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ANTIBIOTIC RESIDUES IN COMMERCIAL LAYER HENS IN KHARTOUM STATE, SUDAN, 2007-2008.

BY

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SUMMARY

The prevalence of antimicrobial residues in table eggs produced in Khartoum State, Sudan was estimated and determined. All available producing layer farms in the state were sampled in April, June and August 2008. For each layer house three egg samples were randomly collected to increase the sensitivity of antimicrobial residue screening test detectability. In total, 933 egg samples were analyzed, collected from 175 layer farms (335 layer houses) in three periods of collection. An in-house residue detection test using *Geobacillus stearothermophillus var calidolactis* was the analytical procedure used for the analysis. Data were analysed using Survey Toolbox to calculate the true prevalence and confidence intervals. The proportion of layer farms with antimicrobial residues in April, June and August was 61.1%, 60.2% and 68.7% respectively. The proportion of layer houses affected in April, June and August were 56.0%, 54.1% and 57.1% respectively. The results showed insignificant variation among the three periods of the surveillance (p = 0.57).

A census covering all three localities of the state (Khartoum, Bahry and Omdurman) was carried out in late 2007 and early 2008. Data were recorded on areas where farms occur, number of houses per farm, total capacity of birds and farming systems. The census showed that there were 252 layer farms in the state distributed in 31 different areas with a total population of 2 221 800 birds.

A structured questionnaire survey was carried out in April 2008 in the state, to assess and collect data on risk factors associated with the presence of antimicrobial residues in table eggs. The questionnaire investigated antibiotic usage patterns for each layer farm as well as the basic knowledge and understanding of farmers about public health concerns associated with antibiotic use in food producing animals. Questions were closed ended and data was obtained through direct interviews with farm owners and managers. Descriptive statistical analysis was carried out on the information captured; calculating frequencies, graphs and measures of association, using the

EpiInfo™ statistical package. Ninety two farms were surveyed 98% of which comprised open-sided houses. It was found that 48.9% of the farms surveyed were on antibiotic treatment when the survey was conducted, while 58.7% of the farms had used antibiotics within the last three months. There was a significant association between having disease on the farm and using antibiotics ($P<0.001$).

The study showed that there is a serious lack of knowledge about the dangers of using antibiotics in animals and their potential impact on human health. In addition, Sudan lacks any type of formal control of veterinary drugs in terms of legislated residue limits or monitoring and surveillance programmes. This leads the authors to the conclusion that all Sudanese consumers are at risk for ARs in eggs.

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LIST OF ABREVIATIONS

ADIs	Acceptable Daily Intakes
AI	Avian Influenza
AP	Apparent Prevalence
AR	Antimicrobial Residue
ATCC	American Type Culture Collection
AU	Animal Unit
C	Celsius
CAMHB	Cation-adjusted Mueller-Hinton Agar
CDC	Centre for Disease Control and Prevention
CLSI	Clinic and Laboratory Standard Institute
CRD	Chronic Respiratory Disease
CVMP	Committee of Veterinary Medicinal Products
DVTD	Department of Veterinary Topical Diseases
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
FAO	Food and Agriculture Organization
FCR	Feed Conversion Rate
FDA	Food and Drug Administration
FPM	Four Plate Method
FSIS	Food Safety and Inspection Service
GC-MS	Gas Chromatography-Mass Spectrometry
Ha	Hectares
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin-Layer Chromatography
IAC	Immuno-affinity Chromatography
ILSI	International Life Science Institute
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KGy	Kilo Gray
km	Kilometer
LC-MS	Liquid Chromatography-Mass Spectrometry
µg	Microgram
mg	Milligram

mL	Milliliter
mm	Millimeter
MHB	Mueller-Hinton Broth
MIC	Minimum Inhibitory Concentration
MRL	Maximum Residue Limit
NaOH	Sodium Hydroxide
NARM	National Antibacterial Residue Minimization
NDKT	New Dutch Kidney Test
NOEL	No observed effect level
NRS	National Residue Survey
PBS	Phosphate Buffered Saline
®	Registered Trademark
Se	Sensitivity
Sp	Specificity
SE	Standard Error
TP	True Prevalence
UK	United Kingdom
UN	United Nations
USA	United States of America
USDA	United States Drug Administration
VMD	Veterinary Medicine Directorate
WHO	World Health Organization
WTO	World Trade Organization

CHAPTER I - INTRODUCTION

The world-wide commercial poultry industry is well-developed and is the largest supplier of animal protein in the form of meat and eggs. Its significance is even greater in developing countries where poultry are relatively cheap and can be kept in a small area, usually providing both protein and some income for a family (Law & Payne, 1996).

The Sudanese poultry industry is principally located in Khartoum State (Table 4) which is responsible for almost 90% of the country's production. The total poultry population in Sudan is estimated at 45 million. The commercial sector comprises around 30 million chickens of which about 20 million are layer hens. It contributes 45% of the agricultural income of the State, while the latter (agricultural income) contributes 7% of the total income (Ministry of Agriculture and Animal resources, Khartoum State, 2005).

Antimicrobial usage has facilitated the efficient production of poultry, allowing the consumer to purchase, at a reasonable cost, high quality meat and eggs as well as reduce the impact of disease outbreaks (Al-Ghamdi *et al.*, 2000). They are used by the poultry industry and poultry veterinarians to enhance growth and feed efficiency and reduce bacterial disease (Donoghue, 2003). In layer hens antimicrobials are only used to treat and prevent bacterial infections. Antimicrobial classes used to treat chickens (broilers, layers and breeders) include: aminoglycosides, tetracyclines, beta-lactams, quinolones, macrolides, polypeptides, amphenicols and sulphonamides (Stolker and Brinkman, 2005).

The heavy reliance on antimicrobials in animal production has resulted in bacterial resistance to many modern antibiotics used for life-threatening diseases in humans. As a consequence the transfer of antimicrobial resistance from food animals to humans or the presence of antimicrobial residues in food of animal origin is now perceived to be a threat to human health (Hughes & Heritage, 2007). The role that the poultry industry may have played in the transfer of resistant bacteria to humans was evidenced by the fact that the numbers of cases of vancomycin resistant enterococcal infections dramatically increased when the vancomycin analogue avoparcin was used as a performance enhancer in poultry (Bager *et al.*, 1997). The banning of avoparcin in the European Union stopped the production of avoparacin with the result that vancomycin resistant enterococci are now less than 5% (above

25% before the banning) of the enterococci isolated from poultry (Casewell *et al.*, 2003).

Of even greater risk are residues of either the parent-drug or its metabolites that can be found in meat, milk and eggs for a variable period of time after the antimicrobials have been administered. Residues may have a direct toxic effect on consumers, e.g., allergic reactions in hypersensitive individuals (Dayan, 1993; Ormerod *et al.,* 1987; Woodward, 1991), or they may cause problems indirectly through induction of resistant strains of bacteria (Stolker & Brinkman, 2005). Therefore it has become necessary that regulations are in place to ensure that antimicrobial residues are not present at levels that may affect human health in animal products for consumption.

Internationally recognized organizations such as the World Health Organization (WHO), Food and Agriculture Organization (FAO), Veterinary Medicine Directorate (VMD) of the European Union (EU) as well as the Food and Drug Administration in the USA (FDA) have set maximum tolerance levels or, acceptable daily intake (ADIs) for humans and withholding times for pharmacologically active substances including antimicrobial agents prior to marketing (Al-Ghamdi *et al.,* 2000). Together with these regulations, surveillance systems should be in place to ensure that these standards are being met. Analyses used must therefore be able to detect antimicrobials at the maximum residue levels.

Currently Sudan has no regulations regarding the use of antimicrobials, the maximum allowable antimicrobial concentrations in food nor any systems to monitor the presence of antimicrobial residues in animal products. Thus, the objective of this study was to investigate the prevalence of antimicrobial residues in commercial layer eggs in Khartoum State, Sudan and assess or suggest guidelines concerning policies and regulations of antibiotic residues control in food of animal origin, with emphasis on egg production in Sudan.

CHAPTER II- LITERATURE REVIEW

1. Agriculture in The Sudan

The Sudan is a vast country with a variety of resources; the most important being agricultural resources. The country has an agricultural potential of 105 million hectares (Ha), of which only 16.7 million Ha are cultivated. According to the Nile Waters Agreement with Egypt, total actual renewable water resources of the country amount to 64.5 km^3/yr. Furthermore, the country has a high reserve (464 to 564 km^3) of underground water of which only about 0.4% is presently utilized. Moreover, more water may be secured for irrigation purposes from the largest flood plain in Africa (Sudd region in Southern Sudan) which covers an area of 100 000 km^3 but loses by evaporation and spills about 50% of annual surface flow (www.sudanimals.com).

The Sudan is endowed with a large number of livestock such as cattle, sheep, goats and camels, which include breeds unique to this region. Unfortunately very little has been done to identify and characterize the genotypes existing in the country. Although, the productivity of indigenous breeds is low compared to temperate breeds, their ability to survive and produce in the harsh and mostly unpredicted tropical environment is remarkable (www.sudanimals.com).

1.1 Khartoum State

Khartoum, the capital of The Sudan, is located in the semi desert zone in between latitudes 15.08° and 16.39° North and longitudes 31.36° and 34.25° East and divided into three major localities (Khartoum, Khartoum North (Bahry) & Omdurman). The topography is flat, except for some scattered mountains. It is hot and dry with rains in summer and cool and dry in winter, the annual rainfall ranges from 75 to 160 mm, falling mainly in July and August. Generally the dry period extends for 8-10 months. The data from the daily average minimum temperature is 21.6℃; the maximum temperature in summer exceeds 40℃, while the minimum temperature in winter is 5℃. The evaporation (Penman) is 7.7 mm/day but during April it reaches 9.3 mm/day. The daily average relative humidity is 38% at 8am and 21% at 12 noon. The wind speed is generally about 14.48 km/hour. Dust storms prevail in the State with speeds of about 17.7 km/ hour.

The population of Khartoum State has grown rapidly in recent years and today is estimated at more than 7 million people, including 2 million refugees from neighbouring countries such as Ethiopia and Chad.

A wide range of production systems can be found ranging from household subsistence to large-scale commercial farming. Intensive livestock production systems for milk, meat, and poultry are operational within and around Khartoum city (El-siddig *et al.,* 2006). The resident livestock are about 728 559 animal units (An animal unit (AU) is one mature cow of approximately 453.6 Kg and a calf up to weaning, usually 6 months of age, or their equivalent). In addition some 1.5 million animal units pass through the State for export and trade purposes (El-siddig *et al.,* 2006).

2. The Poultry Industry

2.1 Global Poultry Industry

The poultry industry is based on the production of two types of products, namely eggs and meat. There are many interconnections between the egg and poultry meat industries. A few breeding enterprises produce day-old chicks of both egg and meat types. The layer type is used to produce table eggs while the meat type produces broilers. The two industries may operate from a common base of layer or broiler breeders, stock-feed mills, equipment and pharmaceutical suppliers (Henry & Rothwell, 1995).

2.1.1 Broiler Production

Poultry is one of the world's fastest growing sources of meat, representing 31.5% of all meat produced in 2007 (www.thepoultrysite.com). The modern broiler industry started in the 1930s, when flock size was seldom greater than a few hundred birds. By the 1950s, the flock size had increased to a few thousand and by the 1980s many broiler houses had a capacity of 100 000 or more (Hubbert *et al*., 1996). Globally as shown in Table 1, there are about 60 billion broilers at any one time, of which 26.6 percent are in the USA, 16.9 percent in the People's Republic of China, 16.4 percent in Brazil and 12.3 percent in the European Union (USDA-FAS, 2007). China consumes almost 17.5 percent of global production compared to the USA which, consumes 23.5 percent and the EU which consumes 12.3%. Table 2 shows global broiler consumption (USDA-FAS, 2007).

Table 1: Selected Countries Broiler Production (1 000 Metric Tons)
(http://www.fas.usda.gov/dlp/circular/2007/livestock_poultry_11-2007.pdf)

Production	2001	2002	2003	2004	2005	2006	2007
USA	14 033	14 467	14 696	15 286	15 870	16 233	16 076
China	09 278	09 558	09 898	09 998	10 200	10 350	10 850
Brazil	06 567	07 449	07 645	08 408	09 360	10 035	10 105
European Union	07 883	07 788	07 512	07 627	07 625	07 540	08 035
Mexico	02 067	02 157	02 290	02 389	02 510	02 635	02 656
India	01 250	01 400	01 500	01 650	01 900	02 200	02 200
Argentina	00 870	00 640	00 750	00 910	01 080	01 180	01 300
Japan	01 074	01 107	01 127	01 124	01 165	01 150	01 235
Thailand	01 230	01 275	01 340	00 900	00 950	01 100	01 050
Canada	00 927	00 932	00 929	00 946	01 000	01 020	00 995
Malaysia	00 813	00 784	00 835	00 862	00 896	00 920	-
Others	06 311	06 598	05 760	05 852	06 165	06 538	07 067
Total	**52 303**	**54 155**	**54 282**	**55 952**	**58 721**	**60 901**	**62 919**

Table 2: Selected Countries Broiler Consumption (1 000 Metric Tons)
(http://www.fas.usda.gov/dlp/circular/2007/livestock_poultry_11-2007.pdf).

Production	2001	2002	2003	2004	2005	2006	2007
USA	11 558	12 270	12 540	13 080	13 455	13 878	13 657
China	09 237	09 556	09 963	09 931	10 150	10 325	11 010
Brazil	05 341	05 873	05 742	05 992	06 622	07 135	07 200
European Union	07 359	07 417	07 312	07 280	07 370	07 270	07 885
Mexico	02 311	02 424	02 627	02 713	02 883	03 029	03 070
India	01 250	01 400	01 496	01 648	01 900	02 199	02 200
Russia	01 588	01 697	01 680	01 675	01 949	02 095	02 540
Japan	01 797	01 830	01 841	01 713	01 877	01 905	01 925
Argentina	00 881	00 618	00 719	00 845	00 973	01 034	01 154
Canada	00 924	00 925	00 933	00 972	00 984	00 995	-
South Africa	00 786	00 830	00 928	00 959	00 997	01 026	01 090
Others	07 822	07 995	07 099	07 367	07 746	08 021	08 518
Total	**50 854**	**52 835**	**52 880**	**54 175**	**56 906**	**58 912**	**61 219**

2.1.2 Egg Production

Before World War II, eggs were produced commercially in small farmyard flocks, rarely exceeding 400 hens, during the war there was an increased demand by the military for powdered egg products. Because of labour shortages, the number of small operations decreased while the remaining farms became large and more efficient. Today flocks of 400 000 layers are not uncommon and some exceed 1 million birds. The principal egg-producing nations are the United States, China, Russia and Japan (Hubbert *et al*., 1996). By 2005, world egg production had increased to 60 million tons with China as a leading producer (Fig.1). Table 3 shows selected egg producers globally.

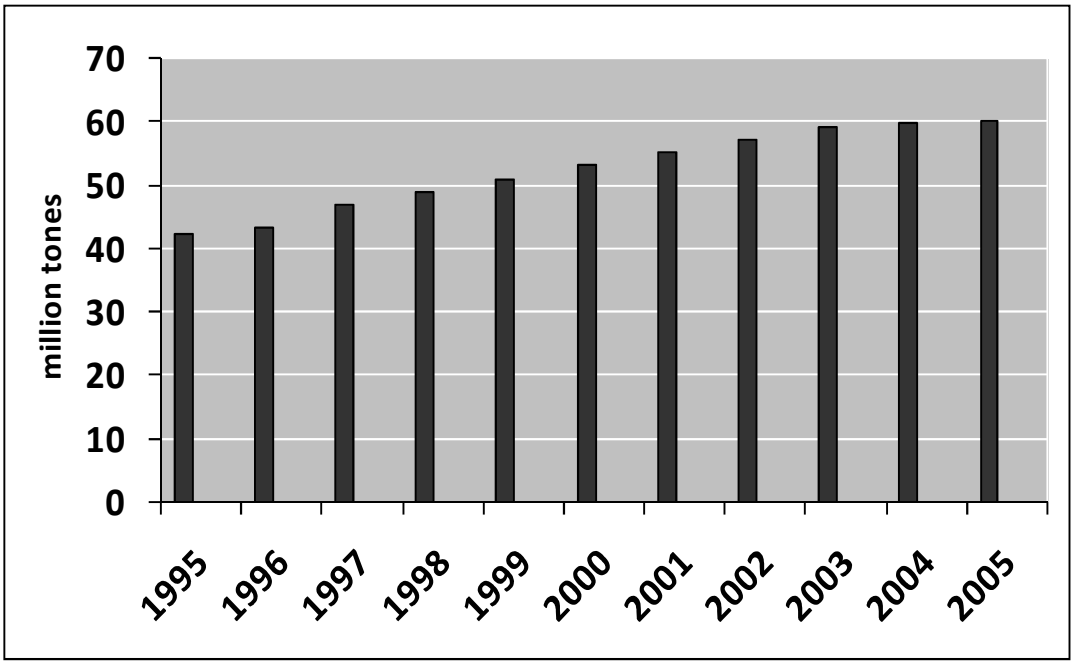


Figure1: World egg production

(Source: http://www.watt-digital.com)

Table 3: 2005 selected countries Egg Production (1 000 tones)
(Source: FAO Database)

Country	Production (1 000 tonnes)	Share (%)
China	24 348	41.1
USA	5 333	9.0
India	2 492	4.2
Japan	2 465	4.2
Russia	2 054	3.5
Mexico	1 906	3.2
Brazil	1 560	2.6
France	1 045	1.8
Indonesia	876	1.5
Turkey	830	1.4
World	59 233	100.0
Developed countries	19 170	32.4
Developing Countries	40 063	67.6

2.2 The Sudanese Poultry Industry

The total poultry population of Sudan is estimated at 45 million birds. The commercial sector contains around 30 million birds of which about 20 million are egg producing chickens. In 2003, 30.5 million chickens were slaughtered, equivalent to 30.5 metric tonnes chicken meat (www.evd.nl). Poultry producers can be divided into three main groups: household poultry keeping (Fig. 2), traditional open house poultry farming (Fig. 3) and modern intensive poultry farming (Fig. 4). The number of farms in Khartoum State in each category is shown in Table 4.

Table 4: Poultry farms in Khartoum State:

Locality	Total number of farms	
	Open system	Intensive closed system
Khartoum	316	2
Khartoum North (Bahry)	171	6
Omdurman	30	2
TOTAL	**517**	**10**

The two most important constraints to household poultry keeping and traditional open house producers are inadequate health care and inappropriate housing. Most poultry farms suffer from Newcastle disease and infectious bursal disease in broilers which markedly decrease the productivity on these farms (Khalafalla *et al.*, 2001). The small open house producers cease production during summer as a result of high ambient temperature (Fig. 3), which negatively affects productivity. Heat stress begins when the ambient temperature climbs above 27°C and is readily apparent above 30°C.

Figure 2: Household poultry keeping

Figure 3: Traditional open-house poultry farming

Intensive poultry keeping, as an agri-business, started in Sudan in the middle of the 1970's when the Sudanese Kuwaiti Company established a poultry farm south of Khartoum. The modern intensive broiler operations keep broilers in an evaporated cooled housing system which enables them to produce all year round. Only the largest broiler producers have an integrated operation including parent stock, hatchery and slaughterhouse. Broiler producers without a hatchery buy one-day-old chicks from local hatcheries or import one-day-old chicks; mainly from Egypt and more recently from EU countries due to the avian influenza (H5N1) outbreaks in Egypt (Ministry of Agriculture and Animal Resources and Irrigation, Khartoum State, 2005).

A common phenomenon in the Sudanese poultry market is the fluctuation in the supply of chicken. During the hot season there is a substantial drop in supply. This is mainly due to the unfavourable circumstances for broilers in open housing systems which causes poor growth results and high mortality. The drop in supply affects the prices of poultry meat during the hot season (www.evd.nl).

Figure 4: Modern intensive poultry (layer) keeping

3. Antimicrobials

3.1 Discovery of antimicrobials

Antimicrobial drugs have greatly enhanced human life expectancy, reduced mortality, and improved quality of life and almost won the war against many infectious diseases. An antimicrobial is a substance that is able to inhibit or destroy micro-organisms, with the largest group being those that are effective against bacteria (Prescott *et al.,* 2000). It was the discovery by Fleming in 1929 of the antibiotic penicillin, a fungal metabolite, and its later development by Ernst Chain and Howard Florey during World War II that lead to the antibiotic revolution with the subsequent discovery and development of many other classes of antibiotics. Antibiotics are the "miracle drugs" that are extensively used for the treatment and prevention of infectious diseases in humans and pets, as well as in food-producing livestock, poultry and fish. Today, antibiotics play a major role in modern agriculture and livestock industries and their use has been on the rise in many developing nations

(Sarmah *et al.,* 2006). The Centres for Disease Control and Prevention (CDC) estimates that approximately 22 700 Kg of antibiotics are produced in the United States alone each year, with roughly 40% used in agriculture. Europe gradually started decreasing the use of antibiotics in food producing animals, especially performance enhancers, Sweden and Denmark banned avoparcin in 1986, followed by the European Union (EU), in 1995. In 1999, the growth-promoting use of bacitracin, spiramycin, tylosin and virginiamycin were banned in the EU (Phillips, 1999).

