staphylococcus aureus*, *E.coli*, *Proteus* and *Corynebacterium bovis* are the most important isolates from apparently healthy uteri of cows, while Shouman *et al.* (1983) and Olson *et al.* (1984) reported that *Corynebacterium pyogens*, *E.coli*, *Streptococcus pyogens*, *Staphylococcus aureus* and *Pasteurella* are the main pathogens isolated from cow suffering from endometritis and pyometra. Many authors recorded the relation between repeat breeder and fungal infection. Singh *et al.* (1993), Verma *et al.* (1999) and Megahed *et al.* (2000) isolated various fungal species from repeat breeder buffaloes as *Aspergillus*, *Penicillium* and *Fusarium*. However, most clinician, during their handling to this problem, paid their attention only to the bacterial infection with excessive use of antibiotic or antiseptic which may aggravate the case.

The objective of the present study was to investigate microbial infection (bacterial and fungi) associated repeated breeders in buffaloes and cows and to test the sensitivity of the bacterial agents to some different antibiotics.

MATERIALS and METHODS

Animals:

This study was carried out on 50 buffaloes and 70 cows (pluriparous and 4-8 years old) from dairy farms at EL-Minia and Assiut Provinces, Egypt. These animals were suffering from repeat breeding (they were served naturally 3-5 times at successive periods without conception).

Clinical examination:

Every animal was examined rectally and vaginally according to the scheme given by Zemjants (1970) and Roberts (1971). Animals were examined by one examiner and with the same conditions under which most veterinarians deal with such cases. According to the size, consistency of the tubular tract and presence of pathological discharge, the examined animals were classified into two categories either subclinically infected without apparent discharge and no palpable abnormalities in their tubular tract (rectal examination) or clinically infected in the uterus and/or cervix, with or without presence of vaginal discharge as in table 1

Table 1: Number of animals examined and reproductive status

Animal species	No. Examined	Repeat breeder			
		Subclinical		Clinically infected	
		No.	%	No.	%
Buffaloes	50	10	20.0	40	80
Cows	70	15	21.4	55	78.6
Total	120	25	20.8	95	79.2

Bacteriological examination:

A total number of 120 cervico-vaginal and uterine swabs were collected by vaginal swabs and a sterile aluminum tampon after sterilized in hot air oven at 160°C for 2 hours for bacteriological examination according to Sharaf *et al.* (1963), Osman and Abou-Gabal (1975) and Zaki and Fouad (1963).

a) Isolation and Identification of isolates: - according to Buchanan *et al.* (1975), Wilson and Miles (1985) and Cruickshank *et al.* (1980)

The vaginal swabs were taken from the external os using sterile gauze swabs, while uterine swabs were taken by aluminum tampons which passed into uterus and left inside the uterine lumen with frequent rotations for few seconds before withdrawal the samples. The obtained swabs were placed directly into screw-capped bottles containing sterile

nutrient broth for bacteriological examination (Erich and Morrow 1980). The screw-capped bottles containing the samples were incubated at 37°C for 24 hours to enhance growth and multiplication of microorganisms.

For aerobic microorganism isolates, a loopful from each sample was streaked on MacConkey's agar plates, Blood agar plates, Nutrient agar plates, Violet Red Bile Glucose agar plates and Baired-Parker agar plates. These plates were incubated at 37°C for 24-48 hours. Different colonies were picked up and purified by subculturing on selective media, then kept on nutrient agar sloops to identify the microscopical appearance, culture character, motility, biochemical and serological tests. For anaerobic microorganisms isolates, a loopful from each sample was inoculated into Thioglycolate broth medium "Oxoid, GM10) and then streaked on to Cooked meat medium ("Mast DM 120"), Neomycin blood agar medium (neomycin sulphate solution was added to the media just before the addition of blood to make final concentration of 150ug/ml). The inoculated solid media was incubated anaerobically at 37°C for 24-48 hrs by using (Gas-pack anaerobic jar "BBL-814-12"). Strick anaerobic isolates were examined and identified for microscopically appearance, culture character, motility, then transferred to cooked meat medium for other biochemical tests as described by Koneman *et al.* (1992)

For fungal isolates, swabs from the same samples were cultivated onto plates of Sabouraud, dextrose agar medium (SDA) supplemented with chloramphenicol (50 mg/l) and incubated at 28°C for 7-10 days till fungal growth was observed. The growing fungi were identified based on their macro and microscopic characteristics the isolated fungi were identified according to Cruickshank *et al.* (1980), Domasch *et al.* (1980) and Nirenberg (1989).

b) Sensitivity test:

The important isolates were tested for sensitivity to some chemotherapeutic agents. One ml of 24hrs.-broth cultures was spread on the surface of nutrient agar. Antibiotic sensitivity discs were placed on the surface seeded agar. Plates were incubated aerobically at 37°C for 24hrs. The sensitivity was judged according to the diameter of clearance zone around the discs according to Quinn *et al.* (1994). Ten different antibiotic discs, supplied by Oxoid were used. These antibiotics were Neomycin (30ug), Gentamycin (10ug), Chloramphenical (30ug), and Oxytetracycline (30ug), Nalidixic acid (30ug), Kanamycin (30ug), Ampicillin (10ug), Penicillin (10ug), Erythromycin (15ug), and Enerofluxacin (10ug).

RESULTS

Results of the present study are presented in Tables from 2-7.

200 different microbial infections (25 microbial infections with incidence of 12.5% for subclinically infected and 175 microbial infections with incidence of 87.5% for clinically infected cases) were detected in this study. The total microbial infections were 120 (60%) for single bacterial isolates, 40 (20%) for mixed bacterial infection, 25 (12.5%) for fungal infection and 15 (7.5%) for mixed infection (bacterial and fungal). The incidences of different single bacterial isolates are shown in Table (3). The most common aerobic microorganisms isolates were *E.coli* 19 (11.9%) followed by *Croynebacterium pyogenes* 15 (9.4%) and *Staphylococcus aureus* 15 (9.4%). The most obligate anaerobic isolates found were *Clostridium perfringenes* with incidence of (3.7%) and *Eubacterium lentum* 5 (3.1%) for both subclinical and clinical cases. For mixed bacterial isolates, *E.coli* + *Croynebacterium pyogenes* + *Proteus spp* (22%) and *Staphylococcus aureus* + *Croynebacterium bovis* (20%) were prevalent as in Table (4). 25 fungal isolates with incidence of (12.5%) from total microbial isolates were detected (Table 5). The most common fungal isolates were 6 *Aspergillus spp* with incidence of (24%) and 6 *Candida* with incidence of (24%). *E.coli* + *Aspergillus spp* (33.3%) were the prevalent mixed infection found in all studied cases (Table 6).

Sensitivity test:

Sensitivity test: was carried out for estimation of the sensitivity of the 160 bacterial isolates to different antibiotics. The results of Sensitivity test are shown in Table (7). It was observed that most isolates 142 (88.75%) were found sensitivite to Enerofluxacin followed by Oxytetracycline 135 (84.37%), Gentamycin 125 (78.13%) and Nalidixic acid 118 (73.75%), while were resistant to Neomycin 132 (82.50%) followed by Erythromycin 130 (81.25%) and Ampicillin 128 (80.00%).

Table 2: Incidence and type of single and mixed infections of samples recovered from repeat breeder buffaloes and cows (subclinical and clinical cases)

Type of infection	No. of isolates		Repeat breeder			
			Subclinical (25)		Clinical cases (95)	
	No.	%	No.	%	No.	%
Single bacterial infection	120	60.0	15	7.5	105	52.5
Mixed bacterial infection	40	20.0	5	2.5	35	17.5
Fungal infection	25	12.5	2	1.0	23	11.5
Mixed bacterial and fungal	15	7.5	3	1.5	12	6.0
Total	200		25	12.5	175	87.5

Percentage infection relative to total number of microbial infection

Table 3: Incidence of total bacterial isolates from 120 cases of repeat breeder (subclinical and clinical cases) buffaloes and cows

Types of bacterial isolates	No. of isolates		Repeat breeder							
			Buffaloes				Cows			
	No.	%	Subclinical (10)		Clinical (40)		Subclinical (15)		Clinical (55)	
A) Aerobic bacteria	No.	%	No.	%	No.	%	No.	%	No.	%
Staphylococcus aureus	15	9.4	1.0	0.63	4.0	2.5	1.0	0.63	9.0	5.6
Staphylococcus epidermidis	6	3.7	0.0	0.0	2.0	1.3	1.0	0.63	3.0	1.9
Streptococcus pyogenes	11	6.9	0.0	0.0	4.0	2.5	1.0	4.8	6.0	3.7
Streptococcus bovis	12	7.5	0.0	0.0	4.0	2.5	2.0	1.3	6.0	3.7
Streptococcus faecalis	13	8.1	4.0	2.5	3.0	1.9	2.0	1.3	4.0	2.5
Klebsiella oxytoca	8	5.0	2.0	1.3	4.0	2.5	0.0	0.0	2.0	1.3
Croynebacterium bovis	11	6.9	0.0	0.0	3.0	1.9	2.0	1.3	6.0	3.7
Croynebacterium pyogenes	15	9.4	0.0	0.0	6.0	3.7	1.0	0.63	8.0	5.0
E.coli	19	11.9	2.0	1.3	6.0	3.7	3.0	1.9	8.0	5.0
Sarcina spp.	4	2.5	0.0	0.0	1.0	0.63	1.0	0.63	2.0	1.3
Citrobacter spp.	5	3.1	0.0	0.0	1.0	0.63	1.0	0.63	3.0	1.9
Pseudomonas spp.	9	5.6	2.0	1.3	2.0	1.3	1.0	0.63	4.0	2.5
Proteus spp.	8	5.0	1.0	0.63	3.0	1.9	0.0	0.0	4.0	2.5
Enterobacter aerogens	10	6.3	1.0	0.63	2.0	1.3	1.0	0.63	6.0	3.7
B) Obligate anaerobic										
Clostridium perfringens	6	3.7	0.0	0.0	1.0	0.63	0.0	0.0	5.0	3.1
Eubacterium lentum	5	3.1	0.0	0.0	0.0	0.0	1.0	0.63	4.0	2.5
Bacteriodes spp.	3	1.9	0.0	0.0	1.0	0.63	0.0	0.0	2.0	1.3
Total	160		13	8.1	47	29.4	18	11.3	82	51.2

Percentage isolates relative to total number of bacteria isolates

Table 4: Type and incidence of mixed bacterial infection (samples recovered from 120 cases of repeat breeder buffaloes and cows)

Types of bacterial isolates	No. of isolates		Repeat breeder			
			Subclinical		Clinical	
	No.	%	No.	%	No.	%
E.coli + Streptococcus pyogenes + Streptococcus faecalis	6	15.0	1	2.5	5	12.5
Staphylococcus aureus + Croynebacterium bovis	8	20.0	0.0	0.0	8	20.0
E.coli + Croynebacterium pyogenes + Proteus spp.	9	22.5	0.0	0.0	9	22.5
Pseudomonas spp. + Proteus spp.+ Sarcina spp	5	12.5	1	2.5	4	10.0
E.coli + Citrobacter spp + Streptococcus faecalis	4	10.0	1	2.5	3	7.5
Enterobacter aerogens + Klebsiella oxytoca	3	7.5	2	5.0	1	2.5
Croynebacterium pyogenes + Streptococcus bovis	5	12.5	0	0.0	5	10.0
Total	40		5	12.5	35	87.5

The percentage was calculated in relation to the total number of mixed bacterial infections.

Table 5: Incidence of fungal isolates from different cases of repeat breeder (subclinical and clinical cases) buffaloes and cows

Types of fungal isolates	No. of isolates		Repeat breeder							
			Buffaloes				Cows			
			Subclinical (10)		Clinical (40)		Subclinical (15)		Clinical (55)	
	No.	%	No.	%	No.	%	No.	%		
Aspergillus spp	6	24.0	0	0.0	2	8.0	1	4.0	3	12.0
Penicillium	3	12.0	0	0.0	1	4.0	0	0.0	2	8.0
Candida	6	24.0	1	4.0	2	8.0	1	4.0	2	8.0
Mucor	2	8.0	0	0.0	1	4.0	0	0.0	1	4.0
Fusarium	3	12.0	0	0.0	1	4.0	0	0.0	2	8.0
Absidia,	1	4.0	0	0.0	0	0.0	0	0.0	1	4.0
Rhizoous	1	4.0	0	0.0	0	0.0	0	0.0	1	4.0
Yeast	3	12.0	1	4.0	1	4.0	0	0.0	1	4.0
Total	25		2	8.0	8	32.0	2	8.0	13	52.0

Percentage isolates relative to total number of fungal isolates

Table 6: Incidence of mixed infection (bacterial and fungal) of samples recovered from different cases of repeat breeder buffaloes and cows

Mixed infection isolates	No. of mixed isolates		Repeat breeder							
			Buffaloes				Cows			
			Subclinical		Clinical		Subclinical		Clinical	
No.	%	No	%	No	%	No	%	No	%	
Staphylococci spp. +Aspergillus spp.	4	26.7	0	0.0	1	6.7	1	6.7	2	13.3
E.coli + Aspergillus spp.	5	33.3	0	0.0	2	13.3	1	6.7	2	13.3
E.coli + Fusarium spp.	3	20.0	0	0.0	1	6.7	0	0.0	2	13.3
Proteus spp. + Fusarium	1	6.7	0	0.0	1	6.7	0	0.0	0.0	0.0
Yeast + Streptococcus faecalis +Candida	2	13.3	0.0	0.0	1	6.7	0.0	0.0	1	6.7
Total	15		0.0	0.0	6	40.0	2	13.3	7	46.7

The percentage was calculated in relation to the total number of mixed infection (bacterial and fungal).