Previously, in developing countries antimicrobial drugs were used as performance enhancers on a limited scale, nowadays, many developing countries such as India, China and South Africa use huge quantities of antibiotics as growth promoters. Many antimicrobial drug classes are used in animals for prophylaxis and therapy. This use tends to increase where farm management is not optimum or when endemic diseases are not properly controlled. Several guidelines are available for appropriate use of antimicrobial drugs in animals, but very little is being done in developing countries (Byarugaba, 2004).

3.2 Antimicrobials used in the global poultry industry

Antibiotics have been widely used in the poultry industry since their discovery more than 50 years ago. They represent an extremely important tool in the efficient production of animal products such as meat and eggs (Phillips *et al.*, 2004). They are used by the poultry industry and poultry veterinarians to enhance growth and feed efficiency and reduce bacterial diseases (Donoghue, 2003).

Antimicrobial classes used as therapeutics in the poultry industry include: aminoglycosides, tetracyclines, β-lactams, fluoroquinolones, macrolides, polypeptides, amphenicols, sulphonamides and trimethoprim (Stolker & Brinkman, 2005).

3.2.1 ß-Lactams (cephalosporins and penicillins)

Penicillin G is an effective antimicrobial for Gram-positive bacterial infections in poultry. The Gram-negative bacteria causing respiratory tract infections in birds, namely *Pasteurella multocida, Avibacterium paragallinarum, Escherichia coli* and *Gallibacterium anatis* (previously called *Pasteurella anatis* causing septicaemic lesions in chickens*)* can also be treated with ampicillin and amoxicillin. However,

penetration of the respiratory tract with this hydrophilic antibiotic may be poor. The broad-spectrum β-lactams such as amoxicillin are more effective for Gram-negative infections such as *E. coli* airsacculitis. Ceftiofur is the only cephalosporin approved for use in poultry in the United States (Silvers *&* Spires, 2002). It is commonly administered with Marek's disease vaccine to day-old chicks (Kinney & Robles, 1994).

3.2.2 Aminoglycosides and Aminocyclitols

Three aminoglycosides are used in poultry: gentamicin, neomycin and streptomycin. Neomycin is commonly used to treat enteric infections and is administrated either in feed or water. Gentamicin is the most widely used aminoglycoside and it is used sub-cutaneously in day-old chicken or turkey chicks. Streptomycin is partially absorbed from the intestine and therefore can be used to treat systematic *E. coli* infections. Spectinomycin and hygromycin are the two poultry approved aminocyclitols. Spectinomycin is highly effective for *E. coli* infections when combined with lincomycin (Smith *et al.*, 2007).

3.2.3 Quinolones and Fluoroquinolones

Quinolones are an important group of synthetic antibiotics with bactericidal action that results from the selective inhibition of bacterial DNA synthesis. They are used in poultry against many Gram-negative bacteria (Stolker & Brinkman, 2005). The fluoroquinolones are second generation quinolones that are highly effective against Gram-positive, Gram-negative and *Mycoplasma* infections. Enrofloxacin a fluoroquinolone with a good respiratory tract distribution can eliminate *Mycoplasma gallisepticum* infection in laying hens. Its use is banned in the USA as it readily induces resistance to it in the zoonotic *Campylobacter* spp.

3.2.4 Tetracyclines

The tetracyclines are the most widely used antimicrobials in poultry. This is largely due to their affordability, a wide margin of safety and broad-spectrum (*Mycoplasma*, Gram-positive and Gram-negative bacteria) and intracellular activity. They are easily administered *en mass* in either feed or water. The three tetracyclines most commonly used in poultry are chlortetracycline, oxytetracycline and doxycycline (Smith *et al.*, 2007).

3.2.5 Polypeptides

Zn Bacitracin is the only poultry approved polypeptide antimicrobial. Zn Bacitracin is very effective for treatment of Gram-positive enteric infections such as enteritis caused by *Clostridium perfringens* (Hofacre, 1998). It is also used as a performance enhancer in broilers (Phillips, 1999).

3.2.6 Sulphonamides and trimethoprim

Sulphonamides are bacteriostatics that are used as veterinary drugs for prophylactic and therapeutic purposes; they also act as growth-promoting substances and are commonly administrated in drinking water as coccidiostats. Trimethoprim is a potentiator when administered together with sulphonamides as both act on different enzymes in the folic acid metabolic pathway (Balizs, *et al.*, 2003).

3.2.7 Amphenicols

Chloramphenicol (CAP) is active against a variety of pathogens. Although CAP was, previously, widely used in veterinary and human medicine, reports of aplastic anaemia in humans arising from its use led to its ban for use in food-producing animals throughout most of the world. Thiamphenicol and Florfenicol, which have structures similar to CAP were permitted as substitutes, the later is used to treat *E. coli* airsacculitis infections in poultry (Corcia, *et al.,* 2002).

3.2.8 Macrolides and Lincosamides

Erythromycin is most frequently used in poultry to treat *Staphyloccus aureus* arthritis. Tylosin and Tiamulin are considered to be highly effective in the treatment of *Mycoplasma* infections in laying hens to restore egg production and reduce transovarian transmission. The only poultry approved lincosamide is lincomycin, it is primarily used to treat infectious coryza and infectious synovitis. It is commonly used to treat *Clostridium perfringens* induced necrotic enteritis and also to enhance poultry performance (Smith *et al.*, 2007).

3.2 The use of antimicrobials as performance enhancers

The earliest evidence of the growth promoting effects of antibiotics became apparent in chickens exposed to small doses of chlortetracycline which grew more rapidly than non-exposed chickens (Stockstad, 1950). The growth enhancing effect of this broad-

spectrum antibiotic class seems to be more marked (Jukes & Williams, 1953) than those, e.g. bacitracin and virginiamycin, with a primarily Gram-positive spectrum (Jukes, 1955). However, tetracyclines are considered to have a negative impact on the commensal microflora of the intestine and therefore their use as performance enhancers is not recommended. In poultry, performance enhancers, such as bacitracin and virginiamycin can also control *Clostridium perfringens* infections, which are potentially fatal. Estimates suggest that the average benefit of such products is an improvement in feed conversion rate (FCR) of approximately 3%, with a range of 0-5 % (Bedford, 2000). The mechanism of action of antibiotics as growth promoters is related to interactions between the antibiotic and the gut microbiota, thus the direct effects of antibiotic growth promoters on the microflora can be used to explain decreased competition for nutrients and reduction in microbial metabolites that depress growth (Dibner & Richards, 2005).

Before the middle of 1980s in Europe, antibiotics which were authorized to be included in poultry feeds without a veterinary prescription were tetracyclines, avoparcin, flavophospholipol, avilamycin, bacitracin methylene dislicylate, zinc bacitacin, lincomycin, spiramycin and virginiamycin (Castanon, 2007). Because of the risk concerning residues of antibiotics in edible tissues and products that can produce allergic or toxic reactions to consumers and the potential risk for humans, the WHO (1997) and the Economic and Social Committee of the European Union (1998) concluded that the use of antimicrobials in food animals is a public health issue (Castanon, 2007). This led to the total banning of antimicrobials, with the exception of sulphonamides, as performance enhancers in poultry by the EU in 2006 (Anadon, 2006).

4. The Role of Antimicrobial Residues and Antibiotic Resistance in Food Safety

Residues in food of animal origin result from the feeding or application of antimicrobials, other therapeutic agents, pesticides and heavy metals in livestock (Oehme, 1973). In 1983, a group of internationally renowned experts convened jointly by the Food and Agriculture Organization of the United Nations (FAO) and WHO concluded that “illness due to contaminated food was perhaps the most widespread health problem in the contemporary world,” and “an important cause of reduced economic productivity”. In 1992, the U.N. Conference on Environment and Development recognized that food was a major vehicle for the transmission of

environmental contaminants, both chemical and biological, to human populations throughout the world, and urged countries to take measures to prevent or minimize these threats. In 2000, the World Health Assembly, the supreme governing body of the WHO, unanimously adopted a resolution recognizing food safety as an essential public health function (Unnevehr, 2003).

Residues may have a direct toxic effect on consumers, *e.g.*, allergic reactions in hypersensitive individuals (Dayan, 1993; Ormerod *et al.*, 1987; Woodward, 1991), or they may cause problems indirectly through induction of resistant strains of bacteria (Stolker & Brinkman, 2005). In humans, the triggering of allergic reactions in sensitized individuals by penicillin residues is well documented (Dewdney *et al.*, 1991). A rare fatal blood dyscrazia in sensitized individuals can also be triggered by chloramphenicol residues in food (Settepani, 1984).

Since the human outbreak of the zoonotic, multi-antimicrobial resistant *Salmonella typhimurium* DT104 in 1986, the use of antibiotics in food-producing animals has become a public health issue. The concerns are that not only could humans become infected with difficult to treat bacteria, but that commensal enteric bacteria such as *Enterococcus faecium* can transfer resistance to the intestinal bacteria of humans. Thus concerns about use of antibiotics in animals and their possible impact on human health cover two major issues: the antibiotic agent that are used and the way in which they are used.

The knowledge on the occurrence, fate and dissemination of antimicrobial residues and antibiotic resistant bacteria is increasing. However, a significant gap still exists in our understanding of the relationship between antibiotic residues, their metabolites and antibiotic resistant bacterial populations after their excretion. To avoid possible extinction, the bacteria have adapted their own defences against antimicrobials (Levy, 1992). The populations of bacteria with this ability tend to be enhanced when antimicrobials are used to treat disease and can lead to certain infections becoming untreatable *e.g.* multi-resistant *Mycobacterium tuberculosis* infections (Davies, 1997). Antimicrobials can also have a marked effect on commensal microflora resulting in either an increase in the antimicrobial resistance of these bacteria or replacement of the bacterial populations by more resistant bacteria (Levy, 1992). Of particular interest in the latter is the effect that the ingestion of food of animal origin may have on the intestinal microflora of humans, either via colonisation with multi-resistant bacteria or the effect of antimicrobial residues (WHO, 2000) (Fig.5).

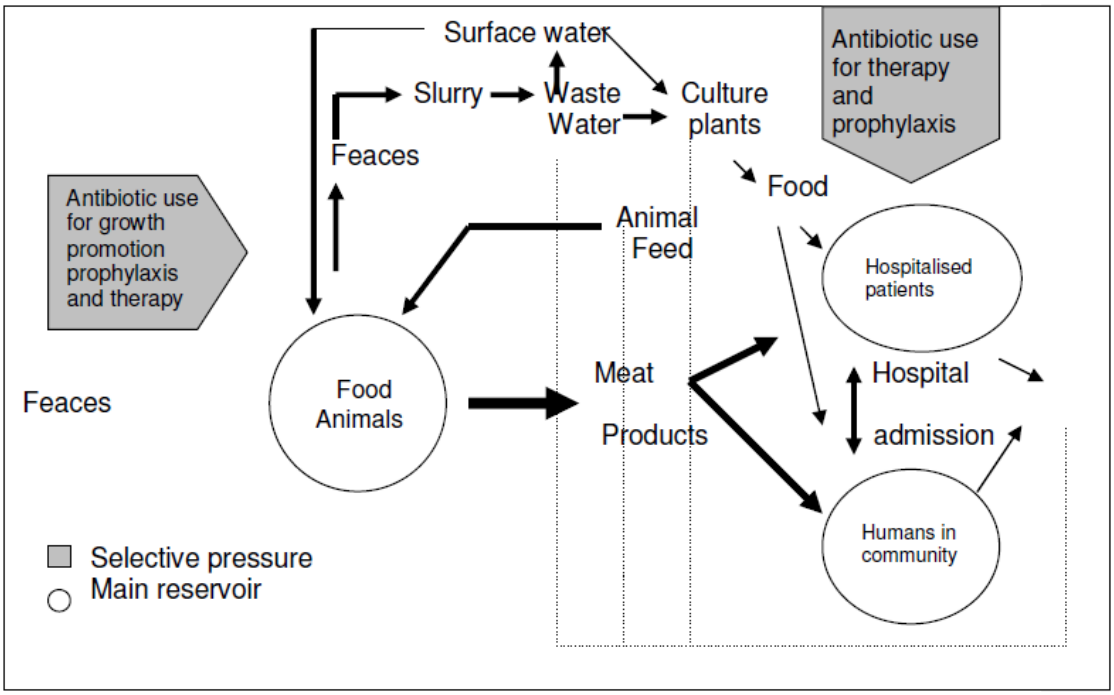


Figure 5: Schematic representation of the use of antimicrobials in human clinical and veterinary medicine (Nawaz *et al.*, 2001)

The worldwide increase in antimicrobial resistant bacteria (Morris & Masterton, 2002) has led to social and scientific concern that the over-prescription and misuse of human prescribed antibiotics and the increased and widespread use of sub-therapeutic doses of antibiotics in agriculture are responsible for this trend (Smith *et al.*, 2002).

5. Rules and Regulations of Antimicrobials Used in Poultry Production

Governments in many countries have established new institutions, standards, and methods for regulating food safety and have increased investments in hazard control. The policies of antimicrobial residues control in developing countries are mainly aimed at addressing food safety issues. The regulation of the use of antimicrobials in food animals vary from country to country. For example the European Union (EU) has strictly regulated control of the use of veterinary drugs, including performance enhancing agents in food-animal species by issuing several Regulations and Directives.

The primary consumer safety consideration is addressed via Maximum Residue Limits (MRL), established by Council Regulation EEC/2377/90. The MRL defines the maximum level of residues of any component of a veterinary medicine that may be

present in foodstuffs of animal origin without presenting any harm to the consumer. The EU definition is virtually the same as that adopted by the Codex Alimentarius Committee for Residues of Veterinary Drugs in Foods and the approach to evaluation of residues of veterinary medicinal products within the European Union is very similar to that employed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) that evaluates for Codex Alimentarius.

The MRL for any substance is determined from data submitted by manufacturers or suppliers to the Safety of Residues Working Party, a sub-committee of the Committee for Veterinary Medicinal Products (CVMP). This determination is ratified by the CVMP and adopted into law by a Regulatory Committee in the form of Commission Regulations. For veterinary medicines used in food animal species in the EU the MRL is determined by an iterative process from a range of safety data, the most important of which is the Acceptable Daily Intake (ADI). The ADI is defined as the level of a substance that may be consumed daily without presenting a hazard to the consumer. It is based on a suitable no observed effect level (NOEL) or from observations in humans, divided by a safety factor, often 100. (Woodward, 1996; European Commission, 2001). Even if efforts have been made to harmonize the MRL at world level [under the aegis of World Trade Organization (WTO)] and the Codex Alimentarius), it must be acknowledged that the latter still strongly differs from one geographical area to another. Thus, due to these MRL differences, the same chlortetracycline-based medicine is granted, for a given species, a withdrawal time of 7 days in Canada and zero in the USA.

Agricultural use of antimicrobials in the USA and Canada is also regulated. There are three categories of use: as feed antimicrobials, as over-the counter drugs and as veterinary prescription drugs. Feed antimicrobials include performance enhancers, coccidiostats and therapeutic antimicrobials and are licensed for specific purposes in the case of broilers, young pigs or calves or feedlot cattle (Prescott, 1997).

In the United Kingdom (UK) and other European Union (EU) countries, antimicrobials are authorized as either veterinary medicinal products or zootechnical feed additives. Veterinary medicinal products and performance enhancers are subject to assessment for safety, emergence of antimicrobial resistance, cross-resistance to therapeutic antimicrobials and transferable resistance (Rutter, 1997). Regulating the use of these veterinary drugs in the UK is primarily the responsibility of the Veterinary Medicines Directorate (VMD) (Al-Ghamdi *et al.*, 2000).

China has regulated the use of antimicrobials in animal feed since 1989 and only non-medical antibiotics are permitted as feed additives. Antimicrobials used include monensin, salinomycin, destomycin, bacitracin, colistin, kitasamycin, enramycin, and virginiamycin (Jin, 1997). In Russia: bacitracin, grizin, flavomycin, and virginiamycin are registered for use as performance enhancers (Panin *et al*., 1997).

Most African countries recognize the importance of food hygiene particularly with regards to meat safety, and have laws and regulations that govern food production and processing including such aspects as meat inspection and drug residue levels. However, enforcement of such laws and regulations is usually poorly done. Farmers, for instance, can buy veterinary drugs and administer them without a prescription. Firstly, financial resources are usually inadequate for law enforcement agencies to carry out their work effectively. Secondly, support facilities such as laboratories are usually ill-equipped in both equipment and personnel. This is further compounded by the fact that in terms of prioritization for resource allocation, livestock production compared to public health issues is a low priority for most African governments (FAO/WHO Regional Conference, October 2005).

Although, the use of veterinary drugs in The Sudan is regulated by the Pharmacist and Toxics Act, approved in 2001, there are no specific regulations for antimicrobial usage in food producing animals. The act mentioned above deals with the licenses of drug sale and the authority of veterinarian on veterinary drugs.

6. Antimicrobial residues monitoring and surveillance

6.1 Monitoring and Surveillance

In order to address and manage food safety, it is imperative to have knowledge on the current situation and trends with regard to the occurrence and spread of residues in the food chain. This knowledge needs to be updated continuously so that appropriate responses can be prepared. Activities involved in such a system are gathered under the terms 'monitoring' and 'surveillance'. Monitoring is the performance and analysis of routine measurements, aimed at detecting changes in the environment or health status of populations, while, surveillance can be defined as the ongoing systematic collection, collation, analysis and interpretation of data, followed by the dissemination of information to all those involved so that directed actions may be taken (Wong *et al*., 2004).

The presence of inhibitory substances and residues of veterinary drugs in food should be continually monitored in both veterinary and human medicine (Popelka *et al.*, 2001). Most livestock products are subject to some form of monitoring and surveillance for antibiotic residues to eliminate health risks to consumers, as well as a negative impact to the environment and the technology of food production (Lohajova *et al.*, 2006). Monitoring activities are designed to provide profile information on the occurrence of residue violations in specified animal populations on an annual national basis. The focus is on violations; therefore, only compounds with tolerances or action levels are included in the monitoring plan (Cordle, 1988).

Many developed countries monitor and survey tissues from animals for antimicrobial residues. In the USA, the United States Drug Administration (USDA) and Food Safety and Inspections Service (FSIS) monitor and surveys for antimicrobial residues and operates under the Federal Meat Inspection Act, the Poultry Products Inspection Act, and the Egg Products Inspection Act (www.fsis.usda.gov). Australia has had a programme to monitor antibacterial residues since the 1960s, the National Residue Survey (NRS), and a programme for surveillance of high-risk animal categories, the National Antibacterial Minimization (NARM) programme, since 1990 (Nicholls *et al.*, 1994).

6.2 Antimicrobial residues analysis

Analysis of antimicrobial residues (AR) in food of animal origin is a developing field of research. The methods used for analysis and their efficiency depend mainly on the final results gained from the test used. Regulatory bodies are interested in the following when a test is evaluated:

- Are animals treated legally with antibiotics?
- Is the concentration of residues below the MRLs?
- Are the samples compliant?
- Is the law respected? (De Brabander *et al.*, 2009).

The availability of simple and reliable screening systems for the detection of antibiotics is an essential tool to ensure food safety (Lohajova *et al.*, 2006). Methods for surveillance testing of antimicrobial residues may be subdivided into screening methods and confirmatory methods. Screening methods are tools that are used to

detect the presence of an analyte or class of analytes at the level of interest. These methods have the capacity for high sample throughput and are used to sift large numbers of samples for potential non-compliant (or positive) results. They are specially designed to avoid false compliant results, in other words they would be highly sensitive, but may have a low specificity (De Brabander *et al.*, 2008). A screening method should allow the detection of all the suspect samples, preferably using a simple routinely applicable procedure (Aerts *et al.*, 1995). In addition to that, control laboratories face a large number of samples, with a variety of analytes that have to be analysed in relatively short periods of time. This means that high throughput methods with low cost and high repeatability must be available (Van Peteghem *et al.*, 2001). These methods must be able to detect an analyte or class of analytes at the level of interest. Some false positives (false compliant) are acceptable, as they will be further submitted for confirmatory analysis but the method must avoid or reduce to a minimum the number of false negative results (non-compliant) because they will not be further analysed.

There are different techniques available for the screening of residues in animal foods as shown in Table 5 and all these stated screening techniques have advantages and disadvantages shown in Table 6 (Fidel & Milagro, 2006).

Methods of analysis of antimicrobials may also be grouped into three classes on the basis of the principle used: microbiological, immunochemical, or physico-chemical. Microbiological methods for detection of antimicrobial residues are fast screening tests. Immunochemical methods fall into two groups, immunoassay and immunoaffinity chromatography (IAC).