Table 7: The results of Sensitivity test for the isolated microorganisms

Type of antibiotics	Degree of sensitive					
	Sensitive		Intermediate		Resistant	
	No.	%	No	%	No	%
Neomycin (30ug),	10	6.25	18	11.25	132	82.50
Gentamycin (10ug)	125	78.13	15	9.37	20	12.50
Chloramphenical (30ug)	107	66.87	25	15.63	28	17.50
Oxytetracycline (30ug)	135	84.37	17	10.63	8	5.00
Nalidixic acid (30ug)	118	73.75	32	20.00	10	6.25
Kanamycin (30ug)	85	52.13	28	17.50	47	29.37
Ampicillin (10ug)	30	18.75	12	7.50	128	80.00
Erythromycin (15ug)	13	8.12	17	10.63	130	81.25
Penicillin (10ug)	45	28.13	36	22.50	79	49.37
Enerofluxacin (10ug).	142	88.75	10	6.25	8	5.00

DISCUSSION

In the present study, 200 different microbial infections (30 microbial infections with incidence of (15%) from subclinical and 170 microbial infection with incidence of (85%) from clinical cases) were isolated. Healthy uterus has its own saprophytic bacteria but under unfavorable conditions might become pathogenic and causes clinical or sub-clinical signs of endometritis (Gunter *et al.*, 1955, Dawson, 1950 and Roberts, 1971 and AboEl-Ata, 1973). According to the present findings, it was clear that infection with single bacterial isolate prevailed among repeat breeding cows with palpable abnormalities in their genitalia upon rectal examination (52.5%) followed by the mixed bacterial infection (17.5%). In agreement with the present results,

Yousef (1984) isolated seven types of bacteria from 10 normal cows and 10 (29.4%) different types of bacteria from 34 cases of repeat breeder cows. Deka *et al.* (1979) reported that 36 (51.4%) out of 70 infertile cows were positive for pathogenic bacterial infection compared with only 6 (20%) out of 30 were positive for non-pathogenic bacteria from apparently normal infertile cows which reported by Metwelly (2002) who isolated 56 (65.1%) positive cases for bacteria from 148 buffalo-cows suffering from repeat breeding. HassabEl-Naby and El-Ekhnawy (2004) reported that mixed and single bacterial infection in repeat breeding cows were 50.8 and 49.1% respectively, while it is lower than obtained by Awad *et al.* (1977). Osman *et al.* (1984) reported that many bacteria were isolated from the healthy and diseased genitalia of buffalo cows and mixed infections were frequently isolated.

In the present investigation, the most predominating aerobic microorganisms isolates were *E.coli* 19 (11.9%) followed by *Croynebacterium pyogenes* 15 (9.4%) and *Staphylococcus aureus* 15 (9.4%). Similar isolates were found and isolated from the uteri of cows with history of repeat breeding, retained placenta and metritis (Gunter *et al.*, 1955, Namboothripad and Raja, 1976, Zafrucas, 1976, Osman, 1984, Eduvie *et al.*, 1984, El-Azab *et al.*, 1988 and Ramakrishna, 1996). *Staph.epidermidis*, *Anthracooid* and *E.coli* were the most bacterial infection isolated from cervices of typical repeat breeder cows and buffaloes (El-Azab *et al.*, 1980, Messier *et al.* 1984, Selim *et al.* 1998 and Hassab El-Naby and El-Ekhnay 2004).

It is noteworthy that *E.coli*, *Citrobacter*, *Proteus*, *Staphylococci*, *Pseudomonas spp.* And *Klebsiella spp.* were isolated from the uteri of normal cows and buffaloes (Zerb, *et al.*, 2001 and Metwelly 2002). Some workers believe that the uterus of the cows at the time of first inseminations is nearly always sterile while several others described that repeat breeding is mainly due to the presence of subclinical infections in the uterus form the opportunistic uterine microflora (Javed and Khan 1991).

E.coli + *Croynebacterium pyogenes* + *Proteus spp* (22%), *Staphylococcus aureus* + *Croynebacterium bovis* (20%) and *E.coli* + *Streptococcus pyogenes* + *Streptococcus faecails* (15%) were the most encountered mixed infections isolated. This was in conformity with previous reports pointed that *E.coli* with *Proteus* and *Citrobacter* (Shouman *et al.*, 1977), *E.coli* + *Klebsiella* with incidence of 25.0% and *E.coli* + *Staphylococci* with incidence of 14.3% (Metwelly, 2001) as well as *E.coli* + *Strep. faecalis* + *Klbs.pneum*, *E.coli* + *Staph.aureus* +

Proteuss .pp, Strept. agalactiae + Staph.aureus and Pseud.aeruginosa + Strept.faecalis (Hassab El-Naby and El-Ekhnawy, 2004) were isolated from repeat breeder cases.

In the present study, 25 fungal isolates (12.5%) from total microbial isolates (23 (11.5%) from the clinically diagnosed repeat breeder cows and 2 (1.0%) from subclinically affected animals) were identified. Some of the affected animals studied here subjected to treatment with repetitive doses of disinfectants and antibiotics. Intra-uterine infusion of disinfectants as well as antibiotics suppresses natural defense mechanisms (Frank *et al.*, 1983). Also, excessive prolonged intra-uterine infusion of antibiotics in treatment of chronic endometritis is usually followed by establishment of fungi and yeasts in the genital tract of mares and cows (Cited after Ramoun *et al.*, 2002).

The most common fungal isolates found in this study were *Aspergillus* spp (24%), *Candida* (24%), *Penicillium* (12%) and *Fusarium* (12%). These results are in accordance with previous studies (Sinha *et al.*, 1980, Singh *et al.*, 1993 and Verma *et al.* 1999) but with lower incidences. Megahed *et al.* (2000) isolated *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp. and *Drechslera* spp. with incidences of 69.81%, 18.87%, 5.66% and 5.66%, respectively.

For the mixed infection (bacterial and fungal), 15 mixed infections (7.5%) from the total microbial infection were found. These results are lower than that obtained by Sinha *et al.* (1980) who recorded 17 (29.3%) mixed infections out of 58 repeat breeders and Metwelly (2002) isolated 30 (34%) cases mixed infections (bacterial and fungal) from 148 buffalo-cows suffering from repeat breeding.

E.coli + *Aspergillus* spp (33.3%), *Staphylococci* spp. + *Aspergillus* spp (26.7%), *E.coli* + *Fusarium* spp (20%) were the most mixed infections isolated here. Similarly, Metwelly (2002) isolated 12 (40%) for (*E.coli* + *Aspergillus* spp), 4 (13.3%) for (*E.coli* + *Aspergillus* spp + *Fusarium* spp), 8 (26.7%) for (*E.coli* + *Penicillium* spp) and 6 (20.0%) for (*Staphylococci* spp. + *Aspergillus* spp) from repeated breeder buffalo cows.