Table 5: List of main techniques available for screening antimicrobial residues

Microbiological methods	Immunological methods	Chromatography methods
STOP test (Dey *et al.*, 1998)	ELISA test kits (Stead, 2000)	High performance thin-layer chromatography (HPTLC) (Stead, 2000)
FAST test (Dey *et al.*, 1998)	Radioimmunoassay (Stead, 2000)	High performance liquid chromatography (HPLC) (Stead, 2000)
CAST test (Dey *et al.*, 1998)	Multiarray (Toldra & Reig, 2006)	Mass spectrophotometry
Four Plate Method (FPM) (Kilinc & Cakli, 2008)	Biosensors (Stead, 2000)	Gas Chromatography
New Dutch Kidney Test (NDKT) (Nouws *et al.,* 1988)		
Premi®Test[1] (Cantwell & O'Keeffe, 2006)		

Confirmatory methods are methods that provide full or complementary information enabling the analyte to be identified unequivocally, and if necessary quantified, at the level of interest. Confirmatory methods must fulfil the criteria listed in Commission Decision 2002/657/EC (Verdon *et al.,* 2006) and must be based on molecular spectrometry providing direct information concerning the molecular structure of the analyte under examination, such as GC-MS and LC-MS.

Immunoassays can be rapid, selective, and sensitive and have proved of considerable utility in some areas of residue analysis. Physico-chemical methods are based on chromatographic purification of residues followed by spectroscopic quantification such as UV, fluorescence or MS detection (De Brabander *et al.*, 2009). Using bioreceptors from biological organisms or receptors that have been patterned after biological systems, scientists have developed a new means of chemical analysis that often has the high selectivity of biological recognition systems. These biorecognition elements in combination with various transduction methods have helped to create the rapidly expanding fields of bioanalysis and related technologies known as biosensors and biochips (Vo-Dinh & Cullum, 2000).

[1] DSM (DSM Food Specialties, P. O. Box 1, 2600 MA, Delft, The Netherlands.

Up to date, screening for antimicrobials has been done with microbiological inhibition tests, and it is unlikely that these tests will be replaced by other techniques in the near future. Indeed, such tests are cheap, fairly broad-spectrum and permit analysis of a large number of samples in a short time compared to immunological and chromatography methods, provided that no extraction is included in the procedure. Microbiological screening relies on the common property of all antibacterials: they inhibit growth of microorganisms. Inhibition tests have been considered non-specific, but the microorganisms used as test bacteria are of course not equally sensitive to all antibiotics. As a consequence, they detect some substances better than others. In the past, many efforts have been done to develop a simple method that detects a large range of antibiotics (Žvirdauskienė & Šalomskienė, 2007). Combinations of different test bacteria, each in an optimal medium, are now considered as the best tool to detect a large range of antibiotics up to the MRL levels in animal tissues; for example the optimal or very good detection capabilities of β-lactam antibiotics and cephalosporins are obtained with *Geobacillus stearothermophilus* bacteria (Gaudin *et al.*, 2004).

Microbial screening tests include

1. The STAR method

Five plates with different combinations of media and microorganisms have been selected in order to detect most of the representatives of all the main antibiotic groups. This test has been called STAR and is used for screening for antibiotic residues.

2. The FAST Test

In 1989, an in-plant screen test was developed to improve the capability of the Antibiotic Residue Detection Program of the USDA/FSIS. The test, which uses *Bacillus megaterium* (ATTC 9885) as a test organism, was called the Fast Antimicrobial Screen Test (FAST). It was able to detect a wide range of antimicrobials and sulphonamides. It was implemented in cattle and swine abattoirs (Dey *et al.,* 1998). As stated by Schneider & Lehotay (2008), the test is currently used by FSIS but it has never been used to detect AR in eggs. Therefore it was decided to test this bacterium using the broth microdilution and agar dilution tests as described by the Clinical Laboratory Standards Institute (CLSI, 2008) to determine the minimum inhibitory concentrations or in other

words the lowest detectable concentration of a range of antimicrobials. This method is based on the principle that if animal tissue contains a residue of a previously administered antimicrobial, fluid from the tissue will inhibit the growth of a sensitive organism on a bacterial culture plate. In this test, cotton swabs saturated with tissue fluid from a suspected carcass are placed on a culture plate whose surface has been seeded with spores of a harmless organism *Bacillus megaterium* (Dey *et al*., 1998).

3. The Premi®Test

This test is based on inhibition of growth of the test microorganism *Geobacillus stearothermophilus var.calidolactis*, a thermophilic bacterium highly sensitive to many antibiotics and sulphonamides (Lohajova *et al.*, 2006).

In addition, there are several microbial tests such as the STOP test and Delvotest-P which are used for residue detection in milk, and the Live Animal Swab Test (LAST) for detection of potential antibiotic residues in meat before the animal is slaughtered (Seymour *et al*., 1988).

To avoid the long incubation period inherent to microbiological inhibitor tests, enzymatic, receptor and immunological tests were developed for a rapid screening of foodstuffs of animal origin for the presence of antimicrobials. The first test developed for that aim was the Penzym test mostly specific for ß lactams, an enzymatic carboxypeptidase colorimetric test, giving a result in 20 minutes (Knight *et al*., 1987). Several screening tests with the total test time below 10 minutes (Receptor test SNAP, Charm MRL Beta-lactam Test (ROSA) and Beta-s.t.a.r and immunoassay Lactek and Parallux) became commercially available for monitoring of raw milk on beta-lactams (Žvirdauskienė & Šalomskienė, 2007). More recently, some rapid tests (Charm MRL-3) were adapted to give a test result within three minutes allowing screening of milk at the farm before collection (Reybroeck & Ooghe, 2008).

7. STUDY AIMS AND OBJECTIVES

Although poultry eggs are an important source of animal protein in Sudan, there are no regulations governing the use of antimicrobials in poultry production. It is possible that the Sudanese public is not aware of the inherent risks that are associated with the eating of eggs that may contain residues. Furthermore, as eggs are mainly consumed locally, the study aims to investigate the farmers and Sudanese government awareness and understanding of international regulatory procedures for the authorization, use and control of antimicrobials, or the standards for residues. One of the hypotheses of the study, there is insufficient veterinary service and extension programmes that would serve to disseminate this information, supporting poultry farmers. In addition to that, the lack of knowledge and understanding may had led to a widespread misuse of antimicrobials by the poultry producers in Sudan. Thus it is critical that scientifically based data be collected and provided as evidence to assist in farmer and public education, as well as provide a basis for which the Sudanese regulatory authorities can formulate regulations that are suitable for their country.

In this study an in-house test using *Geobacillus sterothermophilus var. calidolactis* as a test organism was used to screen for antibiotic residues in egg samples. Since there were concerns regarding the ability of one test to detect all the antibiotics that may be found in the eggs of poultry, it was decided to include in addition to *Geobacillus (Bacillus) stearothermophilus* (ATCC7953) in the testing procedure. *Bacillus megatarium*, *Staphylococcus aureus* (ATCC29213) and *Escherichia coli* (ATCC 25922) would for some of the bacteria act as quality controls to ensure that the MIC tests performed optimally (CLSI, 2008).

Table 6: Main advantages and disadvantages of some antibiotic screening tests
(Source: Modified from Fidel & Milagro, 2006)

TEST	ADVANTAGE	DISADVANTAGE
ELISA test kits	Easy to use	Increased costs since 2002 (more than €650 per kit)
	Available kits for a good number of specific compounds (e.g. clenbuterol, zeranol, etc.)	Limited storage (few months) under refrigeration
	Availability of kits for families of compounds (i.estilbenes, sulphonamides, etc.)	Expensive and need for waste disposal
	Large number of samples (42) per kit for a single analyte	Interferences giving some false positives
	Reduced time (few hours) to obtain the results: about 2–2.5 h for most kits.	Only one kit per residue searched
	High sensitivity & high specificity	
	Possibility to use within the food-processing facility	
Biochip array biosensors	Easy to use	High initial investment (equipment)
	Results available in short time	High operative costs chips and equipment ()
	Multiples residues analysed in one shot (as many as chips in an array)	Analysis restricted to available chips
	Full automatisation: higher productivity	
	High through-put technique: up to 120 samples per hour and array	
HPTLC	High number of samples for a single analyte	Expertise and specialised equipment required
	Reduced time (few hours) to obtain the results	Need of sample preparation (extraction, filtration, etc.)
	Possibility of automatisation for higher productivity	Interferences giving some false positives
	Sensitive	Only one thin-layer plate per residue searched
	Specificity depending on the detection technique	
	Separated sample can be recovered for further confirmatory analysis	
HPLC	Short time (few min/sample) to obtain the results	Expertise required
	Sensitive	Need of sample preparation (extraction and filtration, addition of internal standard, etc.)
	Specificity depending on detector	High initial investment (equipment) Cost of column
	Automatisation leading to higher productivity	
	Possibility to find more information from spectra when using diode array detector	
Microbial methods	Can be used for large scale surveillance programmes	Difficult to standardize preparation procedure (FPT)
	Slowest of the methods	Some test could not insure MRLs compliance (NDKT)
	Broad spectrum	Sample preparation required to remove false positives due to protein bacterial inhibitors i.e. lysozyme in eggs.
	Easy to use	Low sensitivity
	Economical	
	Basic laboratory equipment	

CHAPTER III- MATERIALS AND METHODS

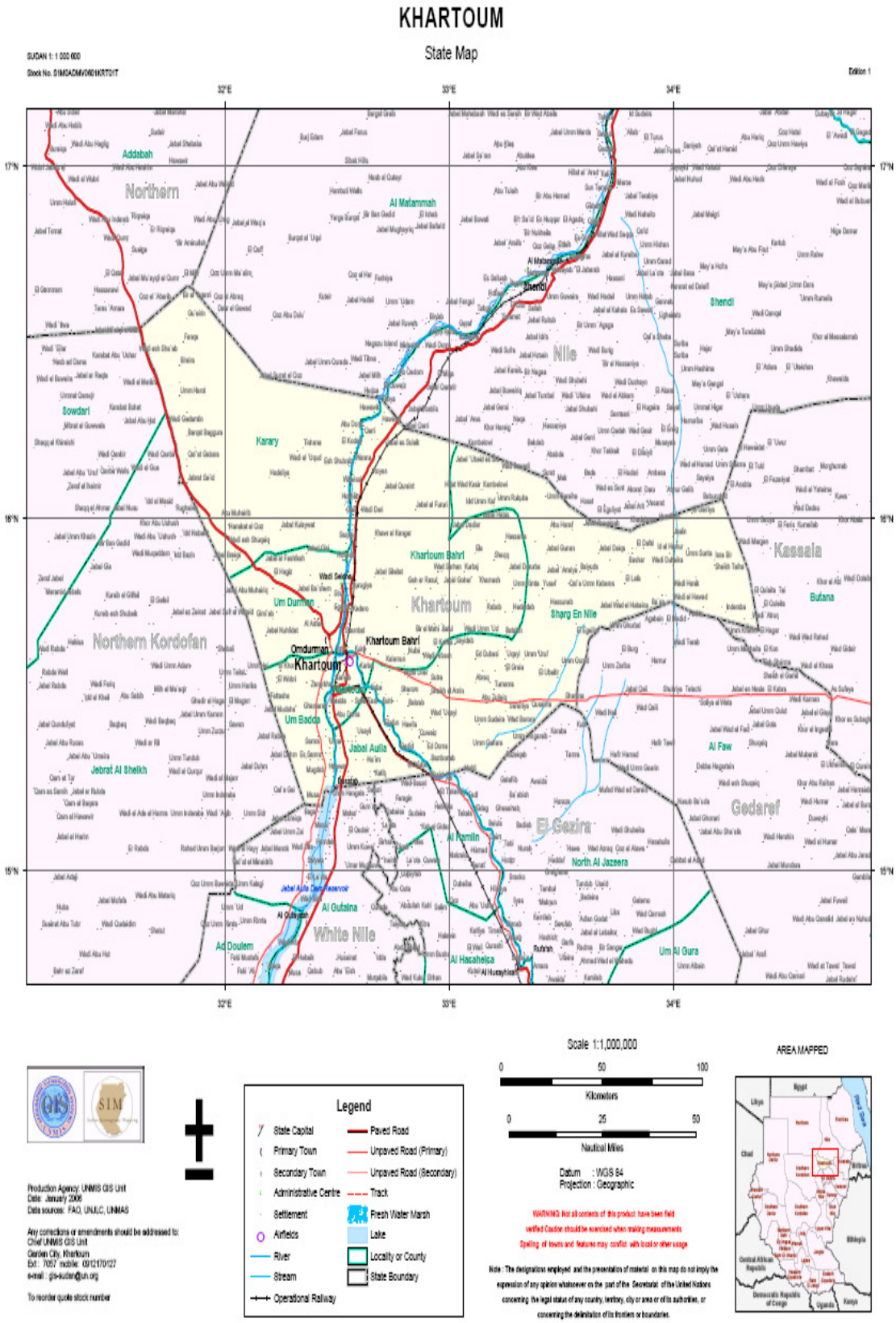
1. Introduction

A survey focusing on the use of antibiotics was carried out on commercial layer farms in Khartoum State, Sudan during late 2007 and early 2008. The survey was conducted in Khartoum State (Figure 6) because it produces approximately 90% of the country's eggs and more than 20% of the Sudanese population resides in Khartoum State. A census for all commercial layer farms in Khartoum State was carried out in late 2007 and January 2008. A questionnaire survey was conducted to collect data on the risk factors associated with antimicrobial residues. About 70% of the laboratory analytical work for the presence of antimicrobial residues was done at the Bacteriology Laboratory of the Department of Veterinary Tropical Diseases (DVTD), Faculty of Veterinary Science University of Pretoria and the rest of the samples were analysed in the Research Laboratory of the Microbiology Department, Faculty of Medical Laboratories, Sudan University of Science and Technology.

2. Census of Layer Farms in Khartoum State

A census to determine the size and structure of commercial layer farms in Khartoum State, Sudan was conducted between December 2007 and January 2008. The sampling frame consisted of all known layer farms in the three localities of Khartoum State (Appendix VI). Identification of farms was based on data from the State Ministry of Agriculture and Animal Resources and Irrigation (Internal publications, 2005), day old chick suppliers (Inmaa Company) and from poultry veterinarians. In addition to that, information from farm owners about other farms was quite beneficial. The lack of formal data concerning the names and boundaries of each area within a locality was a reason to depend on personal interviews and unpublished reports, beside the guidance of the field veterinarians and farm owners.

The sampling unit at the time of the survey was a layer farm producing eggs or pullets or layer farms not currently in production. For each area the following was recorded: the location of each farm; the number of farms in each area; the number of poultry houses per farm; the capacity of each poultry house and the farming system used.



Source: UNMIS GIS UNIT
www.unsudanig.org/library/mapcatalogue/north/data/planning/Map%20267%20Khartoum%20State%2018x17.pdf

Figure 6: Khartoum State Map

3. Questionnaire Survey

A structured questionnaire delivered in the form of an interview was designed to collect data (risk factors) associated with antimicrobial residues. The aims and objectives of the questionnaire were to collect data on farm management procedures used on each layer farm, beside investigating local knowledge and understanding of issues surrounding antibiotic usage in food-producing animals.

The sampling frame for the questionnaire was all known layer farms that were producing eggs at the time of the survey in the three localities of Khartoum State (Fig. 6). Data was obtained on antibiotics recently used, antibiotics used in the last three months, reasons for using the antibiotics, diseases currently on the farm, diseases recorded in the last three months, withdrawal period, how antibiotics were stored, quality control and policies of antibiotics usage in the poultry industry. Perceptions on the public health risk of antibiotic residues in table eggs were also investigated. In addition to that, the farming system, chicken breed, breeding system, number of chicken per house, number of houses per farm and current age of the flock was recorded (Appendix VI). To determine the antibiotics used at the time of the survey, labels and empty bottles of antibiotics were collected and the data recorded.

All elements of the questionnaire were categorical variables, structured as closed ended questions. The only continuous questions were age of the flock, number of chickens per house and number of houses per farm, these were also later coded and and recorded as categorical variables.

The questionnaire was not subjected to pretesting or repeatability testing, it was designed in the English language and the contents were translated to Arabic during the interview. The validity of the questionnaire was assessed by comparing the results of the questionnaire with a reliable criteria; i.e. the related questions in the same questionnaire form and known facts such as the absence of rules and regulations of antibiotic usage in The Sudan. The survey was done in April 2008 covering the whole State and all information needed in the questionnaire form was captured through direct interview conducted by the study. The respondents were the owners or managers of the farms.

4. Sampling

4.1. Sampling design

The sampling frame comprised three localities of Khartoum State, Khartoum, Khartoum North (Bahry) and Omdurman. Egg samples were collected from farms on

four occasions namely, January (winter), April (mid summer), June (start of rainy season) and August (rainy season) 2008 to take seasonal and environmental changes as well as treatment regimens into account. Only three eggs were collected randomly from each poultry house (sampling unit) regardless of the number of houses on the farm. The sampling was based on the assumption that mass treatment is the method of choice when treating layers and therefore AR should be equally present in all the eggs in the house at a specific time. There was no adequate information on the farm locations and distribution in the State. Therefore, the information on farm locations was dependent on the census done in this study prior to the sampling process (see above).

Each periodic egg collection was done as a separate survey as it was difficult to resample the same farm in different periods for several reasons, including; the same farm was now being used as a layer rearing farm, the farm was no longer used for breeding layers, broilers were being reared on the same farm, the owner was not willing to participate again and the owner having left the poultry business for economical reasons.

4.2. Sample handling and preservation

Each egg sample was labeled with a permanent marker and the date of collection, farm origin and the entire house number was clearly stated. The eggs were transported in carton storage trays at room temperature and were processed within 72 hours of collection. In a clean, dust-free room, the eggs were sprayed with 70% ethanol, allowed to air-dry and then the tip of the shell at the air-sac removed with a scissors using aseptic technique. The contents were then poured into Whirl plastic bags[1] labeled with farm origin, poultry house number and date of collection.
The samples were stored in a freezer (-18℃) until processed in Sudan or couriered to South Africa in batches corresponding to seasonal sampling. Prior to sending samples to South Africa an export permit was issued from the Chief Veterinary Officer of Sudan, Ministry of Animal Resources and Fisheries (Appendix V) and an import permit was issued from Department of Agriculture, South Africa (Appendix II). After they had arrived in South Africa the samples were gamma-irradiated by Isotron South Africa (Appendix IV) at 2.4 Kilo Gray (KGy) to sterilise the contents and prevent importation of potential pathogens into South Africa such as Avian Influenza (AI).

[1] Guth Group (Pty) Ltd., 79 st Georges Street, Newlands, 2114, South Africa.

Samples were then transported, still sealed, to the Bacteriology Laboratory of Department of Veterinary Tropical Diseases (DVTD), Onderstepoort which also serves as a quarantine area, where they were stored in a freezer (-18ºC) until processed. It was stated by Farkas 2006, that irradiation between 2 and 4 (KGy) has a good effect on decontaminating egg samples, without affecting the quality of the samples.

5. Laboratory analysis

5.1. Validation of newly developed In-House analytical Procedure Used for Screening Antibiotic Residues in Commercial Layer Eggs in Khartoum State; Sudan[1].

5.1.1. The Kirby-Bauer test:

To test whether the bacteria were effective against a wide range of antimicrobials, it was decided to first perform the Kirby-Bauer disk diffusion test making use of standard operating procedures as described by the CLSI, 2008. The bacteria *Bacillus megaterium* (ATCC 9885), *Staphylococcus aureus* (ATCC 29213) and *E. coli* (ATCC 25922) were checked for viability and purity by culturing colonies that had been frozen at -84℃ on Columbia blood agar[2] containing 5% citrated horse blood for 18 hours at 37℃. Phenotypic tests including Gram's stain, catalase, oxidase, spot indole tests and a range of sugar fermentation tests were then used to confirm the identity of the bacteria (Slepecky & Hemphill, 2006). The following procedure was repeated for each bacterium. A single colony on blood agar was picked up using a sterile cotton tipped swab and then diluted to a MacFarland standard of 0.5 in Normal saline containing 0.02% Tween 80. This swab was then used to coat a 90mm Petri dish[3] containing Mueller-Hinton agar[1] with the bacteria. A dispenser[2] was used to place the antibiotic discs. The antibiotic disks used[2] are shown in Table 7. The plates were then incubated in air at 37℃ for 18 hours and the zones of inhibition read using a caliper[4].

Table 7: Antibiotic used for Kirby-Bauer disk diffusion test5

No	Antibiotic	Concentration (µg)
1	Neomycin	30

1 Tests were carried out at the Bacteriology Laboratory of the DVTD, University of Pretoria, Faculty of Veterinary Science, Pretoria, South Africa; under supervision of Dr. J. Picard.

2 Oxoid Limited, Wade Road, Basingstoke, Hampshire, RG24 8PW, United Kingdom.
3 Orb Diagnostics, CC., P.O. Box 763, Edenvale, 1610, Johannesburg, South Africa.
4 BioMérieux South Africa Pty. 7 Malibongwe Drive, 2125 Randburg, South Africa.
5 Oxoid Limited, Wade Road, Basingstoke, Hampshire, RG24 8PW, United Kingdom.

2	Doxycycline	30
3	Poteniated sulphonamides	25
4	Lincospectin	109
5	Sulphamethoxyazole	25
6	Ampicillin	10
7	Enrofloxacin	5
8	Phosphomycin	50
9	Colistin	300 I.U.
10	Erythromycin	15
11	Gentamicin	10
12	Tylosin	15
13	Tiamulin	30

5.1.2. Minimum Inhibitory concentration (MIC)

The microbroth dilution test was performed on the following bacteria: *B. megaterium, S. aureus and Geobacillus (Bacillus) stearothermophillus var. calidolactis* ATTC 7953[1] using the method prescribed by the CLSI (2008). Two different tests were performed namely, one using only cation-adjusted Mueller-Hinton broth (CAMHB) as the growth medium and one using a 50% mixed egg yolk and albumin suspension in Mueller-Hinton broth. The latter was done to find out if egg contents had any effect on the MIC values.

5.1.2.1. Preparation of antibiotic dilutions

The following analytical grade[1] antimicrobials were tested using a 2-fold dilution series performed in 96-well microtitre plates[2]: ampicillin, trimethoprim, lincomycin, phosphomycin, tylosin, tiamulin, ciprofloxacin, doxycycline, enrofloxacin, oxytetracycline and sulphamethoxyazole.

Preparation of antibiotic stock solutions according to CLSI (2008) was performed for all selected antibiotics; the following formula below was used to determine the weight of antibiotics needed for a standard solution:

Weight (mg) = Volume (mL) of stock solution x Concentration (µg/mL) wanted

[1] Merck Chemicals (Pty) Ltd., 259 Davidson Road, P. O. Box 1998, Halfway House, 1685, South Africa.
[2] Sterilab Services cc., 1 Foreman St., 1620 Kempton Park, South Africa.

Potency of the drug (µg/mg)

The potency of the drug was calculated by using the product of the purity of the product (on the certificate of analysis) and relative molecular weight of the active molecule. The volume of the stock solutions was set at 100ml and was equivalent to 10X the working solutions (shown in Table 8). Working solutions were calculated and prepared using sterile, de-ionised water, with the exception of ampicillin where phosphate buffered saline (PBS, pH 7.2) was used. For phosphomycin 1% fructose was added to the solution. Furthermore a 1:19 dilution of trimethoprim:sulphamethoxyazole was made.

Using a multichannel pipette[1] 100µl of CAMHB was added to each well of a 96-well round-bottomed plate[2]. A 100µl of the working antibiotic solution of each antimicrobial was added consecutively to a well of the first column and then serially diluted from well 1 to well 12 by 2-fold dilution with the final 100 µl being discarded (Table 9). Note that 2 wells in the last column were allocated for a growth and broth control respectively.

Table 8: Antibiotic preparation

Antibiotic	Solvent for stock solution	Weight of Stock solution in 100mL solvent	Working solution 4X starting concentration

1 Biohit Plc, Laippatie 1, 00880, Helsinki, Finland.
2 Sterilab Services cc., 1 Foreman St., 1620 Kempton Park, South Africa.

		(mg)	µg/ml
Ampicillin	PBS (pH 7.2)	46.435/mL	16
Colistin	water	640	64
Doxycycline	water	128	32
Enrofloxacin	Dropwise 1M NaOH until dissolution and then water	160	2
Erythromycin	90% alcohol	128	16
Phosphomycin	Water containing 10% fructose	512	1024
Gentamicin	water	41.375mL	32
Lincomycin	water	128	16
Spectinomycin	water	256	16
Neomycin	water	128	32
Trimethoprim	Dropwise 1M acetic acid until dissolution and then water	640	16
Spectinomycin	water	256	128
Sulphamethoxyazole	Dropwise 1M boiled NaOH until dissolution and then water	1024	1024
Tiamulin	water	128	32
Tylosin	water	128	32

Table 9: Dilutions and example of the recording sheet of MIC measured in µg/mℓ

Enrofloxacin	16	8	4	2	1	0.5	0.25	0.125	0.0625	0.03	0.015	0.008
Norfloxacin	32	16	8	4	2	1	0.5	0.25	0.125	0.0625	0.03	0.015
Neomycin	64	32	16	8	4	2	1	0.5	0.25	0.125	0.0625	0.03
Tylosin	64	32	16	8	4	2	1	0.5	0.25	0.125	0.0625	0.03
chlortetracycline	1028	512	256	128	64	32	16	8	4	2	1	0.5
florfenicol	128	64	32	16	8	4	2	1	0.5	0.25	0.125	0.0625
spectinomycin	128	64	32	16	8	4	2	1	0.5	0.25	0.125	growth con
ampicillin	16	8	4	2	1	0.5	0.25	0.125	0.0625	0.03	0.015	broth contr
gentamicin	16	8	4	2	1	0.5	0.25	0.125	0.0625	0.03	0.015	0.008

5.1.2.2. Bacterial preparation

A bacterial suspension was made of each purified bacterium in the same way as for the Kirby-Bauer test. An exception being that *G. stearothermophillus* was cultured at 65℃. Thereafter 10 µL of the bacterial suspension was added to 10 mL of MHB[1] with 2ml of $CaCl_2.2H_2O$ (3.68g +100ml) and 1ml of $MgC_2.6H_2O$ (8.36g +100ml) added (CLSI, 2008).

Thereafter 100 µl of each bacterial suspension was added to each well with the exception of the broth control well where 100 µl of broth was added instead. 100 µl of the broth containing the test organism was plated onto blood agar as a concentration and purity control. The plates were then incubated overnight in air at 37℃ in the case of *B. megaterium* and *S. aureus* and at 65℃ in the case of *G. stearothermophillus*. The test was done in triplicate. The highest dilution that had no visible growth was considered to be the lowest detectible concentration of the antimicrobial.

5.1.2.3. Testing for the effect of egg contents

To determine whether substances within the egg will affect the detection levels of antimicrobials the test was repeated (previous steps) using eggs known to be free of antibiotics i.e. originating from untreated hens. One percent (1%) glucose plus phenol red ($C_{19}H_{14}O_5S$; 354, 38)[2] was added to the Mueller-Hinton broth to act as an indicator of fermentation. Furthermore, the test was run in duplicate with the exception that 0.5g/litre tetrazolium salt 3'-{1-[(phenylamino)-carbonyl]-3,4-tetrazolium}-bis (4-methoxy-6-nitro)benzene-sulfonic acid hydrate (XTT), which turns from colourless to a deep red when reduced, was used as an indicator instead. Tetrazolium iodide salt was added after overnight incubation and incubated for a further two hours to allow for maximum colour change. The reason for the addition of

1 Oxoid Limited Wade Road, Basingstoke, Hampshire, RG24 8PW, United Kingdom.
2 SARCHEM (Pty) Ltd., P.O. Box 144, Muldersdrift, 1747, South Africa.

an indicator was that the cloudy egg solution masked the growth of the bacteria. Later on this test was modified and repeated using the macrodilution medium in 3 mL plastic tubes[1] using the method described by the CLSI (2008).

Working in a Biosafety type II cabinet [2] the eggs were arranged with pointed ends up in a plastic tray, sprayed with 70% alcohol and allowed to dry. The ends were cut open using a scissors and the contents poured into a Whirl-Pak bag[3]. The eggs were homogenised using a stomacher lab-blender 400[4]. Initially whisking had been attempted, but this lead to uneven mixing and contamination of the sample. A 50% egg suspension was made by adding 50 µL of the homogenized egg to 50 µl of CAMHB containing either 1% glucose and phenol red or no indicator to each well. The antibiotics and serial dilution was then done in the same way as when no antibiotics were added, as described in 5.1.2.1. In the macrodilution test 100 µl of the egg was added to 900 µl cation-adjusted Mueller-Hinton broth containing 1% glucose and phenol red ($C_{19}H_{14}O_5S$; 354, 38)[5] to each plastic tube[6]. One mL of each antibiotic (as shown in Table 9) was added to the first tube of a 12-tube series and two fold dilution made from tube 1 to tube 12. One mL of the bacteria suspension was added to each tube. A growth control and negative control tube were allocated for each bacterium where in place of bacteria suspension 1 mL of the Mueller-Hinton broth with the aforementioned additives was added to the negative control tubes. The tests were repeated in triplicate. The tubes were incubated overnight at 37℃ in the case of *B. megaterium* and *S. aureus* while *G. stearothermophillus was* incubated for 3-4 hours at 65 ºC.

5.1.3. Agar diffusion test

The Agar diffusion test was performed on the following bacteria: *B. megaterium and G. stearothermophillus* using the method prescribed by the CLSI (2008). A bacterial suspension was made of *B. megaterium* and *G. stearothermophillus* purified bacteria in the same way as for the Kirby-Bauer test.

The media was prepared as follows:

1 Plastpro Scientific (Pty) Ltd, PO Box 3192, Edenvale, 1610, South Africa.
2 Labaire (Pty) Ltd., Corporate Park, 2 Gazelle, Ave, Midrand, South Africa.
3 Guth Group (Pty) Ltd., 79 st Georges Street, Newlands, 2114, South Africa.
4 Seward Medical, UAC House, Black, Friars Road, London Model No. BA 6021, Britain.
5 SAARCHEM (Pty) Ltd., P.O. Box 144, Muldersdrift, 1747, South Africa.
6 Plastpro Scientific (Pty) Ltd., PO Box 3192, Edenvale, 1610, South Africa.

28g of nutrient agar[1] was poured into a glass bottle and mixed in 1L of de-ionized water with a magnetic stirrer[2]. The solution was autoclaved[3] at 121ºC for 30 minutes, then removed and kept till it cooled down to 60℃. Thereafter 50 µL of the bacterial suspension was added to 50 mL nutrient agar[2] and aliquoted into a 90mm Petri dish[4]. These Petri dishes were punched using a sterile metal puncher to obtain wells with a diameter of 2 cm. Homogenized samples were diluted with CAMHB 1:1 to reduce the coagulation of the egg samples, then incubated in a water bath[5] at 80ºC for 10 minutes to destroy the lysozyme and other inhibitory substances in the egg material and removed. Using a micropipette, 200µL of the processed eggs were added to each well and incubated overnight at 37℃ in the case of *B. megaterium* and 65ºC *for G. stearothermophillus.* Zones of inhibition were measured as an indicator of the presence of antimicrobial inhibitors.

5.1.4 Live hen trial

The aim of the hen trial was to determine if the test could detect specific antibiotics in the eggs of treated hens. Two trials were run; the first one had 38 hens in it and the second 36 hens. The reason for two trials was that the FAST test was used on the first batch of eggs. Probably lyzozymes present in the eggs lead to non-specific inhibition in this test as well as a lower sensitivity of *Bacillus megaterium compared* to *Geobacillus steareothermophilus* resulting in it being considered unsuitable. Furthermore, not all the eggs had been stored at the time the second test was available. Procedures applied in this trial were the same as explained below in the second trial.

In the second trial, 36 hens fed on commercial layer pellets without antibiotics or sulphonamides where divided into 12 groups, each containing 3 hens. All groups were separated from each other in separate cages. Hens were weighed individually and each group was allocated to a specific antibiotic (12 antibiotics) while the remaining control group were given water. Individual birds were treated by oral gavage with one of the antibiotics based on the therapeutic dosage and body mass. Table 10 shows the antibiotic used, weight of the chickens, dose used and withdrawal period. Eggs were collected and labeled 2 days prior to treatment and also on the day of dosing (just before dosing to check for possible presence of inhibitory substances) and thereafter, daily during dosing for 7 days. After dosing was

2 Labretoria CC/BK., P. O. Box 95777, Waterkloof ,0145, Pretoria, South Africa.
2 Optolabor (Pty) Ltd., P.O. Box 31208, Braamfontein, Johannesburg, South Africa.
3 Speedy autoclave model HL-340. HLMC Co., Taipei, Taiwan.
4 Orb Diagnostics, CC., P.O. Box 763, Edenvale, 1610, Johannesburg, South Africa
5 Labotec South Africa .www.labotec.co.za (there is a problem with the number formatting.)

completed, eggs were still collected for another 7 days to check for a decrease in excretion to the point where the tests could no longer detect antimicrobial residues in the eggs.

The eggs were disinfected, opened and stored as previously described (Section 5.1.2.3.) and only tested once all the eggs had been collected. They were then diluted 1:10 with nutrient broth containing 1% glucose and phenol red, inactivated at 80℃ and tested in the laboratory for antibiotic residues using *B. megaterium*, *S. aureus* and *G. stearothermophilus* in a broth macrodilution test as described in Section 5.1.2. Results were recorded and a summary report was written.

Table 10: Antibiotics used for the hen trial

Antibiotic	Dose	Weight of birds(Kg)	Withdrawal Period (days)	Supplier
Sterile Water (control)	5ml water	2.0 1.5	-	-
Avimox 10 (Amoxycillin 10% each g contains amoxicillin 100mg)	250	2.4 1.9	1	Immunovet SA[1]
Baytril 10% (Enrofloxacin 100mg/ml (Benzyl alcohol 1.35%))	5	2.0 2.0 2.0	7	Immunovet SA (Bayer)
ESB3 (Sulphachloropyrazine 300g/kg, con.0.03%)	300g/kg	2.0 1.8	3	Immunovet SA
Tyloveto-s 100% (Tylosin tatrate 1000mg)	50	2.0 1.3	3	Immunovet SA
Terramycin (55mg/g oxytetracycline hydrochlorate)	50	2.0 2.0	4	Immunovet SA
Fosbac (Each 100g contains: calcium Phosphomycin 25g, fructose 1.6 diphosphate 18g, Vit. E 3000 iu)	160	2.1 1.7	7	Immunovet SA
Tiamun 10% feed premix (Tiamulin hydrogen fumarate 100g/kg)	30	2.1 2.0 2.3	3	Immunovet SA
Lincocin TM sterile solution (Lincomycin hydrochloride, monohydrate equivelant to licomycin base 100mg: benzyle alcohol 0.9%)	100	1.8 1.6	2	Pharmacia SA[2]
Ciprofloxacin	5	2.3 1.8 2.1	7	Sigma[3]
Trimethoprim	100	1.9 2.0 2.0	10	Sigma[3]
Doxycycline	50	2.0 2.0 2.0	3	Sigma[3]

1 Immunovet Services, Vervoer Street, Kya Sand, Randburg 2194, South Africa.
2 Pharmacia (Pty) Ltd. Alphen West G, George Street, Midrand, 1685, South Africa.
3 Sigma Aldrich (Pty) Ltd., P. O. Box 4853, Atlasville, 1465, South Africa.

5.2. The test procedure used on eggs

Egg samples were removed from the freezer and allowed to defrost. Thereafter each sample was homogenized using a stomacher lab-blender 400[1]. Samples then were organized according to their labels and were ready for processing.

5.3 Test Organism preparation

Geobacillus sterothermophillus var. calidolactis ATTC 7953[2] was used as a test organism. The bacteria were removed from the freezer and using a sterile loop one drop was added to 1ml of normal saline[3] and vortexed. The bacteria were cultured on Muller Hinton Agar (Oxoid Products)[3] and incubated at 65℃ overnight and the growth of colonies and formation of spores was identified. The purity of the culture was proven by the use of a Gram's stain.[4] Only if one colony type and a monoculture of Gram positive rods were noted was the culture accepted as pure.

5.4. Broth Medium preparation

An amount of 8g of nutrient broth base[5] and 10g of glucose[2]and 0.02g of phenol red[2] were poured in a glass bottle and mixed together in 1L de-ionized water with a magnetic stirrer[6]. The solution was autoclaved at 121℃ for 15 minutes[7], then removed and kept till it cooled down and aliquoted into 100 mL sterile Schott bottles.[8] These were stored in a household refrigerator[9] at ± 5 ℃ until required.

5.5. Analysis

Single colonies on Muller Hinton agar was picked up using a sterile pipette tip and then diluted in 1 mL normal saline containing 0.02% Tween 80 to a turbidity equivalent to a 0.5 MacFarland standard. The mixture was then vortexed and added to 1 000mL nutrient broth. Using a micropipette, 900µL of the mixture of the medium and test organism was pipetted into 45 test tubes. Thereafter 100 µL of the sample (homogenized egg contents) was added into the tubes to give a total volume of 1 000 µL. Each tube was labeled separately with the sample number using a permanent marker pen. Each sample was repeated in three tubes for quality control. One tube in

1 Model No. BA 6021, Seward Medical, UAC House Black, Friars Road, London,, Britain.
2 Merk Chemicals (Pty) Ltd., 259 Davidson Road, P. O. Box 1998, Halfway House, 1685, South Africa.
3 Quantum Biotechnologies (Pty) Ltd., Bactlab House, Mikro Industrial Park, 1 Abcon Close, Strydompark, Randburg, 2169, South Africa.
4 www.ltdiagnostics.com
5 Labretoria CC/BK., P. O. Box 95777, Waterkloof, 0145, Pretoria, South Africa.
6 Optolabor (Pty) Ltd., P.O. Box 31208, Braamfontein, Johannesburg, South Africa.
7 HLMC Co., Taipei, Taiwan.
8 Labotec, South Africa .www.labotec.co.za
9 Club Refrigerating CC, South Africa. (number formatting is a problem.)

each rack was kept as a positive growth control using an egg free from an untreated hen.

Samples were incubated in a water bath[1] at 80ºC for 10 minutes to destroy lysozyme and other protein inhibitors and removed. The water bath was cooled to 65℃ and the samples were incubated for 2-4 hours depending on the reaction of the growth control (complete change from red to yellow means the test was completed). The time was checked several times and it was found that 2-4 hours was the suitable time for the test to complete. After the test was completed, samples were removed from the water bath and the results were recorded. A colour change from red to yellow indicated glucose fermentation and therefore no inhibition of growth, whereas retention of the red colour indicated inhibition of growth and hence the presence of inhibitors (see Figure 7).

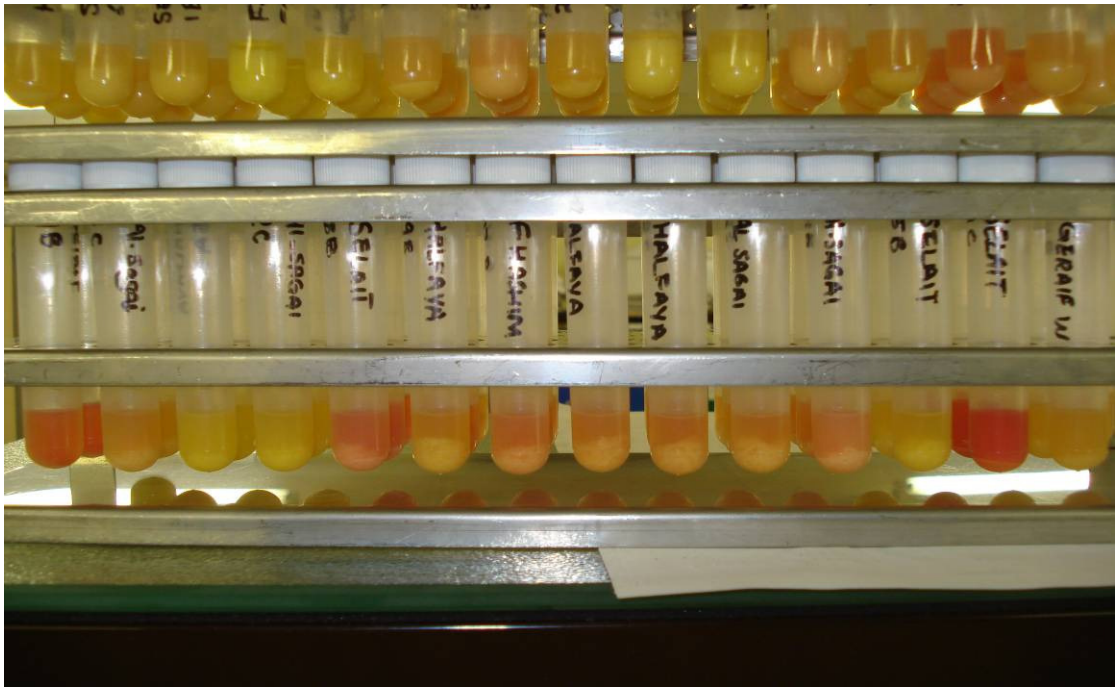


Figure 7: Complete test reactions

5.6 Antibiotics used in poultry industry in Sudan

The information on the types of antibiotics used in the poultry industry in The Sudan were collected through direct visits and interviews and records of Khartoum and Omdurman veterinary hospitals, and from veterinary drug companies in Khartoum. A report stating all types of antibiotics was compiled.

1 Labotec, South Africa .www.labotec.co.za

6. Data Analysis

6.1 Calculation of Prevalence

Surveys are a common way of data collection. The validity of any sampling scheme is based on the assumption that a population is divided into representative subunits from which characteristics of the population can be estimated (Thrusfield, 1995).

An important application of surveys in epidemiology is estimation of the prevalence of clinical diseases, infection or sero-positive animals from samples of an animal population. The apparent prevalence (AP) derived from the survey, which is the number of test positive eggs divided by the total number of eggs sampled, does not take into account the possibility of false positive and false negative results and does not consider any uncertainty in the survey results. For the calculation of the true prevalence (TP), equation 1 was used, which adjusts for the sensitivity and specificity of the diagnostic test. (Thrusfield, 1995).

$$TP = \frac{AP + Sp - 1}{Se + Sp - 1} \quad \text{.....(1)}$$

Where, TP means the true prevalence, AP the apparent prevalence, Se the sensitivity of the test and Sp the specificity.