Sensitivity test:

The most active antibiotics against the bacterial isolates were Enerofloxacin (88.75%) followed by Oxytetracycline (84.37%), Gentamycin (78.13%) and Nalidixic acid (73.75%). Similarly, Vicek and Savobodova (1985) reported that the bacterial isolates were susceptible to Oxytetracycline and Chloramphenicol. Similar results were obtained by Megahed (1986), Ramakrishna (1996). In accordance

to our results, Metwally (2001) found that, the in-vitro antimicrobial susceptibility of bacterial isolates from cows with endometritis to Enrofloxacin, Oxytetracycline, Gentamycin and Ampicillin were 96.0, 89.0, 85.0 and 85.0% respectively. Karwani and Aulakh (2004) reported that, out of total 155 isolates from repeat breeder cattle and buffaloes, maximum isolates 146 (94%) were found sensitive to Ciprofloxacin followed by Gentamicin 115 (74%) and Chloramphenicol (67%). Hassab El-Naby and El-Ekhnawy (2004) concluded that the bacteria causing repeat breeding in cattle and buffaloes were more sensitive to Enrofloxacin, Gentamicin and Chloramphenicol while other antibiotics have moderate to less effectiveness against most pathogens. The majority of bacterial isolates were resistant to Neomycin (82.50%), Erythromycin (81.25%) and Ampicillin (80.00%). These results were nearly similar to those obtained by Awad *et al.* (1977) and Megahed (1986). Refaat (1980) reported that the isolated bacteria from buffaloes-cows suffering repeat breeding were moderately sensitive to Erythromycin. Karwani and Aulakh (2004) found that isolates from repeat breeder cattle and buffaloes were resistant to Penicillin, Ampicillin, Neomycin and Naledixic acid with varying degrees of drug resistance.

The present findings, in addition to the aforementioned reports are of great importance to direct the veterinarians' attention for the subclinical cases that neglected without correct diagnosis and proper treatment. Keeping in view the present findings and cited statement reported here, it is suggested that, of the repeat breeding animals, clinical examination along with isolation of micro-organisms and sensitivity test be routinely performed to ascertain the cause and prognosis of the case. Mixed infections with bacteria and fungus must be taken in consideration upon dealing with repeat breeding problem.

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SUMMARIES OF THESIS

Sylvia Osama Ibrahim, Ph.D., Zoonoses, 2006.

Seroepidemiological Studies on Hydatid Disease in Animals and Man.

Hydatidosis is one of the most important endemic zoonotic parasitic diseases with a wide spread distribution in the Middle East. In the present study, a survey was made for hydatidosis among slaughtered animals in Assiut and Bani-Adi abattoirs. The prevalence rates were 10 (8%) out of 125 camels, 4 (0.4%) out of 1032 sheep, 4 (0.4%) out of 1158 cattle and 1(0.1%) out of 1057 buffaloes. Concerning Echinococcosis in dogs, in the present study 60 stray dogs were examined, 10 of them from around Bani-Adi abattoir and no *Echinococcus granulosus* tapeworms were found with a zero prevalence. Concerning hydatidosis in human beings, for the serodiagnosis of hydatidosis the respective tests were indirect haemagglutination test (IHA) and enzyme linked immunosorbent assay (ELISA). Out of 92 serum samples from patients admitted to Assiut University Hospital examined for detection hydatidosis antibodies, 6 cases proved to be positive with a prevalence of 6.5% by the indirect haemagglutination test. The ELISA technique applied to the same serum samples of the patients indicated a prevalence rate of hydatidosis amounting to 4.3%.

Hoda Fathy Ahmed, Ph.D., Pathology, 2006.

Morphopathological studies on the female genital system of infertile buffalo cows.

During the period of 3 years, 350 buffaloes cows were slaughtered in El-Wasta and Moisha slaughterhouses of Assiut Governorate. Their age ranged from 6-10 years. All of these buffaloes had a case history of infertility problems. The genital tracts and ovaries were collected and examined grossly. Samples from cervix, uterus and fallopian tube were processed for histopathological studies. Some samples from cervix and endometrium were chosen for S.E.M. study. Ovarian affections were showed in 104 cases out of 350 cases. These affections are include:

1- smooth ovary 94 cases (26.86%). 2- Paraovarian cysts 4 cases. 3- Ovarian bursal adhesion 4 cases. 4- Ovarian abscess 2 cases. The histopathological changes which observed in the cervix were in form of acute catarrhal cervicitis 36 cases and chronic fibrosing cervicitis 9 cases. According to the histopathological changes in the

endometrium of these cases, the lesions were grouped into: acute catarrhal endometritis 30 cases, eosinophilic endometritis 5 cases, suppurative endometritis 15 cases, chronic catarrhal endometritis 41 cases and neoplastic changes in 4 cases. The histopathological changes which detected in fallopian tube were grouped into three categories: epithelium hyperplasia in 20 cases, adenomyosis in 32 cases and salpingitis in 9 cases.

Araby Mohamed Nassar, Ph.D., Veterinary Surgery, 2006.

Radiographic studies on the development of teeth with special reference to their surgical affections.

The current study was carried out on one hundred dog heads. The head specimens were collected randomly from mongrel dogs of both sexes and of variable ages. In addition 12 newly born dogs were used in this study. A full postmortem examination was performed for each head specimen including registration of different developmental and acquired surgical abnormalities and diseases of the teeth. All affections were recorded, described and illustrated in tables and by coloured photographs. Then, all head specimens were prepared for radiography by separating the mandible from the skull. A ventrodorsal radiographs were taken for the mandibular and maxillary incisors. After that, a median section was performed through the skull and mandible to have two separate equal halves. Radiography was performed in lateromedial view for the hemi-skulls and hemi-mandibles including incisors, canine and cheek teeth. The newly-born dogs were kept under standard ration and were sacrificed periodically at 1, 2, 3 and 6 months of age (each age by two dogs). The heads were prepared and radiographed in the same manner as done for the collected head specimens. After radiography all head specimens were macerated and boiled in water with hydrogen peroxide to have a clean hemi-skulls and hemi-mandibles. The prepared specimens were used to study the topographical anatomy, dental radiography, dental developmental and acquired abnormalities and diseases of the teeth.

Saber Abd El-Motagally Hassanein, Ph.D., Veterinary Hygiene, 2006.

Studies on some environmental and hygienic factors affecting dairy cattle performance.

Our study revealed that high environmental temperature and temperature-humidity index (THI) has drastic effect on milk yield, in which during summer season a sharp reduction in milk yield (6.52 ± 0.21) as compared with winter season (17.42 ± 0.28) was observed.

Blood samples analyses for determination of plasma levels of some milk related circulating hormones (thyroxine and Prolactin) obtained the following results: Thyroxine hormone was negatively correlated with milk yield and positively with temperature-humidity index (THI) and environmental temperature, while prolactin hormone was not correlated with milk yield and correlated positively with temperature-humidity index (THI) & environmental temperature. A total of 480 samples collected from the two examined animal farms in Assiut Province (Dairy farm of Faculty of Agriculture, Assiut University and Abnob El-Hamamm). These samples included 100 air samples from animal yards, 40 air samples from milking rooms, 100 soil samples from animal yards, 40 floor samples from milking rooms, 100 tap-water and 100 water-troughs (half of samples from each examined animal farm). Our bacteriological investigation revealed that a relationship between bacterial count in milk and that in animal environment (air, soil and water). It has been found that premilking udder and teats preparation had significant effect on total colony count; Coliform count and Staphylococcal count in quarter-milk and teats.