Once all samples had been tested, the results were entered into the spreadsheet programme Excel (Microsoft Corporation, USA, 2003). Each egg sample from a house was tested individually, a house was recorded as positive to antimicrobial residues if any of the 3 egg samples tested positive for inhibitors and the farm was identified as positive if any of the houses were positives.

The TP was calculated for the various antibiotic residue positive results using the sensitivity and specificity published for a similar screening test known as the PremiTest®. As yet there are no validated Se and Sp results for the in-house test that was used. The sensitivity done on meat, not egg samples used to calculate TP was (72.5%) (Gaudin *et al.*, 2008) and specificity (98%) (Gaudin *et al.*, 2009). The prevalence was calculated for each period of collection separately, as the sampling was not conducted within the same farms in each seasonal collection.

The 95% confidence intervals for TP were calculated for the population proportions (Prevalence) by applying the apparent prevalence in equation 2.

$$TP \pm 1.96SE(AP) = AP - 1.96\sqrt{\frac{AP(1-AP)}{n}}, AP + 1.96\sqrt{\frac{AP(1-AP)}{n}} \quad \text{.....(2)}$$

Where, AP is the apparent prevalence, SE standard error and n the sample size. Survey Toolbox version 1.04[1] was used to calculate the true prevalence and the 95% confidence interval.

6.2 Questionnaire survey analysis

The data from the questionnaires was captured into and analysed with EpiInfo™[2] statistical package version 3.5.1. Several descriptive statistics including frequencies, means, medians and statistical associations between several factors were measured (Chi-square and Fisher exact test). Odds ratios were calculated to control for any possible confounding effects. The April 2008 results of antimicrobial residues were used to classify the farms either positive (at least one sample was positive for antimicrobial residues) or negative for antimicrobial residues. April results were used because the questionnaire survey was conducted in April and each farm questioned was sampled in that period. The independent variables were tested for bivariable associations with the outcome variables (Presence of antimicrobial residues) using the Fisher Exact test for categorical variables. The final results were summarized in a report.

6.3 Geographic Information System (GIS) and spatial analysis

Google Earth program version 4.3[3] was used to trace the farms and areas of sampling and record the coordinates of all the farms sampled in Khartoum State. The main challenge was to record the coordinates of each farm sampled as they were clustered in each area with a small difference of seconds and even fraction of a second between them. All coordinates were entered into the spreadsheet programme Excel[4]. Necessary conversion of the Excel file for use in ArcView[5] 9.3 was done. Africa, Sudan and Khartoum state shape files (maps in a format suitable to be used by ArcView) were downloaded from www. maplibrary.org[6]

1 Animal Health Services (www.ausvet.com.au/contents.php?)
2 http://www.cdc.gov/epiinfo/epiinfo.htm
3 http://earth.google.com/download-earth.html
4 Microsoft Corporation, USA, 2003.
5 Esri Redlands 2009.
6 www.maplibrary.org/stacks/Africa/Sudan/Khartoum/index.php.

Maps of Khartoum State showing the sampling locations, farm density, farms sampled and prevalence spatial distribution for each period of collection were created using ArcView 9.3 as stated above.

CHAPTER IV- RESULTS

1. Results of the census of commercial layer farms in Khartoum State

The census covered the three localities of the state; Khartoum North (Bahry), Khartoum and Omdurman. The census showed that there were 252 layer farms containing 764 commercial layer houses in the state, with a total capacity of 2 221 800 birds. The census covered 31 different poultry farming areas in the state, 17 areas (54.9%) were in Bahry, 10 areas (32.2%) in Khartoum and four areas (12.9%) in Omdurman. Table 11 shows a summary of the commercial layer farms census and more details are shown in Appendix VI.

2. Questionnaire Analysis

A Questionnaire survey was conducted in April 2008. Ninety two farms participated in the survey. The total number of farms that participated in the survey comprised 52% of the total number of farms in the several areas surveyed. The remaining farms were not surveyed because they were not in production at the time of the survey. Table 12 shows the number and proportions of farms surveyed in each locality and area. The results of the questionnaire survey are shown in Table 13.

Table 11: Summary of layer farms census in Khartoum State

No	Locality	Area	Number of Farms	Number of Houses	Total Capacity of birds
Khartoum North (BAHRY)					
1	BAHRY	EL- SELAIT	13	45	163,400
2	BAHRY	SOBA EAST	4	7	25,000
3	BAHRY	EL- EZBA	6	11	15,000
4	BAHRY	HILAT KUKU	8	15	16,900
5	BAHRY	OMDOUM	5	8	29,000
6	BAHRY	EL-KADARU & DROSHAB	13	57	87,600
7	BAHRY	EL-HAG USIF & EL-SHIGLA (Sharq Elniel)	10	28	32,700
8	BAHRY	SHAMBAT	16	57	49,300
9	BAHRY	EL-KABASHI	12	25	249,500
10	BAHRY	EL-FAKI HASHIM	12	62	135,000
11	BAHRY	EL-SAGAI	13	65	195,000
12	BAHRY	EL-TIBNA & ZAKIAB	18	66	67,300
13	BAHRY	EL-SAMRAB	5	17	20,000
14	BAHRY	EL-HALFAYA	16	82	184,700
15	BAHRY	EL-MAZALAT	3	7	8,000
16	BAHRY	EL-SABABI	5	10	10,500
17	BAHRY	EID BABIKER	7	11	12,600
	Sub Total	**17**	**166**	**573**	**1,301,500**
KHARTOUM					
1	KHARTOUM	BOTRY	6	23	111,000
2	KHARTOUM	SOBA	11	14	52,000
3	KHARTOUM	EID HUSSIEN	6	7	6,500
4	KHARTOUM	TYBA HASANAB (Elselaimania & Traiat albiga)	8	25	482,000
5	KHARTOUM	EL-SHIGAILAB	5	8	38,000
6	KHARTOUM	EL-SALAMA	5	7	25,000
7	KHARTOUM	EL-KALAKLA & DIKHAINAT	17	38	53,000
8	KHARTOUM	EL-KALAKLA NORTH	5	8	8,000
9	KHARTOUM	EL-GERAIF WEST	12	34	44,800
10	KHARTOUM	GEBEL AWLIA	3	8	33,000
	Sub Total	**10**	**78**	**172**	**853,300**
OMDURMAN					
1	OMDURMAN	ABO ROF	1	3	3,000
2	OMDURMAN	EL-GARAFA	2	3	4,000
3	OMDURMAN	EL-SARHA	2	7	10,000
4	OMDURMAN	NEFASHA	3	6	50,000
	Sub Total	**4**	**8**	**19**	**67,000**
	TOTAL	**31**	**252**	**764**	**2,221,800**

Table 12: Number of farms surveyed in each area of Khartoum State, 2008

No	Locality	Area surveyed	Total number of farms	Farms surveyed	Proportion of farms surveyed
1	BAHRY	EL-SELAIT	13	6	46%
2	BAHRY	EL-KADARU & DROSHAB	13	6	46%
3	BAHRY	EL-HAG USIF & EL-SHIGLA (Sharq Elniel)	10	2	20%
4	BAHRY	SHAMBAT	16	8	50%
5	BAHRY	EL-KABASHI	12	7	58%
6	BAHRY	EL-FAKI HASHIM	12	4	33%
7	BAHRY	EL-SAGAI	13	4	31%
8	BAHRY	EL-TIBNA & ZAKIAB	18	12	67%
9	BAHRY	EL-SAMRAB	5	1	20%
10	BAHRY	EL-MAZALAT	3	1	33%
11	BAHRY	EL-SABABI	5	3	60%
12	KHARTOUM	SOBA	11	9	82%
13	KHARTOUM	TYBA HASSANAB	8	1	13%
14	KHARTOUM	EL-SHEGAILAB	5	1	20%
15	KHARTOUM	EL-KALAKLA & DIKHAINAT	17	16	94%
16	KHARTOUM	EL-KALAKLA NORTH	5	5	100%
17	KHARTOUM	EL-GERAIF WEST	12	6	50%
TOTAL		**17**	**178**	**92**	**52%(Farms surveyed/Total)**

Table 13: Results of questionnaire survey, Khartoum State 2008

Variable	No. Farms Surveyed			Farming System			Chicken Breed						
	Bahry	Khartoum	Total	Open	Closed	Total	Unknown	Hi-sex	Bovan	Lohman	Hyline	Mixed	Total
Number	54	38	92	91	1	92	13	47	9	12	5	6	92
Percentage (%)	59%	41%	100%	99%	1%	100%	14%	51%	10%	13%	5%	7%	100%

Table 13: Continued

Variable	Breeding System			No. of chickens/house					No. of Houses/ Farm				
	Multi age	All in all out	Total	100-500	500-1,000	1,000-2,000	>2,000	Total	1	2	3	≥4	Total
Number	34	58	92	6	81	2	3	92	52	25	9	6	92
Percentage (%)	37%	63%	100%	7%	88%	2%	3%	100%	56%	27%	10%	7%	100%

Table 13: Continued

Variable	Age/Month (farm)							Antibiotics in use now			Antibiotic used in the past 3 months			
	Unknown	4-8	8-12	12-16	>16	multiage	Total	No	Yes	Total	Don't Know	No	Yes	Total
Number	4	27	36	6	4	15	92	47	45	92	8	30	54	92
Percentage (%)	4%	29%	39%	7%	4%	17%	100%	51%	49%	100%	9%	33%	58%	100%

Table 13: Continued

Variable	Purpose of Antibiotic usage[1]					Route of administration[2]				Understanding of Withdrawal period		
	1	2	3	4	Total	1	2	3	Total	No	Yes	Total
Number	56	12	22	2	92	89	2	1	92	69	23	92
Percentage (%)	61%	13%	24%	2%	100%	97%	2%	1%	100%	75%	25%	100%

Table 13: Continued

Variable	Selling eggs during and after using drugs			Do drugs pass from chicken body to eggs?			Do drugs in eggs affect humans?			Any quality control measures to eggs?		
	No	Yes	Total	No	Yes	Total	No	Yes	Total	No	Yes	Total
Number	2	90	92	77	14	91	81	10.0	91	87	5	92
Percentage (%)	2%	98%	100%	85%	15%	100%	89%	11%	100%	95%	5%	100%

Table 13: Continued

Variable	Means of Storage of Drugs[3]							Rules and Regulations of antibiotic usage			Governmental body responsible for rule and regulations[4]			
	1	2	3	4	5	6	Total	No	Yes	Total	1	2	3	Total
Number	50	2	28	3	4	5	92	87	5	92	1	88	2	91
Percentage (%)	54%	2%	30%	3%	5%	6%	100%	95%	5%	100%	1%	97%	2%	100%

[1] 1: Therapeutic, 2: Prophylactic, 3: Therapeutic and prophylactic, 4: Don't Know.
[2] 1: Water, 2: Water and feed, 3: Water and eye drop.
[3] 1: Store room, 2:, 3: Chicken house, 4: Pharmacy, 5: Store room and Fridge, 6: Others.
[4] 1: National Standardization and Metrology, 2: No governmental body, 3: State Ministry of Agriculture and Animal Resources and Irrigation.

The results of April 2008 surveillance for antimicrobial residues were used to classify the farms either positive or negative. The questionnaire survey covered 92 farms but only 34 farms were tested for the bivariable associations with the outcome (presence of antimicrobial residues), because samples collected from the rest of the farms were damaged *in transit* (See Chapter III). Table 14 shows the analysis of categorical risk factors for farm levels positive for antimicrobial residues in Khartoum State, two tailed Fisher Exact Test was calculated using EpiCalc* 2000 software.

The results show that there is no significant association between the entire categorical variable in the questionnaire and the presence of antimicrobial residues on the farms. The exception was the significant association between the lack of understanding of withdrawal period and the presence of antimicrobial residues in the egg products.

* EpiCalc 2000, version 1.02, Joo Gilman & Mark Myatt 1998, Brixton books.

Table 14: Fisher exact test for testing the association between factors associated with antimicrobial residues and the presence of antimicrobial residues in eggs

Risk factor	No. of farms tested	No. of farms positive (%)	P-value (Fisher Exact test)
Locality			
Bahry	15	11	1.00
Khartoum	19	14	
Chicken Breed			
Hisex	14	11	
Bovan	5	3	
Lohman	5	3	0.81*
Hyline	2	2	
Mixed	2	2	
Unknown	6	4	
Breeding System			
All-in all-out	11	10	0.21
Multi-age	23	15	
No. of chickens/house			
100-500	4	3	
500-1,000	28	20	1.00*
1,000-2,000	1	1	
>2,000	1	1	
No. of Houses/ Farm			
1	19	13	
2	10	8	0.92*
3	2	2	
≥4	3	2	
Age (Month)			
4-8	8	6	
8-12	17	12	
12-16	2	1	1.00*
>16	2	2	
Multiage	2	2	
Unknown	3	2	
Antibiotics in use now			
Yes	16	13	0.44
No	18	12	
Antibiotics used in the past 3 months			
Yes	21	15	1.00
No	13	10	

* P-value was calculated using Data Analysis and Statistical Software (STATA), version 10.1, StataCorp, College Station, TX, U.S.A).

Table 14: continued

Purpose of Antibiotic usage Therapeutic Prophylactic Therapeutic and prophylactic Others	 22 6 5 1	 15 4 4 1	1.00*
Route of administration Water Feed Water and Feed Others	 33 0 0 1	 24 0 0 1	-
Understanding of Withdrawal period Yes No	 7 27	 3 22	0.06
Do drugs pass from chicken body to eggs Yes No	 5 29	 2 23	0.1
Does drugs in eggs affect humans Yes No	 4 30	 4 21	0.55
Storage of drugs Store-room Fridge Chicken house Others	 22 9 2 1	 17 6 2 0	0.35*
Any quality control measures to eggs ***(Fumigation, Cracked eggs, cleaning….etc*)**: Yes No	 3 31	 2 23	1.00
Rules and Regulations of antibiotic usage Yes No	 1 33	 1 24	1.00
Governmental body responsible for rule and regulations Yes No	 0 34	 0 25	1.00
Diseases on farm now Yes No	 10 24	 6 19	0.39
Diseases on farm in last three month Yes No	 17 17	 12 13	1.00

* P-value was calculated using Data Analysis and Statistical Software (STATA), version 10.1, StataCorp, College Station, TX, U.S.A).

Figures 8 and 9 show the antibiotics mainly used by farm owners and their frequency in the study population

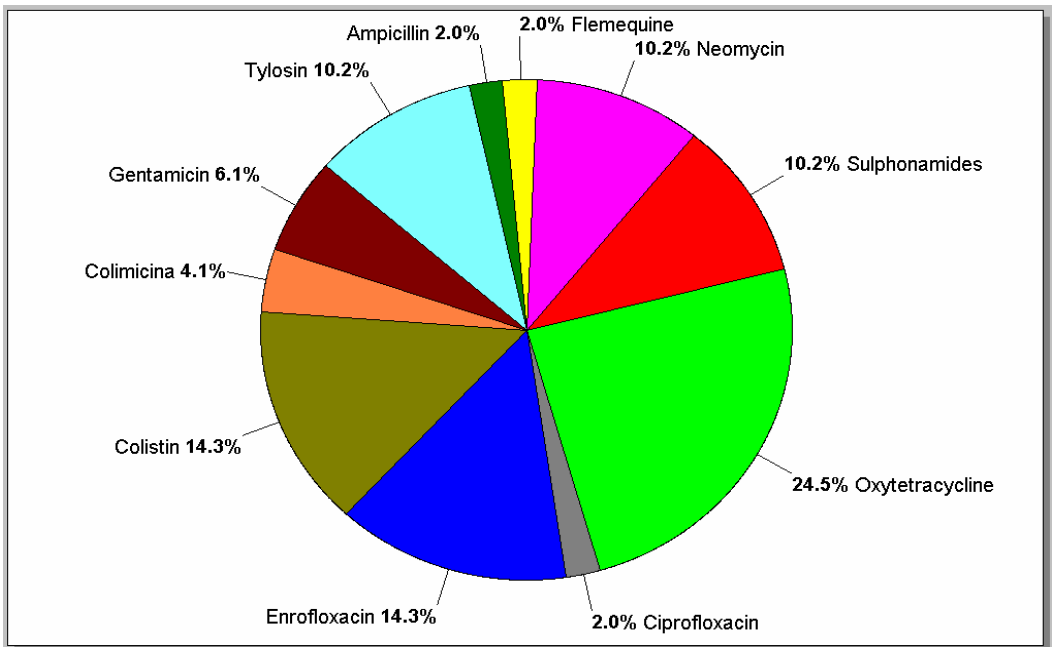


Figure 8: Antibiotics used at the time of the survey, Khartoum State, 2008

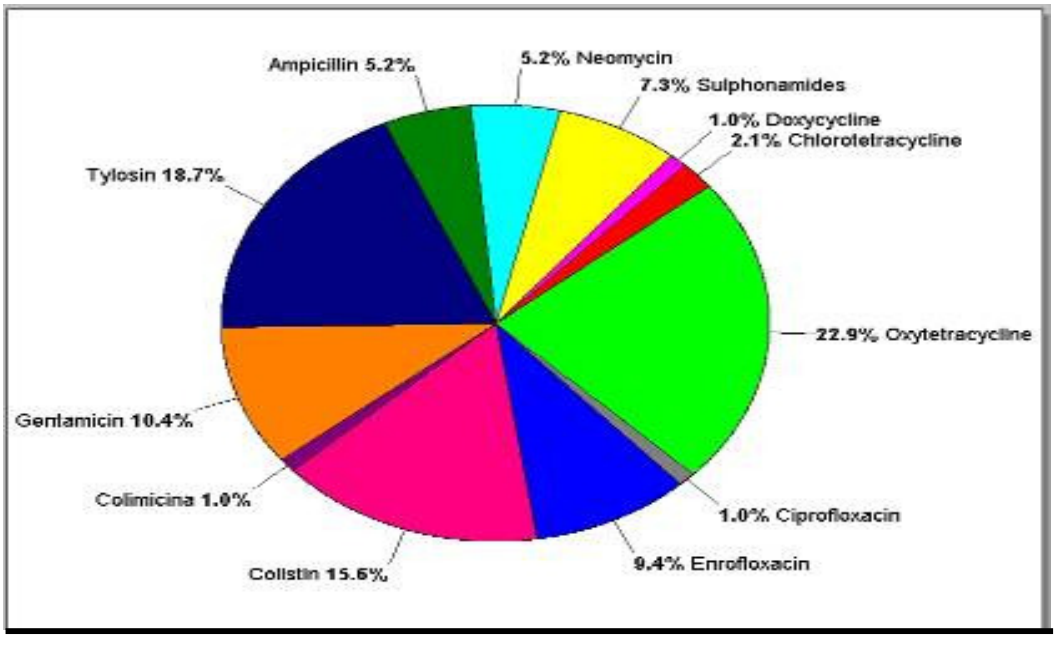


Figure 9: Antibiotics used in the last three month before the survey, Khartoum State 2008

Forty of the questioned farms (43%) reported disease on farms at the time of the survey, while 75 farms (62%) experienced diseases on the farm in the last three months before the survey. Figures 10 & 11 show the frequency of the several diseases reported on the farms by the farmers.

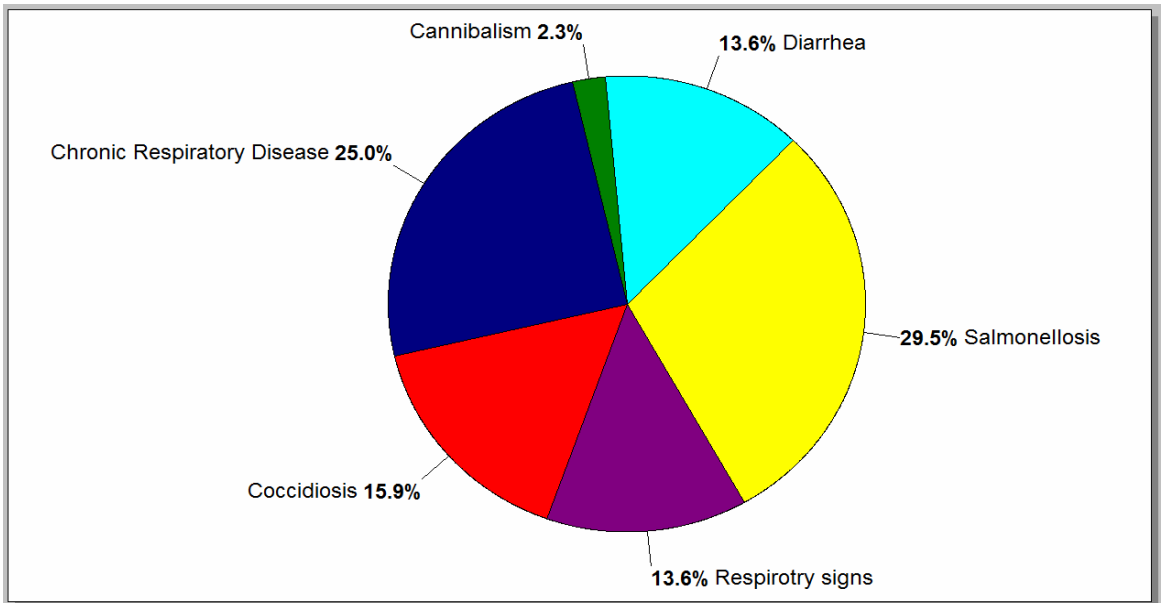


Figure 10: Diseases reported on farms at the time of the survey, Khartoum State, 2008

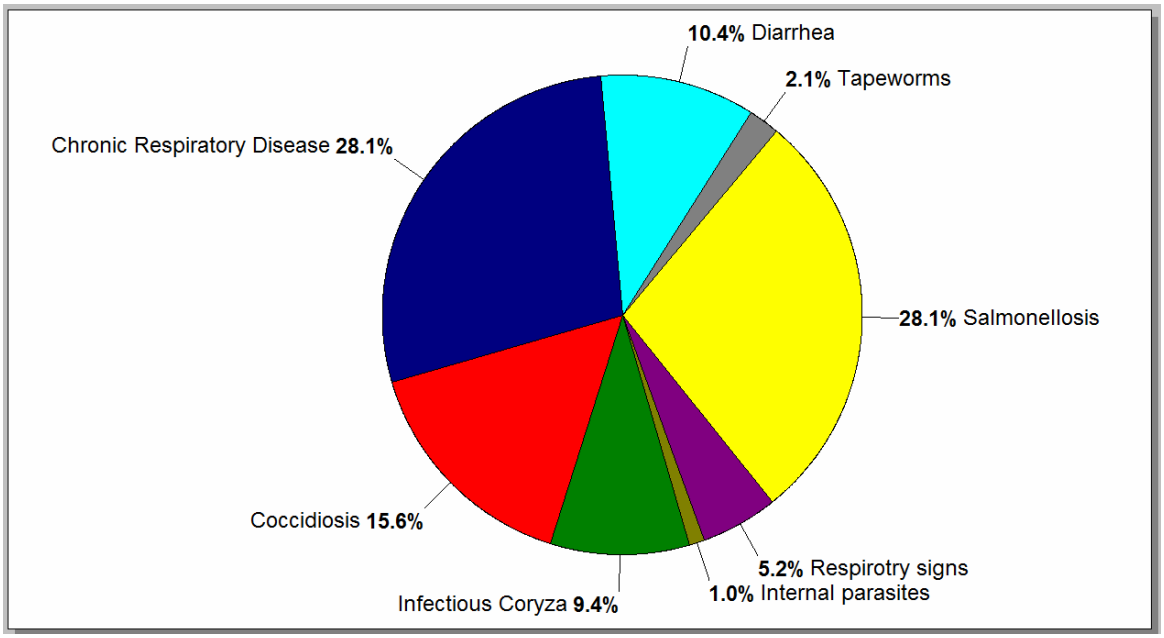


Figure11: Diseases reported in the last three months before the survey, Khartoum State 2008

The association between the presence of disease on the farm and antibiotic usages at the same time is illustrated in a two by two table and the analysis of the table calculating the odds ratio, and chi-square are shown in Table 15.

Table 15: Cross tabulation of (Presence of disease) and (antibiotic usage)

		Antibiotic used now		Total
Disease on Farm now		No	Yes	
	No	41	11	52
	Yes	6	34	40
		47	45	
OR=21; 7< OR< 36 (95% Confidence intervals)				
Chi-square=37; P<0.01				

The odds of antibiotic use (at the time of the survey) were 21 times greater on farms that have diseases than those that do not have diseases.

A cross tabulated table of antibiotics used in the last three months and the presence of disease on the farm in the last three month are shown in Table 16.

Table 16: Cross tabulation of (Presence of disease) and (antibiotic usage in last three months)

		Antibiotic used in the last three months		Total
Disease in last three months		No	Yes	
	No	23	9	32
	Yes	7	49	56
		30	58	
OR=18; 6< OR< 54 (95% Confidence intervals)				
Chi-square=32; P<0.01				

The odds of antibiotics used in the last three month are 18 times greater on farms that have diseases than on those that do not have diseases in the same period.

Table 17 shows the analysis done for the exposure variable (disease on farms) and the outcome variable (antibiotic use). The above two variables were stratified with the variable (purpose of use of antibiotics), which has four strata (therapeutic use, prophylactic use, therapeutic and prophylactic use and unknown purpose). The association between prophylactic use of antibiotics and the presence of diseases on farms was found to be insignificant P-value < 0.15. On the other hand, the association between (therapeutic and prophylactic) use at the same time and with the presence of disease on farms was significant with a P-value <0.02.

The fourth stratum (purpose of antibiotic use unknown) showed insignificant association with disease on farm. The Summary Odds Ratio of the stratified analysis is 22; the adjusted Odds Ratio is 27, Chi-square 37 and P-value < 0. 01. From the result above the confounding effect of (therapeutic purpose of use) on the two variables is seen.

The odds of antibiotics used for therapeutic purpose are 17 times greater on farms that have diseases than on those that do not have diseases.

Table 17: Cross tabulation of (Diseases present on the farms) and (purpose is therapeutic use of antibiotics)

		Antibiotic used now (therapeutic)		Total
		No	Yes	
Disease on Farm now	No	26	5	31
	Yes	6	19	25
		32	24	
OR=17; 4< OR< 62 (95% Confidence intervals)				
Chi-square=20; P<0.01				

Table 18 shows a significant association between the variable (Can drugs affect humans?) and (Do drugs pass from chicken body to eggs) (P <0.02).
The odds of saying drugs in eggs don't affect humans is 28 times greater in those people that say drugs don't pass from the hens body to the eggs.

Most people don't believe drugs in eggs affect humans or that drugs can pass from the chicken body to eggs.

Table 18: Cross tabulation of (Do drugs in eggs affect humans?) and (Do drugs pass from the chicken's body to eggs?)

		Do drugs in eggs affect humans?		Total
		No	Yes	
Do drugs pass from the chicken's body to eggs?	No	74	3	77
	Yes	6	7	13
		80	10	
OR=28; 6< OR< 141 (95% Confidence intervals)				
Chi-square=23; P<0.02				

The information on the types of antibiotics used in the poultry industry in The Sudan was obtained from Khartoum and Omdurman veterinary hospitals, and from veterinary drug companies in Khartoum.

Table 19 shows the available antibiotics, which are used in poultry. They belong to many antibiotic classes; tetracycline, sulphonamides, trimethoprim, β-lactams, macrolides, lincosamides, aminoglycosides, quinolones, polypeptides and combinations of different groups in one compound. There are no growth promoters that are used in the poultry industry in Sudan, but some farmers add tetracycline and coccidiostats to the feed for this purpose

Table 19: Antibiotics used in poultry production in Sudan

No.	ANTIBIOTIC TRADE NAME	CONTENTS
1	OXTETRACYCLINE	Oxtetracycline (OTC)
2	DIMOXAN	Amoxycillin+colistin
3	NEOXYVITAL POWDER	OTC +neomycin+vitamins + minerals
4	UVETRIL	Enrofloxacin 100mg/ml
5	UVE-OXYVIT	Vit AD3E,C,Fe,Mn,Zinc copper sulphates, OTC
6	FLUMESOLE 200	Flumequine
7	AMOXYVETO-50S	Amoxycillin
8	COLIVETO-4800	Colistin sulphate
9	LINCOMYCIN-40S	Lincomycin
10	L-SPEC 100 s	Lincomycin +spectinomycin
11	DIAZIPRIM-48%	Sulfadiazine +trimethoprim
12	AMPISTIN	Ampicillin +colistin
13	COLIDOX	Colistin + doxycycline
14	COLIDAD	Colistin
15	NEW-OXYVIT	OTC, neomycin sulphate+vitamins
16	GENTAMYCIN 20%	Gentamicin
17	GENTADOX	Gentamicin sulphate + doxycycline
18	CHLOR 200	Chlorotetracycline
19	TYLO 200	Tylosin
20	NEOTREAT	Neomycin+oxytetracycline
21	DOXYVEET 500	Doxycycline hyclate
22	NEONOR	Neomycin sulphate
23	VETICOZORIL	Diclazoril
24	SULPHAQUINOZALINSOL	Sulphaquinazoline
25	VETICOTRIMETHIPRIM	(Trimethoprim sulphadiazine +vit
26	VETICOSULPHAMYCIN	Trimethoprim +sulphadiazine +erythromycin
27	OXYNEOVET	Oxytetracyclin +neomycin +vit
28	VETICODOXYSTIN POWDER	Doxycycline +colistin sulphate
29	VETICOAMPIVET	Ampicillin trihydrate +vit.
30	VETICOSYSYPROFLOXACIN	Ciprofloxacin

3. Laboratory analysis

3.1 The Kirby-Bauer test

The results are shown in Table 20. An example of this test is shown in Figure 12.

Table 20: Results of the Kirby-Bauer disk diffusion test

Antibiotic	**Susceptibility (mm)**	**Zone of inhibition (mm)**		
		B. megaterium	***S. aureus***	***E. coli***
Neomycin	-	30	20.5	21.2
Doxycycline	18-25	33.4	24.7	21.9
Trimethoprim + sulphamethoxyzole.	24-32	35.5	26.5	30
Lincospectin	-	24.6	26	25
Sulphamethaxyazole	18-26	35.8	26	0
Ampicillin	23-29	34.4	16.8	20
Enrofloxacin	28-36	35.1	20	28
Phosphomycin	-	14	28.2	31.2
Colistin	-	15.6	34.7	12.7
Erythromycin	0	36.4	0	0
Gentamicin	19-26	31.1	17.5	22.5
Tylosin	-	28.1	24	0
Tiamulin	-	16.3	0	0

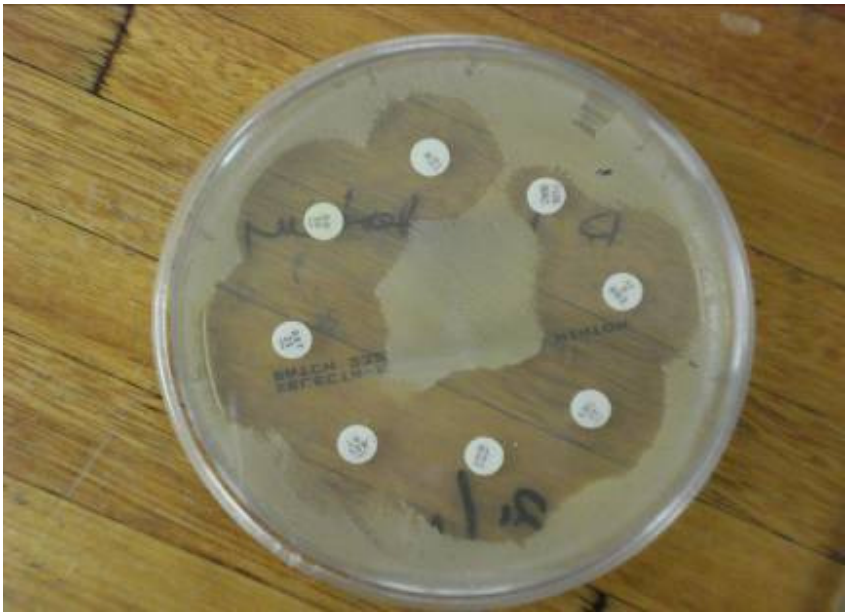


Figure12: Kirby-Bauer disk diffusion test

3.2 MIC test with and without homogenized egg contents

The MIC results are shown for *B. megaterium* and *S. aureus* in Table 21. After reading those tests that contained egg it was found that non-specific inhibition was happening for *B. megaterium* and *B. stearothermophilus*, but not *S. aureus*. This effect was noted in the first well, but not the other wells and was consistent for each antimicrobial tested and was present in the growth control. Figures 13 and 14 show the MIC plates without eggs or an indicator and when eggs were used with tetrazolium salt.

Similarly there were equally-sized zones of inhibition around the wells of dilutions 10^{-1} to 10^{-4}, but not the 10^{-5} to 10^{-8} dilutions of the nutrient agar plates. This result was consistent, regardless of the antibiotic type and it was also present in the control without antibiotics. This indicated the presence of a non-specific inhibitor in the egg mixture that was diluted out at egg mixture concentrations of $\leq 10^{5}$. This effect was thought to be due to the effect of lysozyme and possibly other protein enzymes which are found in varying concentrations in most eggs (Abdou *et. al.*, 2007). Albumin was consistently found to have a higher concentration of the inhibitor (Jambalang A., personal communication 2009). The lowest temperature required to fully inactivate these protein inhibitors in albumin was 80℃ (Jambalang A., personal communication 2009).

The minimum inhibitory concentration (MIC) of the antimicrobials was determined for *G. stearothermophilus* and *B. megaterium* using a 2-fold dilution series of analytical grade antibiotics in nutrient broth containing glucose and phenol red as an indicator of metabolic activity as well as 10% of heat inactivated homogenized egg (Jambalang A., personal communication 2009). The results are compared to the MRL and shown for *B. megaterium* in (Table 21) and for *G. stearothermophilus* in Table 22. Furthermore the latter results were compared to published figures of a similar test known as the Premi®test.

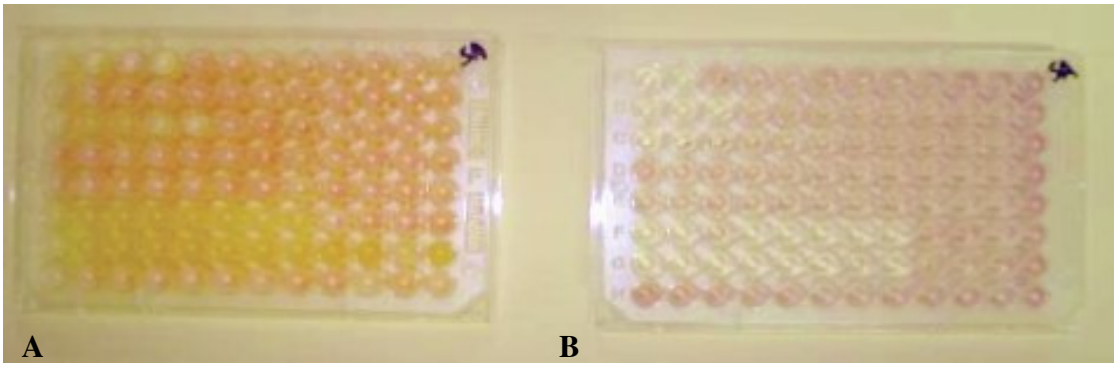


Figure13: MIC test results of *S. aureus* with using A) Phenol red and glucose as a fermentative indicator and B) using tetrazolium salt as an oxidative indicator.

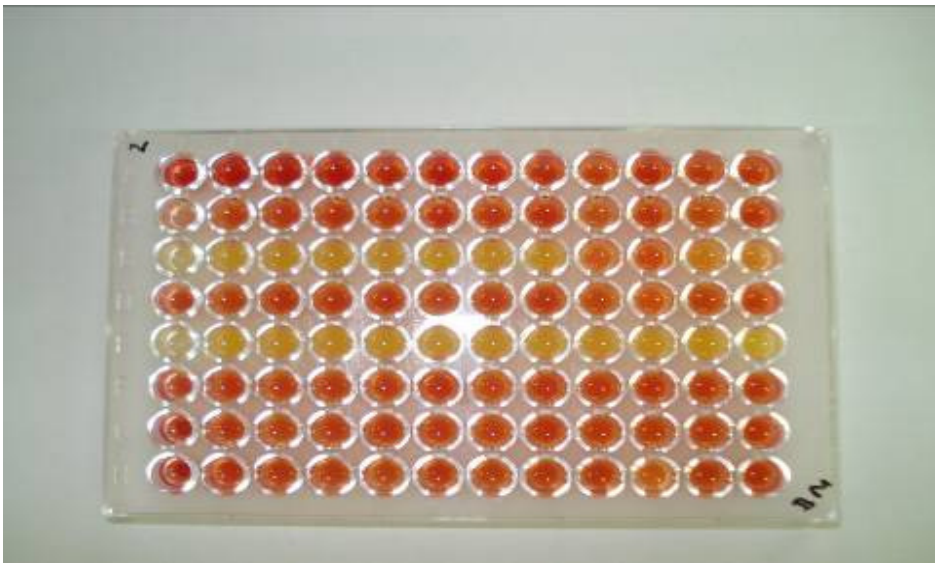


Figure14: MIC plates with eggs and Phenol red and glucose

Table 21: Detection levels of B. megaterium ATCC 9885 and S. aureus ATCC 29213 compared to the required MRLs

Antibiotic	*B. megaterium*		*S. aureus*[*]		MRL	Source
	MIC µg/L		MIC µg/L		µg/kg	
	No egg	Egg[†]	No egg	Egg		
Ampicillin	31.3	1.3	125	2.7	50	SA regulations[‡]
Colistin	2 000	>16 000	>16 000	>16 000	300	EMEA[§]
Doxycycline*	2.7	1 000	125	1 000	300	EMEA
Enrofloxacin*	31.3	62.5	62.5	250	100	EMEA[2]
Erythromycin	62.5	2.7	250	62.5	150	EMEA
Fosfomycin	32 000	2 000	1 000	4 000	100	EMEA
Gentamicin	31.3	125	31.3	250	-	-
Lincomycin	>32 000	>4 000	16 000	250	50	EMEA
Neomycin	62.5	400	62.5	400	500	EMEA
Trimethoprim	250	1 000	250	1 000	50	EMEA
Spectinomycin	4 000	>32 000	>4 000	>4 000	500	EMEA
Sulphadimidine	2 000	2 000	32 000	8 000	100	EMEA
Tiamulin	2.7	2.7	8 000	8 000	1 000	EMEA
Tylosin	125	125	1 000	1 000	200	EMEA

[*] *S. aureus* was used for quality control purposes.
† Inactivated homogenized egg
‡ Regulations governing the maximum limits for veterinary medicine and stock remedy residues that may be present in foodstuffs, 2002, South Africa.
§ European Medicines Agency,
* Not for use in animals that produce eggs (EMEA).

Table 22: Detection levels of G. stearothermophilus when compared to the Premi®test and required MRLs (Jambalang, 2009)

Antibiotic	In-house test *G. stearothemophillus**	Premi®test	MRL
	µg/L	µg/L	µg/L
Enrofloxacin	142	250	100
Norfloxacin	512.6		
Neomycin	22.5	600	500
Tylosin	18.75	75	200
Chlorotetracycline	412	1 000	200
Florfenicol	262.6	-	-
Sulfadiazine	412	70	100
Sulphamethoxyazole	61.67	-	100
Trimethoprim	210.4	50	50
Spectinomycin	416.67		200
Ampicillin	15	5	50
Gentamicin	15	-	-
Phosphomycin	5 625	-	100
Lincomycin	30	150	50
Tiamulin	583.33	-	1 000
Colistin	45	-	300
Oxytetracycline	166.67	400	200
Doxycycline	37	-	200

*** The test was performed in 10% heat-inactivated egg.**

3.3 Hens' trial results

The daily number of eggs collected was inconsistent, possibly because of the process of handling and age of the birds. There were some days when no eggs were even collected.

In experiment 1, all eggs tested, including the controls, showed inhibition when using *B. megatarium*, which was identified as being susceptible to the effect of inhibitors in the eggs as the controls were giving positive results. *S. aureus* was not inhibited and was able to detect antimicrobial residues only for enrofloxacin and sulphonamides at a limit of detection less than the MRL. The main purpose of using this organism was to serve as a quality control bacterium.

G. stearothermophilus was used to detect antibiotics in egg samples collected from experiment 2 and the results showed that the day prior to administration of antimicrobials, as expected, antimicrobials were not detected in all the eggs collected on these days. However, eggs were collected and tested for these days as a way of double checking if the feed did not contain any antimicrobial (feed mill cross-contamination) and to see also if heating the egg samples to 80ºC for 10 minutes truly inhibits natural inhibitors in eggs,. Eggs collected were pooled for the same antimicrobial before testing. The first day after administration of antibiotics, antimicrobial residues were detected in all egg samples collected for that day. Antimicrobial residues were detected in all the eggs collected up the 7th day of dosing. The first day after the end of dosing (8th day), antimicrobial residues were only detected in four (amoxycillin, trimethoprim, tylosin and ciprofloxacin (which is used in Sudan) out of 11 antibiotics tested. By the 2nd day after the end of administration only the presence of trimethoprim could be detected, which was detected till the 9th day after the end of treatment.

4. Sample analysis

The eggs sampled in the January sampling and part of April were rotten when they arrived in South Africa, which didn't allow any analysis to be carried out on them. The reason for the poor state of these samples was an unexpected delay of the samples to be couriered from Sudan to South Africa on top of which the samples were delayed at the airport for irradiation before clearance. The rest of the samples were well managed and the above problems were taken into consideration and solved.