Yasser Mohamed Sabry Helmy Wafy, Ph.D., Milk Hygiene, 2006.

Sanitary improvement of serving milk and dairy products in Assiut University Hospital.

A total of 360 random samples of raw milk, yoghurt, Damietta and processed cheese (90 each) were collected from Food Department in Assiut University Hospitals. The third of samples were collected immediately after arrive to the hospital, the second third after heat treatment of milk and after cutting of damietta cheese and storage in refrigeration of yoghurt, Damietta and processed cheese. The last third from ready of serving milk and dairy products. These samples were examined physically, chemically, sanitary and microbiologically in order to determine their quality and sanitary condition.

- Physical and chemical examination.
- Microbiological examination of raw milk and dairy products before and after heat treatment, cutting, storage in refrigeration and ready for serving.
- Microbiological examination of milk and dairy products contactsurfaces.
- Sanitary status of milk and dairy products before and after cleaning and disinfections of contact surfaces.

Mohammed Mohammed Abd El-Rahman, Ph.D., Milk Hygiene, 2006.
Sanitary status of milk and some milk products marketed in suburbs of Assiut Governorate.

One hundred and sixty random samples (40 each of milk, cooking butter, kareish cheese and Domiati cheese) were collected from different markets and shops in Assiut Governorate suburbs to be examined for their sanitary status. Six pre-milking hygiene treatments mostly practiced were chosen for evaluation as follows:

1- Washing udder and teats with cold water. 2- Washing teats with cold water and drying with paper towels. 3- Washing teats with liquid soap and drying with paper towels. 4- Washing teats with liquid soap using a brush and drying with paper towels. 5- Washing teats with sodium hypochlorite 5%. 6- Washing teats with sodium hypochlorite 5% and drying with paper towels.

The results of the efficiency of various methods of pre-milking udder preparation on milk quality of both types of milking (Machine milking and hand milking) were as follow: A- Machine milking: In case of machine milking the changes in microbial load of milk were +17.3, -42.27, -64.24, - 51.61, -64.61 and -84.17%, respectively.

B- Hand milking: The percentage of change in the microbial load of milk obtained from udders received no treatment and that subjected to pre-milking udder treatments for hand milking were +7.61, -33.86, -70.23, -59.60, -75.99 and 87.35 %, respectively.

Saad Mahrous Faheem, Ph.D., Infectious Diseases (Virology), 2006.
Clinical and laboratory studies on Rift Valley fever among Camels at areas of Assiut and Daraw.

Along 4 years clinical and laboratory studies were carried out on 1186 camels from some villages of Assiut and Daraw quarantine (Aswan) at various ages, sex and seasons for throw light on susceptibility of local and imported camels to infection with Rift Valley Fever virus. Detection to the percentage of infection and isolation of the virus or detection of its antigen were also carried out. Our study cleared that local and imported camels are susceptible to RVF infection and the percentage of RVF antibodies in sera of camels at Assiut villages and Daraw quarantine was 14.75 %, 11.72% and 16.86% using serum neutralization test (SNT), complement fixation test (CFT) and enzyme linked immunosorbent assay (ELISA) respectively. Concerning the clinical signs of the disease the present study revealed that some camels which were positive serologically to the specific antibodies has no abnormal clinical signs

except fever and the percentage of RVF antibodies in sera of feverish camels were 17.5%, 13.75% and 17.5% using SNT, CFT and ELISA respectively. A high percentage of RVF antibodies was obtained during summer with a percentage of 24% using ELISA. Camels in all ages (young, prime, aged) are susceptible to infection. Percentage of RVF antibodies in sera of female and male were 20.52% and 15.13% respectively using ELISA. Percentage of RVF antibodies in sera of vaccinated camels at Assiut villages were 15.18%, 20.74% and 28.88% using SNT, CFT and ELISA respectively while in non vaccinated camels at the same villages were 13.5%, 11.5% and 14.5% using the same pervious tests respectively. The virus or its antigen could not be laboratory isolated or detected from infected or clinically healthy camels using tissue culture, Mice inoculation and Agar Gel Precipitation.

Khaled Ahmed Sayed, Ph.D., Infectious Diseases (Virology), 2006.

Prevalence and diagnostic studies on infectious bovine hinotracheitis at Assiut Governorate.

This study was applied on 535 cattle from which 506 were used in the seroprevalence study and 29 animals used in virus isolation as following:- The 506 serum samples were tested firstly by the serum neutralization test (SNT) with overall prevalence rate of 33%. Out of the 506 serum samples 184 samples were also tested by ELISA and the overall prevalence rate by the ELISA was 38.6%. Comparison between the sensitivity rate of the 2 tests (SNT & ELISA) reveals that the ELISA is more sensitive than SNT. Nasal and ocular swabs which were collected from the 29 diseased animals were subjected to the isolation on the tissue culture and identification by using indirect fluorescent antibody technique and the virus (BHV-1) was successively isolated from 8 cases. Also these 29 samples were tested by the agar gel diffusion test and 5 were found to be positive in such test. The agreement rate between the isolation and the agar gel diffusion test was 89.7%.

Amany Abdel Khalic Sayied, Ph.D., Meat Hygiene, 2006.

Monitoring and control of mycotoxins producing fungi in Assiut city hospitals restaurants.

The present investigation was designed to study the prevalence and population density of mould genera and toxigenic fungi of 400 samples of meat (100 samples of each of raw meat, minced meat (without additives), cooked meat under steam and cooked kofta). The results revealed that the mean counts of the examined raw meat, minced meat

and cooked kofta on Czapek's medium were $5.96 \times 10^3 \pm 5.174 \times 10$, $6.78 \times 10^3 \pm 6.144 \times 10$ and $8.07 \times 10^3 \pm 7.505 \times 10$ while the mean counts of the examined raw meat, minced meat and cooked kofta on Dichloran rose bengal medium were $4.77 \times 10^3 \pm 4.059 \times 10$, $5.47 \times 10^3 \pm 5.089 \times 10$ and $5.94 \times 10^3 \pm 5.111 \times 10$, respectively. The results mentioned that all the samples tested from minced meat, raw meat, cooked meat under steam and cooked kofta were free from mycotoxins. The results showed that chlorine and hydrogen peroxide had highest effect on fungi growth.

Ayman Mounir Ibrahim, Ph.D., Vet. Surgery, 2006.

The bacterial causes of claw and hoof affections and methods of treatment.