A further 1,044 samples were collected, after January sampling. 731 samples were couriered to South Africa in September 2008 and arrived there in a good state. Of these only 620 could be analyzed as 111 had lost their identity due to the erasure of the labels. The 313 remaining samples were processed in the Research Laboratory of Sudan University of Science and Technology. In total 933 samples were analysed. The total number of layer farms sampled was 175 farms (335 layer houses) of which 43 farms (68 houses) were sampled in April 2008, 79 farms (154 houses) sampled in June 2008 and 53 farms (113 houses) sampled in August of the same year. Figures 15 and 16 show the summary of the sampling results.

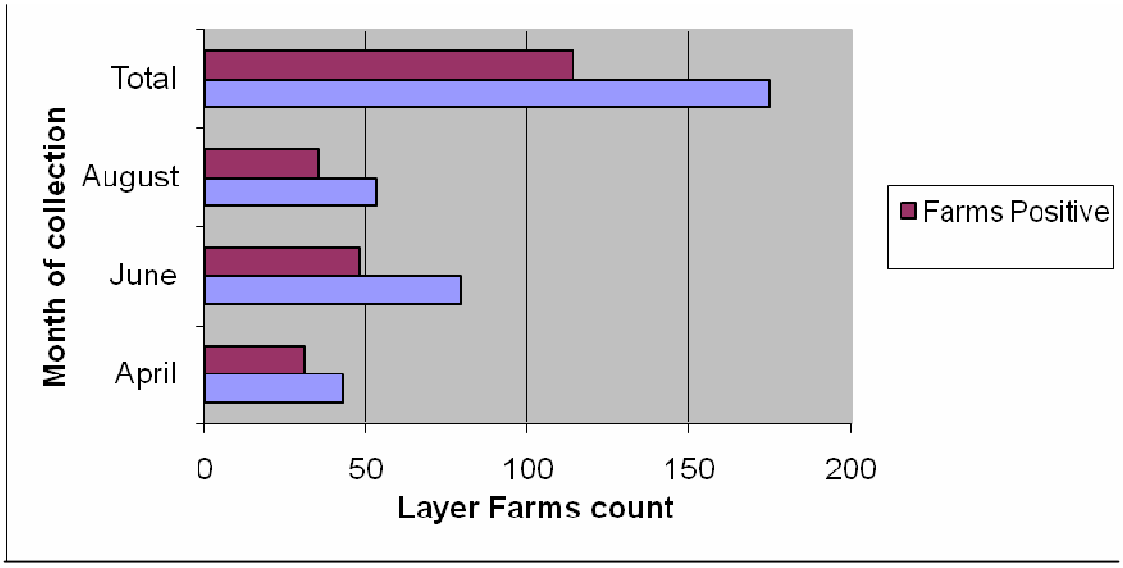


Figure15: Summary of layer farms sampled

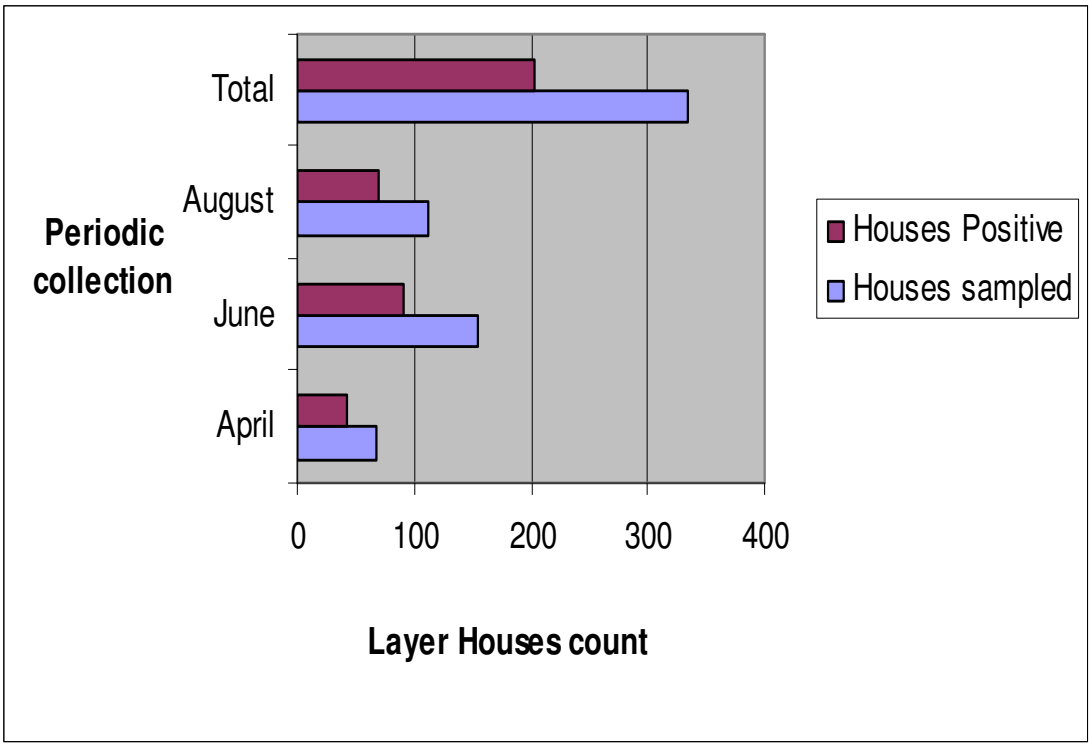


Figure16: Summary of layer houses sampled

Tables 23 and 24 list the proportion of layer farms and layer houses from each area with antimicrobial residues. The prevalence survey tool in the statistical programme Survey Toolbox* was used to calculate the 95% confidence levels of the true prevalence. 61.1 % of the farms sampled in April 2008 had antimicrobial residues in them, while 56% of the layer houses had antimicrobial residues. The proportion of layer houses with antimicrobial residues from farms affected was 91.6%.

* Survey Toolbox version 1.0 beta, by Angus Cameron, 140 Falls Road, Wentworth Falls, NSW 2782, Australia.

Table 23: Proportion of farms with antimicrobial residues, listed by area of collection in Khartoum State, April 2008

No.	Locality	Area	Total Farms	Farms Sampled	AR positive farms	AP* %	TP† %	95%CL LCL, UCL‡
1	Bahry	Shambat	16	8	7	88	100	87, 100
2	Bahry	El-mazalat	3	1	0	0	0	0, 0
3	Bahry	El-selait	13	3	2	67	89	58, 100
4	Bahry	Eid Babiker	7	2	1	50	66	26, 100
5	Bahry	El-tibna & Zakiab	18	3	2	67	89	58, 100
6	Khartoum	Gereif West	12	9	8	89	100	88, 100
7	Khartoum	Soba	11	11	7	64	85	69, 100
8	Khartoum	El-salama	5	2	1	50	66	26, 100
9	Khartoum	El-kalakla & Dekhainat	17	4	3	75	100	75, 100

Table 24: Proportion of layer houses with antimicrobial residues, listed by area of collection in Khartoum State, April 2008

No	Locality	Area	Total houses	Houses sampled	Houses positives	AP %	TP %	95%CL LCL, UCL
1	Bahry	Shambat	57	15	9	60	79	65, 94
2	Bahry	El-mazalat	7	2	0	0	0	0, 0
3	Bahry	El-selait	45	4	3	75	100	75, 100
4	Bahry	Eid Babiker	11	3	1	33	42	12, 73
5	Bahry	El-tibna & Zakiab	66	4	3	75	100	75, 100
6	Khartoum	Gereif West	34	19	14	74	99	87, 100
7	Khartoum	Soba	14	14	8	57	75	60, 90
8	Khartoum	El-salama	7	2	1	50	66	26, 100
9	Khartoum	El-kalakla & Dekhainat	38	5	4	80	100	79, 100

Farms sampled in June, 2008 showed that 60.2% of the farms had antimicrobial residues, while 54.1% of the layer houses had antimicrobial residues. The proportion of layer houses with antimicrobial residues from farms with antimicrobial residues was 89.9%.

* Apparent prevalence
† True prevalence
‡ LCL: Lower confidence limit, UCL: Upper confidence limit.

Table 25: Proportion of farms with antimicrobial residues, listed by area of collection in Khartoum State, June 2008

No.	Locality	Area	Total Farms	Farms Sampled	Farms positives	AP %	TP %	95%CL LCL, UCL
1	Bahry	El-selait	13	7	5	71	95	75, 100
2	Bahry	El-ezba	6	5	4	80	100	79, 100
3	Bahry	Hilat Kuku	8	3	3	100	100	100, 100
4	Bahry	El-kadaro& Droshab	13	5	4	80	100	79, 100
5	Bahry	El-halfaya	16	2	2	100	100	100, 100
6	Bahry	El-haj Usif & El-shigla	10	1	1	100	100	100, 100
7	Bahry	Shambat	16	4	2	50	66	37, 94
8	Bahry	El-kabashi	12	3	1	33	42	12, 73
9	Bahry	El-faki hashim	12	2	1	50	66	26, 100
10	Bahry	Elsagai	13	4	1	25	32	07, 56
11	Bahry	El-tibna & Zakiab	18	5	2	40	52	27, 77
12	Bahry	El-sababi	5	2	1	50	66	26, 100
13	Bahry	Eid Babiker	7	1	0	0	0	0, 0
14	Khartoum	Botry	6	2	2	100	100	100, 100
15	Khartoum	Soba	11	5	5	100	100	100, 100
16	Khartoum	Eid Hussien	6	3	1	33	42	12, 73
17	Khartoum	Tyba Hasanab	8	1	1	100	100	100, 100
18	Khartoum	El-shegailab	5	1	1	100	100	100, 100
19	Khartoum	El-salam	5	1	0	0	0	0, 0
20	Khartoum	El-kalakla & Dekhinat	17	13	6	46	60	45, 76
21	Khartoum	Kalakla north	5	3	2	67	89	58, 100
22	Khartoum	Gerief West	12	5	3	60	79	55, 100
23	Omdouman	Aborof	1	1	0	00	00	00, 00

Table 26: Proportion of layer houses with antimicrobial residues, listed by area of collection in Khartoum State, June 2008

No.	Locality	Area	Total houses	Houses sampled	Houses positives	AP %	TP %	95%CL LCL, UCL
1	Bahry	El-selait	45	18	9	50	66	52, 79
2	Bahry	El-ezba	11	10	9	90	100	89, 100
3	Bahry	Hilat Kuku	15	6	5	83	100	83, 100
4	Bahry	El-kadaro& Droshab	57	20	18	90	100	92, 100
5	Bahry	El-halfaya	82	5	4	80	100	79, 100
6	Bahry	El-haj Usif & El-shigla	28	2	2	100	100	100, 100
7	Bahry	Shambat	57	8	6	75	100	83, 100
8	Bahry	El-kabashi	25	6	3	50	66	43, 89
9	Bahry	El-faki hashim	62	6	3	50	66	43, 89
10	Bahry	El-sagai	65	7	1	14	16	02, 31
11	Bahry	El-tibna & Zakiab	66	8	2	25	32	14, 49
12	Bahry	El-sababi	10	4	2	50	66	37, 94
13	Bahry	Eid Babiker	11	1	0	00	00	00, 00
14	Khartoum	Botry	23	4	3	75	100	75, 100
15	Khartoum	Soba	14	8	7	88	100	87, 100
16	Khartoum	Eid Hussien	7	3	1	33	42	12, 73
17	Khartoum	Tyba Hasanab	25	2	1	50	66	26, 100
18	Khartoum	El-shegailab	8	1	1	100	100	100, 100
19	Khartoum	El-salama	7	1	0	00	00	00, 00
20	Khartoum	El-kalakla & Dekhinat	38	20	7	35	45	33, 57
21	Khartoum	El-Kalakla north	8	6	3	50	66	43, 89
22	Khartoum	Gerief West	34	7	4	57	75	54, 97
23	Omdouman	Aborof	3	1	0	00	00	00, 00

In August 2008 sampling, 68.7% of the farms had antimicrobial residues on them; while 57.1% of the layer houses antimicrobial residues were detected. The proportion of layer houses with antimicrobial residues from farms with antimicrobial residues was 83.1%.

Table 27: Proportion of layer farms with antimicrobial residues, listed by area of collection in Khartoum State, August 2008

No.	Locality	Area	Total Farms	Farms Sampled	Farms positives	AP %	TP %	95%CL LCL, UCL
1	Bahry	Shambat	16	3	2	67	89	58, 100
2	Bahry	El-mazalat	3	1	1	100	100	100, 100
3	Bahry	Soba East	4	2	1	50	66	26, 100
4	Bahry	El-sababi	5	2	1	50	66	26, 100
5	Bahry	El-haj usif & El-shigla	10	2	2	100	100	100, 100
6	Bahry	El-sagai	13	3	2	67	89	58, 100
7	Bahry	El-halfaya	16	3	2	67	89	58, 100
8	Bahry	El-kadaro & Droshab	13	4	3	75	100	75, 100
9	Bahry	El-faki hashim	12	4	4	100	100	100, 100
10	Bahry	Hilat Kuku	8	1	1	100	100	100, 100
11	Bahry	El-samrab	5	2	0	0	0	0, 00
12	Bahry	El-selait	13	3	3	100	100	100, 100
13	Bahry	El-kabashi	12	1	1	100	100	100, 100
14	Bahry	El- tibna & Zakiab	18	3	0	00	0	0, 00
15	Bahry	Eid babiker	7	4	2	50	66	37, 94
16	Khartoum	El-kalakla & Dekhinat	17	4	2	50	66	37, 94
17	Khartoum	El-shegailab	5	4	2	50	66	37, 94
18	Khartoum	Soba	11	5	4	80	100	79, 100
19	Khartoum	Gerief West	12	2	2	100	100	100, 100

Table 28: Proportion of layer houses with antimicrobial residues, listed by area of collection in Khartoum State, August 2008

No.	Locality	Area	Total houses	Houses sampled	Houses positives	AP %	TP %	95%CL LCL, UCL
1	Bahry	Shambat	57	5	4	80	100	79, 100
2	Bahry	El-mazalat	7	2	1	50	66	26, 100
3	Bahry	Soba East	7	5	2	40	52	27, 77
4	Bahry	El-sababi	10	4	1	25	31	07, 56
5	Bahry	El-haj usif & El-shigla	28	5	4	80	100	79, 100
6	Bahry	El-sagai	65	7	4	57	75	54, 97
7	Bahry	El-halfaya	82	9	8	89	100	88, 100
8	Bahry	El-kadaro & Droshab	57	10	6	60	79	62, 97
9	Bahry	El-faki hashim	62	10	5	50	66	48, 84
10	Bahry	Hilat Kuku	15	3	2	67	89	58, 100
11	Bahry	El-samrab	17	5	0	00	00	00, 00
12	Bahry	El-selait	45	10	10	100	100	100, 100
13	Bahry	El-kabashi	25	3	2	67	89	58, 100
14	Bahry	El- tibna & Zakiab	66	3	0	00	00	00, 00
15	Bahry	Eid babiker	11	7	3	43	56	35, 77
16	Khartoum	El-kalakla & Dekhinat	38	8	5	63	84	64, 100
17	Khartoum	El-shegailab	8	5	2	40	52	27, 77
18	Khartoum	Soba	14	8	6	75	100	83, 100
19	Khartoum	Gerief West	34	4	4	100	100	100, 100

The comparison of the overall proportions of farms and layer houses with antimicrobial residues in the three seasonal sampling periods revealed insignificant difference with p-values of 0.57 and 0.88 respectively. These results are shown in Table 29 and 30.

Table 29: Comparison between proportions of farms with antimicrobial residues in the three periodic collections

Month of collection	Proportion	Sample size
April 2008	61.10%	43
June 2008	60.20%	79
August 2008	68.80%	53
Uncorrected chi-square = 1.1		
DF = 2		
P-value= 0.578		

Table 30: Comparison between proportions of layer houses with antimicrobial residues in the three periodic collections

Month of collection	Proportion	Sample size
April 2008	56.00%	68
June 2008	54.10%	154
August 2008	57.10%	113
Uncorrected chi-square = 0.25		
DF = 2		
P-value= 0.884		

A comparison between proportions of layer farms in each area that were identified with antimicrobial residues for each period of collection (April, June and August) is shown in Tables 31 and 32. Only 'El-selait' and 'El-tibna and Zakiab' showed a significant difference between the three periods with p-value 0.02 and 0.08 respectively.

Table 31: Comparison between proportions of layer houses in each area with antimicrobial residues in the three periodic collections

Area	April		June		August		Chi-square	DF	p-value
	AP %	Sample size	AP%	Sample size	AP%	Sample size			
Shambat	60	15	75	8	80	5	0.95	2	0.62
El-selait	75	4	50	18	100	10	7.56	2	0.02
Eid Babiker	33	3	00	1	43	7	0.72	2	0.69
El-tibna & Zakiab	75	4	25	8	00	3	4.88	2	0.08
Gereif West	74	19	57	7	100	4	2.42	2	0.29
Soba	57	14	88	8	75	8	2.46	2	0.29

Table 32: Comparison between proportions of layer houses in each area with antimicrobial residues in June and August collections

Area	June		August		Chi-square	DF	p-value
	AP%	Sample size	AP%	Sample size			
Shambat	75	8	80	5	0.04	1	0.83
El-sababi	50	4	25	4	0.53	1	0.46
El-haj usif & El-shigla	100	2	80	5	0.47	1	0.49
El-sagai	14	7	57	7	2.83	1	0.09
El-halfaya	80	5	89	9	0.21	1	0.64
El-kadaro & Droshab	90	20	60	10	3.75	1	0.05
El-faki hashim	50	6	50	10	0.0	1	1.0
Hilat Kuku	83	6	67	3	0.3	1	0.58
El-selait	50	18	100	10	7.37	1	0.006
El-kabashi	50	6	67	3	0.23	1	0.62
El -tibna & Zakiab	25	8	00	3	0.92	1	0.33
Eid babiker	00	1	43	7	0.69	1	0.4
El-kalakla & Dekhinat	35	20	63	8	1.83	1	0.17
El-shegailab	100	1	40	5	1.2	1	0.2
Soba	88	8	75	8	0.45	1	0.5
Gerief West	57	7	100	4	2.37	1	0.12

Arc View 9.3 was used to illustrate the density and location of layer farms in Khartoum State. Figures 17 and 18 show the density of layer farms and location of layer farms sampled in the entire State.