This study was carried out on 3540 animals of different species. These animals were divided into two categories. The first category included 3508 animals. The bacteriological examination revealed that 172 anaerobic bacterial isolates and 164 aerobic bacterial isolates were identified. The most prevalent M.os.were *Fusobacterium necrophrum* and *Corynebacterium pyogenes*. The second category (treatment groups) included 20 lactating dairy cows with foot rot and 12 donkey showed septic hoof affection, were divided into 4 groups, each of 5 cows and 3 donkeys and treated topically by honey, *Thymus vulgaris*, *Matricaria chamomilla* and *Organum vulgare*. It was found that the best result were obtained with the use of honey and *Thymus vulgaris* followed by *Matricaria chamomillia* and lastly the group by *Origanum vulgare*.

Hoda Ibrahim Mostafa, Ph.D., Clinical Laboratory Diagnosis, 2006.

Clinico-biochemical studies on hypophosphatemia in buffaloes with some therapeutical trials in Assiut Governorate.

This study did on 140 female buffaloes classified into three groups. Group (I) healthy control, group (II) buffaloes suffering from clinical hypophosphatemia (Hburia) classified into three groups, group A treated with dibasic sodium phosphate, group B treated with Tonophos, group C treated with Tonophos + vit. C. Group (III) suffering from subclinical hypophosphatemia. Samples were taken from group II during Hburia and 10 days after treatment. Blood samples for RBCs, WBCs, Hb, PCV and blood serum for in IP, Ca, Mg, Cu, Fe, LDH, uria and creatinine. Statistical analysis showed severe anemia in group II with decreased in IP, Ca, and Cu and increased in Mg, Fe, LDH, uria and creatinine, improvement very well with group C. Group III recorded anemia and decreased in blood serum IP, Ca Cu and Fe.

Motamed El-Sayed Mahmoud Ali, Ph.D., Animal Behavior and Management, 2006.

Studies on some managemental and stress factors relating to sickness behavior in chicken.

These studies were conducted to check the effect of pretreatment with an extract from hot chilli peppers; capsaicin (CAP) on some stress factors like injection of lipopolysaccharides (LPS), exposure to heat and cold and instillation of ammonia in white leghorns chicks. The results showed that chicks preferred CAP (10 ppm) more than tap water. When CAP (10 mg/kg, Bw, IV) was pretreated, chicks showed less hyperthermic effect of heat stress, less hypothermic effect of cold stress in 4, 7 and 10 days and lowered mortalities in 4 days. In addition to the absence of LPS induced monophasic fever, early phases of polyphasic fever and inhibition of inducible gene expression of MHC II in bursa of Fabricius and inducible NO and iNOS gene expression in liver, lung and brain after injection of LPS.

Mohammed Mostafa Mohammed, M.V.Sc., Hygiene and Control of Meat, Fish and Animal By-products, 2006.

Prevalence of *Aeromonas hydrophila* in some types of Nasser lake Fishes.

One hundred freshly caught fish samples of four species including *A. baremoze*, *H. forskalii*, *L. niloticus* and *O. niloticus*, 25 of each, of Nasser lake fishes were examined for the presence of *Aeromonas* species. The organoleptic examination of samples revealed that all the examined samples were accepted, although highly significant differences in the sensory assessment scores between the fish species could be detected. Also, the determination of the flesh pH of the examined samples indicated that there was a highly significant difference between the examined four species and pH values. Detection of *Aeromonas* species by using direct plating method and enrichment technique indicated that most samples were contaminated with *Aeromonas* species and correlation between the *Aeromonas* species count and the fish species resulted in a significant differences. The proteolysis and lipolysis activity of the isolates was detected.

Marwa Sayed Ahmed El-Sayed Khallaf, M.V.Sc., *Animal Behaviour and Management, 2006.*

Behavioural Studies on Pregnant Rabbits and Their Litters Subjected to Some Stressors.

The effect of some factors on maternal behaviour and litter traits in female *NZW* rabbits were studied, including the followings: 1- The effect of transient Doe-litter separation. 2- The effect of light/Dark cycle. 3- The effect of frightening. 4- The effect of water deprivation. It is of interest to reveal that: Only lighting for only 4-h led to decreasing the value of serum cortisol level at day of parturition, water deprivation during pregnancy led to significant increase in mortality rate of does, receptivity and conception rate of doe rabbit affected badly by water deprivation during both pregnancy and lactation and continuous lighting.

Amira Tharoat Mohamed Hosseney Tamer, M.V.Sc., *Meat Hygiene, 2006.*

Studies on *Pseudomonas* Species in some meat products with special reference to its proteolytic and lipolytic activity.

Pseudomonas species could be detected on 100 random samples of frozen (beef burger, kofta, sausage minced meat). *Pseudomonas* species were isolated in different percentages from the samples. Also, the characterization of *Pseudomonas spp.* isolated from the examined meat products samples for production of extracellular virulence factors as proteolytic and lipolytic enzymes were studied. The public health significance of the organism and the precautions, which should be taken to control this organism in meat products industry as well as recommended sanitary measures, were also discussed.

Yasmin Omar Mohamoud Elsaid El-Amir, M.V.Sc., *Animal Pathology, 2006.*

Evaluation of green tea treatment of ultraviolet-B skin photocarcinogenesis with arsenite cocarcinogen in mice. "The role of nucleolar organizer regions and mast cells"

The present study was confirmed to investigate the role of green tea polyphenols as antioxidants in the protection and treatment of ultraviolet B skin photocarcinogenesis and the role of sodium arsenite as cocarcinogen. UVB radiation induced dysplastic changes in epidermis, hyperplasia of hair follicle, trichofolliculoma, trichofolliculocarcinoma, squamous cell carcinoma, basal cell carcinoma, fibropapilloma, rhabdomyosarcoma and mixed tumors. Green tea treatment prevented

induction of hyperplasia of hair follicle, trichofolliculocarcinoma, squamous cell carcinoma, fibropapilloma, rhabdomyosarcoma and mixed tumors. Green tea also reduced dysplastic changes and trichofolliculoma. Arsenite did not express its role as cocarcinogen in this experiment. Green tea treatment has no effect in the UVB and arsenite group. Giemsa stain revealed increased number of mast cells in benign and malignant tumors. They were large in number in relatively differentiated malignant tumors. There was significant increase in number of AgNORs in malignant tumors than in benign tumors. AgNORs can be used in grading squamous cell carcinoma.

Marwah Ahmed Ahmed, M.V.Sc., Pathology and Clinical Pathology, 2006.

Pathological evaluation of environmental pollution with Fluorine and Cadmium Emitted from Mangabad Superphosphate Factory on Goats.

In the present study, blood and tissue samples were taken from goats reared in the vicinity of Mangabad Super phosphate factory to evaluate the toxopathological and biochemical alterations induced by the factory emissions. Chemical analysis revealed significant increase in the levels of cadmium and fluoride in the blood and different tissue samples. Cadmium was found significantly higher in the blood, liver, kidneys, lungs, heart and spleen while fluoride levels were only significantly increased in liver, bone and heart compared to controls. Goats exposed to the factory emissions showed significant increase in the levels of AST and ALT and urea indicating liver and kidney damage, respectively. Histopathological examination showed clear implication of blood vasculature through the body. In this context, it was found blood vessel degeneration, perivascular edema, thrombosis and hemorrhages in different body systems. Most pronounced histopathological findings in the liver were vacuolar and fatty degeneration, necrobiotic changes, activation of Kupffer cells and fibrocytic changes. Activation of Kupffer cells might be playing a major role in the mechanism of cadmium induced hepatotoxicity through releasing some proinflammatory cytokines within the liver. In the Kidney, there were glomerular swelling, periglomerular fibrosis, necrobiotic changes of renal tubular epithelium and fibroblastic changes in the interstitium. Glomerulo- and interstitial nephritis perhaps resulted from immune reaction against cadmium-metallothionein bound complexes. Major pulmonary lesions constituted of alveolar emphysema, bronchiolitis and interstitial

pneumonia. Cardiac lesions formed of degeneration and necrosis of myocardial fibers, myocardiolysis and perivascular and interstitial fibrosis. Periosteal thickening, enlargement of bone trabeculae and narrowing of bone cavities were seen in some of exposed-goats. Brain sections showed neuronal degeneration and necrosis, microglial reaction and demyelination. Skin of some exposed-goats showed epidermal atrophy and hyperkeratosis, partial to complete loss of the epithelial sheath of hair follicle or complete loss of hair follicles in the dermis, cystic dilatation of sweat glands, myxedema and eosinophilic infiltration in the dermis. In spleen, there were some evidence of lymphocytic exhaustion, thickening of follicular artery and hemosiderosis. Testicular epithelium appeared degenerated in some cases and atrophied in others.

Ahmed Mohammed Kotb, M.V.Sc., Animal Anatomy, 2006.

Comparative morphological studies on the vascular tunic of the eyeball in some domestic animals.

The present work was carried out on 16 eyeballs from each of the following animals: donkeys, buffaloes, camels and dogs to elucidate the gross anatomical, light microscopical and scanning electron microscopical features of the vascular tunic. In addition morphometric data were carried out on the latter tunic in all animals under study. The absolute volume of the eyeball is variable among studied animals. The camels have the smallest eyeball among the large animals under study. It can be postulated that the size of the eyeball of an animal is inversely related to the amount of light in the surrounding environment. The *Tapetum lucidum* is either fibrous as seen in donkeys and buffaloes or cellular as observed in dogs consisting of variable layers of well ordered collagen bundles or tapetal cells respectively. The latter structures are of almost constant size and separated by almost constant spaces. The number of tapetal layers varies from animal to another. The *Tapetum cellulosum* of dogs demonstrates the presence of more layers than those of donkeys and buffaloes that may indicate a higher reflectance in the former animal. The anterior surface of the iris is covered by a single layer of flat epithelial cells, while the iris is covered posteriorly by two layers of pigmented epithelial cells continuous with the two layers of retinal epithelium. The deep layer is transformed to the dilator pupillary muscle.

Randa Abd El Hakeim Abd El Raheim, M.V.Sc., Clinical & Laboratory Diagnosis, 2006.

Clinical and laboratory studies on urinary disorders in native breed goats.

The present study included clinical examination of one hundred twenty three cases of goats, aged from six months to over two years. Out of them one hundred animal were proved to be suffered from urinary tract disorders and twenty three animals were clinically healthy and showed no clinical or laboratory abnormalities and taken as control group. Physical, chemical and microscopical examination of urine samples collected from these animals. Besides pathological examination of diseased urinary tissues were also performed. The present investigation declared that, the most common urinary tract disorders in examined diseased goat were cystitis 30%, nephrosis 27%, pyelonephritis 18%, glomerulonephritis 13%, focal intersitial nephritis 11% and nephrolithiasis 1%.

Zeinab Ahmed Basha Ahmed, M.V.Sc., Clinical Laboratory Diagnosis, 2006.

The changes in blood picture and some serum biochemical parameters in clinically anemic cattle.

The present study aimed to: recognizing the changes in blood picture and some serum biochemical parameters in clinically anemic cattle. The sample consists of: 103 cattle (96 adult 3-5 years and 7 calves under one year old) of both sexes were examined in this study. Following results were reached: Changes in blood picture in anemic cattle are closely related to the etiological agent, The changes in serum levels of Fe, Cu, vitamin C and vitamin E are related to the causative agent of the anemia. All types of studied anemia are associated with reduction in the serum levels of vitamins C & E. This refers to: Therapy of anemic condition should involve improving of immune status of the body through supplementation with vitamins C & E. in addition supplementation with adequate amount of Fe and Cu.

Doaa Safwat Mohammed Fahmy, M.V.Sc., Forensic Mmedicine and Veterinary Toxicology, 2006.

Trials to estimate the age of bloodstains.

The present study has been designed to reveal the relationship between the change of some blood constituents and the age of bloodstain. The present study was carried out on 2160 bloodstains samples obtained from cattle, chicken and consenting human volunteers (720 each). The bloodstains

samples of each species were divided into equal four groups (180 each) concerning the four media (cotton fabric, iron, wood and soil). The samples of each media (180) were divided into three groups for the estimation of absorbance, enzymes (LDH, AST and ALT) and total proteins at 24, 48, 72 hours, 1, 2, 3 weeks, 1, 2, 3, 4, 5 and 6 months post-staining. The obtained results revealed negative relationship between the absorbance, enzymes activities and total protein and the age of bloodstains. The final results of the present study indicated that the estimated parameters (absorbance, enzymes activities and total protein) showed insignificant variation between the three investigated species (cattle, chicken and human being). A significant variance was recorded regarding the different studied media. The recorded results were statistically expressed in equations, which is easily to be used for estimation of bloodstains age.

Mohammed Abdelhadi Mohammed, M.V.Sc., Forensic Mmedicine and Vveterinary Toxicology, 2006.

Effect of toxic interaction between oxytetracycline and cadmium in broilers.

450 Ross chicks of both sexes at age of one day old were used in the present study. Chickens were divided into two main groups A and B. After 31 days age, each group was subdivided into three sub-groups A₁, A₂ and A₃ for group A and B₁, B₂ and B₃ for group B. The present investigation revealed the toxic effects of oxytetracycline (OTC) on broiler chickens muscles (pectoral and thigh) and livers due to administration of therapeutic and over concentration according to the design of the experiment. The residual levels of OTC in pectoral muscle at 11-21 days post exposing was within the permissible limits. However, the thigh muscles in-group A₂ and B₂ are not within the limit and highly exceed the MRLs in livers of all studied groups. The highest cadmium (Cd) levels in the kidney was recorded in group B₃ at the 19th day, in the liver was in group B₃ at the 15th day, in pectoral muscle was in group B₃ at the 17th and 23rd day post exposure. The highest Cd level of thigh muscle was recorded in group B₃ at the 11th day in bone tissue was recorded in group B₃ at the 15th day post exposure. Hematological results indicated that: All the investigated blood parameters (RBCs, Hb and PCV) were significantly decreased in group B₂ and B₃ in comparison with B₁. Creatinine was recorded in both groups B₂ and B₃ in comparison with group B₁. The highest level of weight of broiler chickens was recorded in group A₂ at the 23rd day post exposure. The

histopathological changes of the investigated organs of broiler Chickens (liver, kidney, spleen and bursa of fabricius) revealed pronounced, mild to minimum changes or appeared more or less normal depending upon the various handled groups of the experiment and control. OTC concentration in thigh muscles and liver exceeded MRL up to 21 days. Only a significant decrease in body weight gain was recorded in group B₃ which exposed to Cd and over concentration of OTC. The residual level of OTC increased up to 10 times in the presence of cadmium (B₂&B₃). From our results we do not recommend consumption of chicken's liver and thigh muscles within 21 days of stopping therapeutic concentration of OTC administration.

Mohamed Azat Abdel-Gaid, M.V.Sc., Veterinary Hygiene (Zoonoses), 2006.

Epidemiological studies on leptospirosis in some animals and Man in Upper Egypt.

A total of 284 blood samples were collected from different species of animals including cattle (100), buffalo (16), rodents (70) and dogs (98). The cattle samples were collected from two governmental farms in Assiut Governorate including Abnoub EL Hamam (55) and The Military farm (44) with symptoms of infertility and mastitis as well as one sample from New Valley Governorate suffering from jaundice. The examined buffaloes (16) were collected randomly from Moasha slaughterhouse in Assiut. Regarding dogs (98) and rodents (70), they were randomly collected from different areas in Assiut province. Our results revealed that: The serological examination of 100 cattle suffering from infertility, mastitis or jaundice was positive in 14% in Assiut and New Valley Governorates. While our data explained the higher incidence rate of Leptospirosis among apparently healthy buffaloes 18.75% attributed to preferring them to bathing in the water and muddy soils which may be contaminated with the urine of infected dogs and rats. From our results, we observed that the overall Leptospiral infection among human been in Upper Egypt Governorates was 40.87% from examined samples which indicate the following: 1- In relation to occupations, the highest risk of infection was 71.42% among sewer workers and 83.33% in Rice field workers this result may be attributed to the contamination of water by urine of rodents and dogs. 2- Infections among patients suffering from urinary tract infection was 47.16% and among jaundice patients was 12% most of them were farmers, whom contact with animals urine.

**Amal Ahmed Mahmoud Abd-Elmagid El-Khawaga, M.V.Sc.,
Microbiology and Immunology, 2006.**

Incidence of Helicobacter species in milk and some milk products in Assuit City and some factors affecting H. pylori growth.

Helicobacters represent a potential hazard upon human health especially *H. pylori* as it causes many diseases such as peptic and duodenal ulcers, gastric carcinoma and mucosa associated lymphoid tissue lymphoma (MALT). Other *Helicobacters* as *H. heilmanii*, *H. felis*, *H. cinaedi* and *H. pullorum* have been associated with diarrhea and gastric disease in man. Therefore, this study was planned to determine the incidence of *Helicobacter* spp. in milk and some milk products in Assuit city through conventional methods including culture and biochemical identification. *H.pylori* is the best known thus further identification including PCR, and antibiotic sensitivity to the various antibiotics used for its eradication as well as some factors that enhance or retard the growth of *H. pylori* such as temperature, pH, sodium chloride concentration & potassium sorbate have been studied.

**Elham Mahfouz Yousef, M.V.Sc., Clinical Laboratory Diagnosis,
2006.**

Some mineral Profile in sheep serum in New Vally Governorate.

A total number of 484 parasite free sheep were selected. Of these selected animals, a total of 442 sheep were apparently healthy and 42 cases manifested symptoms suggesting mineral disturbances. These cases included steely wool, alopecia, enzootic ataxia and goiter. Blood samples were taken from each sheep by jugular vein puncture in addition to soil and forage samples from these areas for estimation of Ca, P, Fe, Cu, Zn and I in blood serum of sheep and agronomical samples. These estimated values in soil and forages were above the recommended marginal levels for Ca, Fe and Zn but were low for P and I. Minerals in water were within the levels recommended by the WHO, but Fe concentration was high. The over all mean value of blood serum Ca, P, Fe, Cu, Zn and I \pm SD (mg/dl) recorded for 484 sheep at different localities at El Kharga and El Dakhla cities and at different physiological stages including different ages, sex, productive and reproductive stages was 9.26 ± 1.32 , 4.39 ± 1.03 , 139.5 ± 27.9 , 64.55 ± 22.78 , 65.45 ± 23.51 and 6.03 ± 2.28 respectively. These results indicated that the blood serum of sheep reared in the Egyptian oasis were normal and above the deficiency limits for Ca and Fe but it were near the border line for P, Cu, Zn and it were deficient in I. Correlation value (r) between mineral

concentrations in both soil and forage and in blood serum of sheep was non significant for Ca, Fe, Cu, Zn and I while it was positive for P.

Esraa Abd El-khalik Ahmed Mohammed, M.V.Sc., Pharmacology, 2006.

Non opioid analgesic, nefopam, influence on immune system in mice.

The effect of opiate analgesics non-steroidal anti-inflammatory on the immune functions have been reported. The effect of the non-opiate analgesic nefopam on the immune functions has not yet been investigated. Male Swiss albino mice were treated with either heat killed *E.coli* or saline. They were classified into 12 groups. The effect of subacute (15 mg/kg/12 hr. S.C. daily for one week) and chronic (10 mg/kg/12 hr. S.C. daily for one month) treatment with nefopam on the levels of interferon-gamma (IFN- γ) and total immunoglobulin were examined in normal and immunized mice. Also, the effects of chronic administration of nefopam on the phagocytic activity of peritoneal macrophage were evaluated in normal and immunized mice. Our findings postulated that nefopam stimulated the immune system and increased defense mechanisms at least in part, due to its inhibitory effect on the serotonin and norepinephrine reuptake. This information may be of future therapeutic value for nefopam as an immune enhancer.

Mohamed Hamdy Mohamed, M.V.Sc., Hygiene and Control of Meat, Fish and their products and Animal By-products, 2006.

Monitoring of *Helicobacter* species in selected chicken meat products with special reference to *H. pylori*.

Eighty samples of chicken Luncheon and chicken Kofta (40 of each) were collected randomly from different locations, supermarkets and different groceries in Assiut Governorate. The results showed that *Helicobacter* spp. could be detected on HPSPA in 6 samples of chicken Luncheon (15%) and 9 samples of chicken Kofta (22.5%). However the incidence of *Helicobacter* spp. on Columbia agar was 2 samples of chicken Luncheon (5%) and 7 samples of chicken Kofta (17.5%). *H.pylori* was isolated in 2.5% from examined chicken Luncheon samples and 5% from examined chicken Kofta samples on HPSPA and in a percentage of 2.5% from the examined chicken Kofta samples on Columbia agar. *H. pylori* was sensitive to garlic extract and thyme and that was demonstrated by reduced count of *H. pylori* on HPSPA.