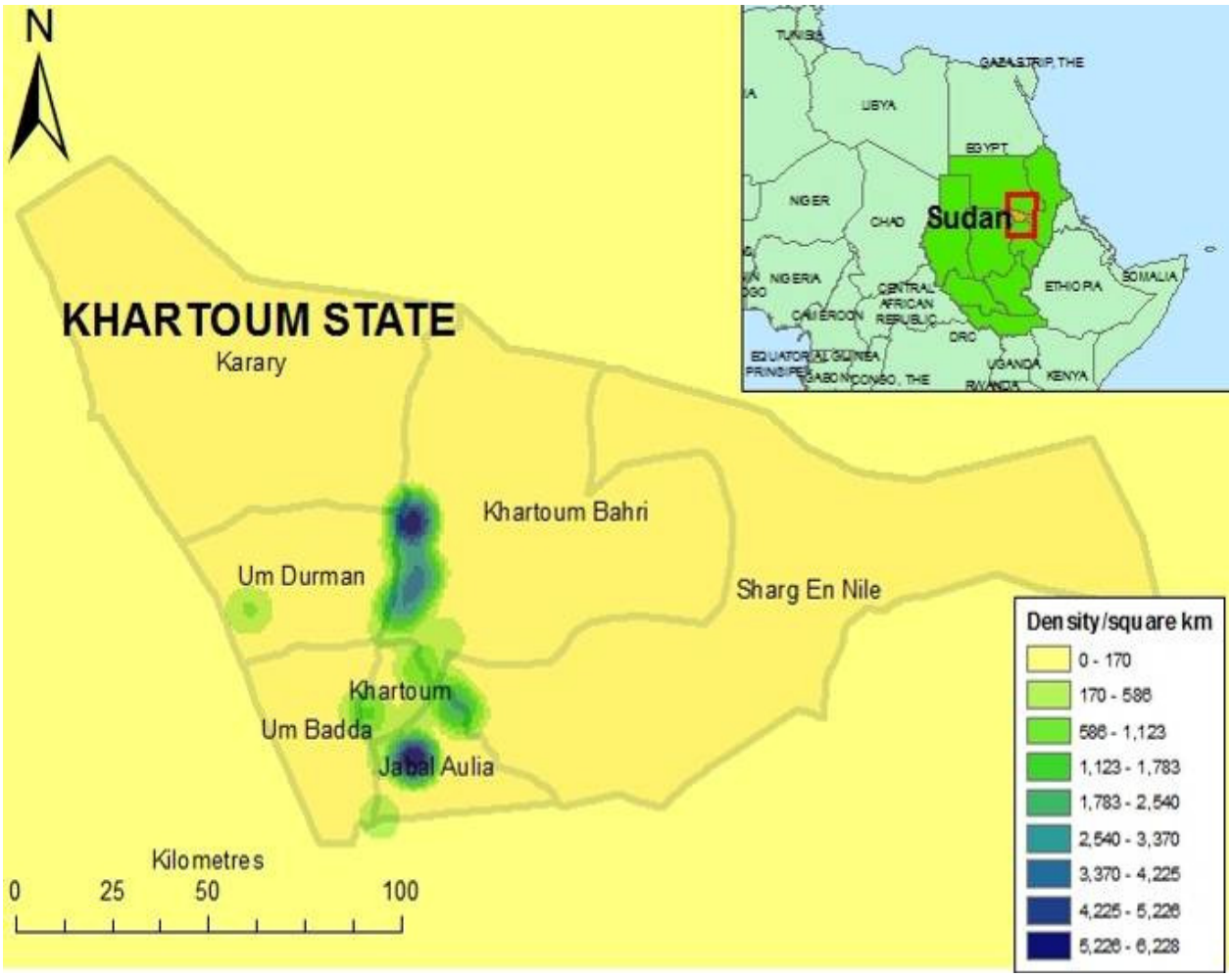


Figure 17: Layer farms density, Khartoum State, 2008

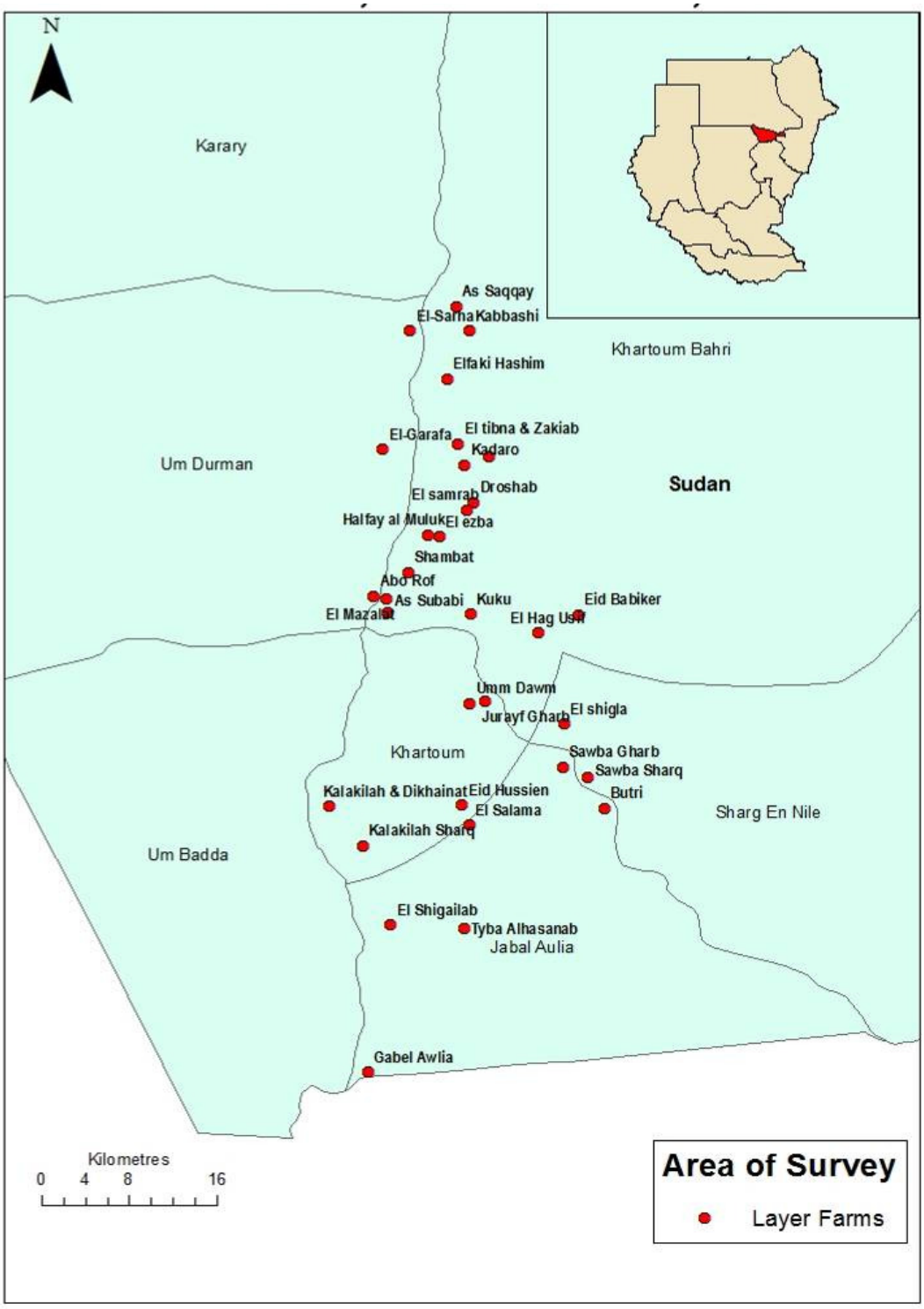


Figure 18: Farm locations, Khartoum State, Sudan

Figures 19, 20 and 21 show the prevalence of antimicrobial residues detected in eggs in the three periods of surveillance April, June and August respectively.

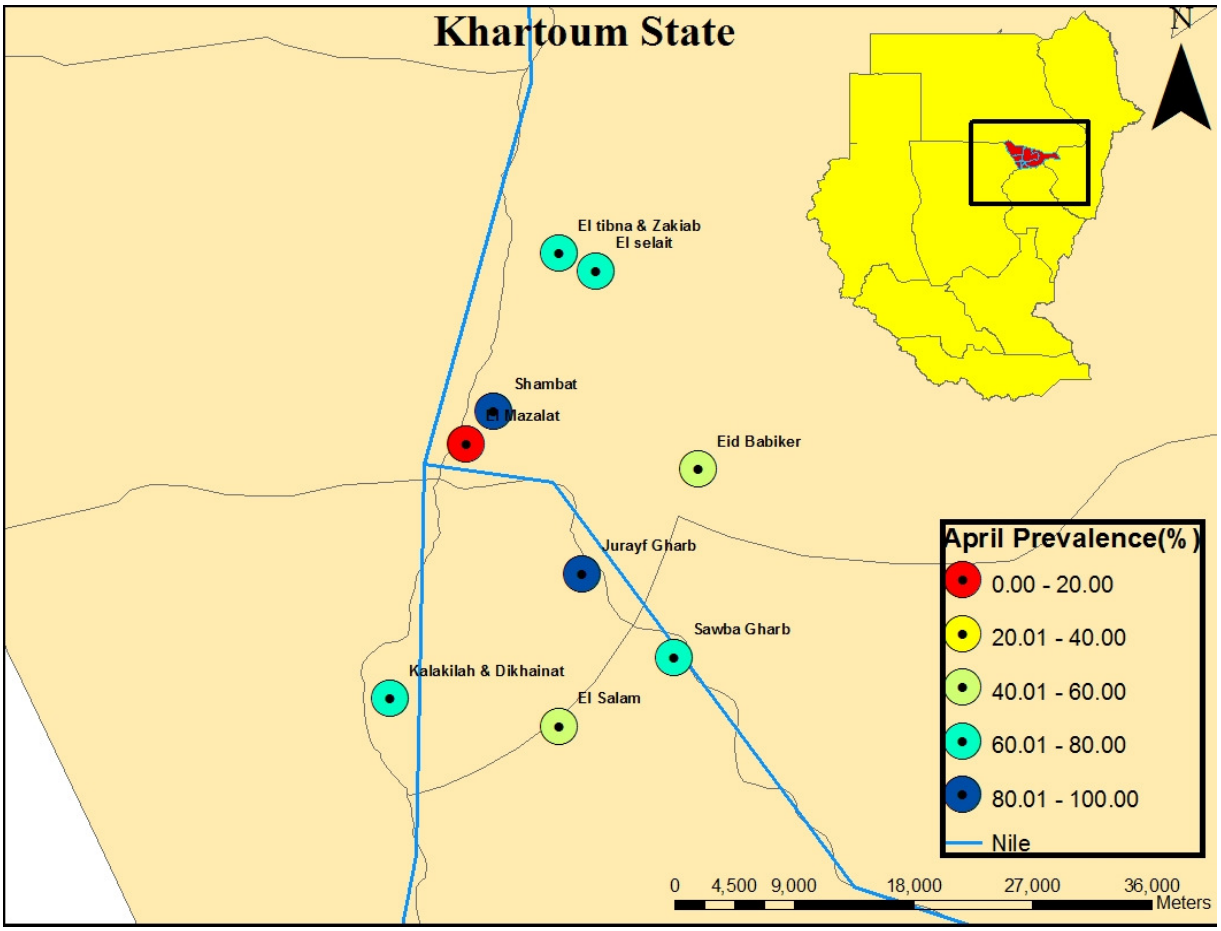


Figure 19: Prevalence of antimicrobial residues, April 2008

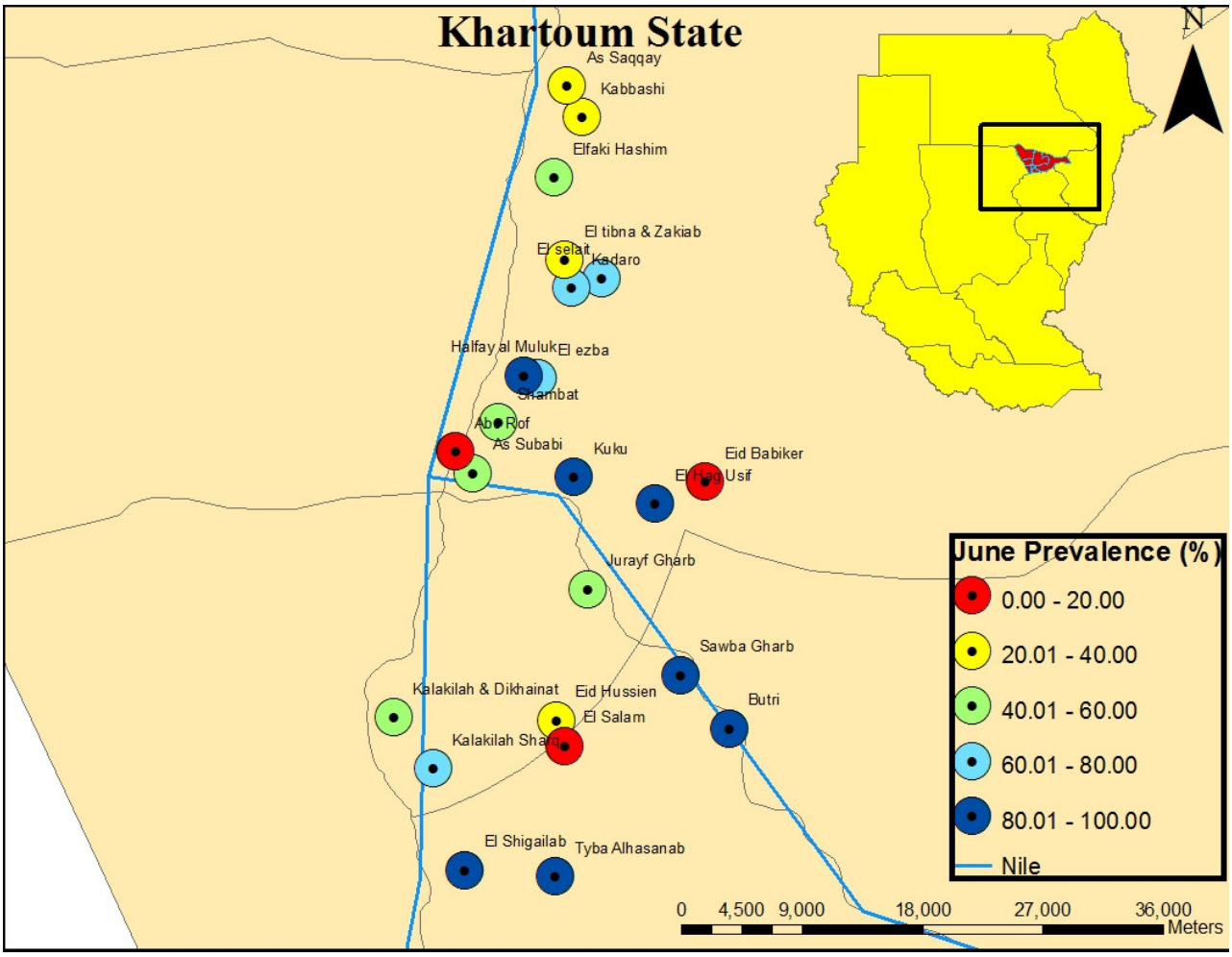


Figure 20: Prevalence of antimicrobial residues, June 2008

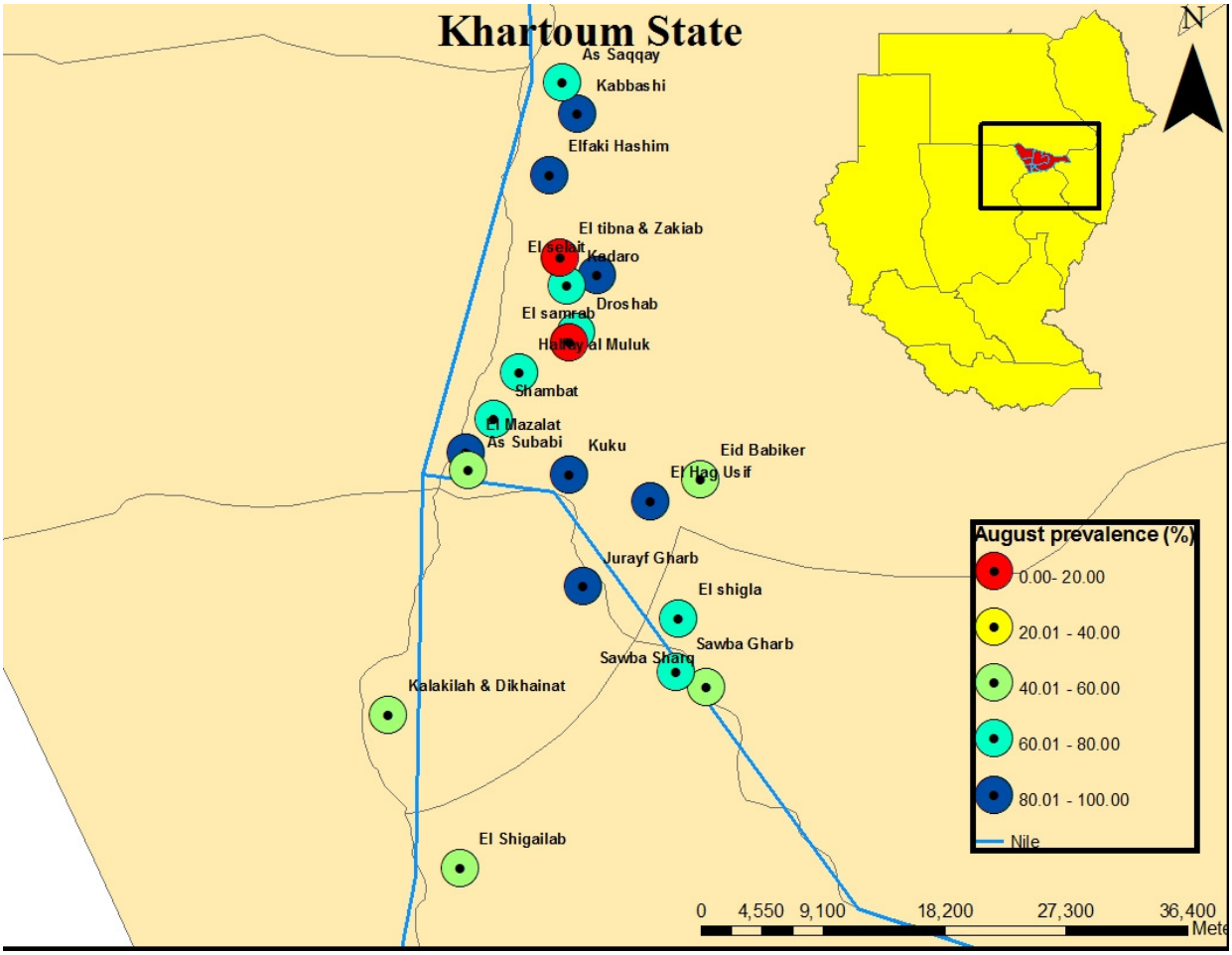


Figure 21: Prevalence of antimicrobial residues, August 2008

CHAPTER V-DISCUSSION

1. Census of layer farms in Khartoum State, Sudan

A census of layer farms in Khartoum State, Sudan, was conducted in the period between December 2007 and January 2008. The aim was to capture data on the total number of layer farms, total number of layer houses on each farm, the total capacity of birds and the farming systems (Appendix VI). This census was necessary as the unpublished census conducted in 2006 after the AI outbreak, only recorded farms affected by Avian Influenza (AI) and did not differentiate between broiler and layer farms. This census proved to be a challenge as most farms are not registered with the local authorities, the land ownership or occupancy was not always recorded and the land use was fluid. Therefore, the investigator had to visit farms individually to confirm their status, taking up valuable time and resources. The main information on farm locations was dependent on the internal data of the State Ministry of Agriculture and Animal Resources and Irrigation, beside the information gathered from field veterinarians and farm owners' guidance to other farms in the area. In addition, the day old chick suppliers provided useful data concerning layer farms in the state. The last census conducted by the State Ministry of Agriculture and Animal Resources and Irrigation showed that there were 527 farms in Khartoum State (Table 4). The present census revealed that there were 252 layer farms in the State, where 166 farms (65.9%) were located in Bahry, 78 farms (30.9%) in Khartoum and 8 farms (3.2%) in Omdurman Locality. Of 764 layer houses, 573 houses (75%) were in Bahry, 172 houses (22.5%) in Khartoum and 19 houses (2.5%) in Omdurman. This was 152 more than the number estimated in 2006 (unpublished census conducted in 2006 after the AI outbreak). The total capacity of houses in the state was estimated at 2 221 800 birds, divided among the three localities as 1 301 500 birds (58.6%) in Bahry, 853 300 birds (38.4%) in Khartoum and 67 000 birds (3%) in Omdurman. The capacity of layer houses was calculated depending on the standard number of birds to be allocated in each meter square in the open system (5-7 birds/ m^2). Worldwide, the number of laying hens is estimated to be 4.93 billion. The numbers of birds in The Sudan is, therefore, very small compared to the major egg producers of the world.

Most of the farms in Bahry Locality were small holdings. About 10-20 farms aggregated closely together in each area along the Nile, with most farms neighboring each other. In Khartoum Locality, most of the large farms (over 50 000 birds) were in the area between the White and Blue Nile Rivers (Figure 6). 60% of the intensive laying farms were located in Khartoum Locality. These areas are separate from urban development and designated for agriculture.

The majority of farms (92.1%) were open traditional housing system (Figure 3), which made use of local materials for construction. Birds in these houses are not as well protected from the rain and high ambient temperatures as controlled environmental housing. Biosecurity systems in this type of farming were very poor or absent and the occurrence of respiratory, enteric and gastrointestinal diseases were common (43% of the farms surveyed reported diseases at the time of the surveillance). Therefore traditional laying houses tended to have a lower carrying capacity compared to semi-closed or intensive closed systems, since the later can control environmental changes and apply better biosecurity measures..

Almost 50% of the farms recorded at the time of the census were not producing eggs for the following reasons:

1. Many farmers lacked the financial resources to restock their farms after the depopulation and condemnation of carcasses resulting from the 2006 Avian Influenza (AI) outbreak. The Sudanese government had only compensated the farmers with 60% of the direct cost (carcass price) divided into three payments. At the time of the census, many farmers had not yet received full compensation. This problem was compounded by the fact that the animal feed prices increased dramatically during 2008.
2. A shortage of day-old chicks because the suppliers were unable to meat the whole demand associated with the partial ban of day old chicks and fertile eggs imports after the AI outbreak. This lead to an increase in the price of day-old chicks making it cost ineffective for small scale producers.
3. The lack of government protection of small-scale producers made them highly vulnerable to the effects of disease, market forces and the weather. Furthermore there were no State or industry run disease control programmes, therefore the introduction of diseases such as Newcastle Disease and salmonellosis caused

massive fatalities and chronic respiratory disease resulted in severe production losses.

5. Farmers making use of traditional housing for breeding layers or broilers can only produce during the cooler winter months (from late October till December).
6. Some farms were in the downtime period preparing for another cycle.
7. The pullets were not yet in lay.
8. Farmers switched from layer to broiler production. Usually in Sudan, farmers raise day-old laying chicks till they start laying and then continue in the same house till the end of their production cycle. After the culling of the batch the farmer may use the same farm for producing broilers or start a new cycle of layers.

The data provided by this census does not therefore cover the full production potential of the Sudanese layer industry. It does, however, provide a baseline and guide for researchers and officials wishing to compile a more complete database concerning the poultry industry. In addition to that it will serve as a primary source of data for all who are related to the Sudanese poultry industry and the country will benefit from the data. The government in Sudan has subsequently established a census forum to create their own database of all livestock farms in the state and they may benefit from the data provided in this research.

2. Questionnaire survey

Ninety two farms participated in the questionnaire survey conducted in April 2008. The participants of the questionnaire were 52% of the total number of farms (178 farms) in the areas surveyed. The main reason for the low participation was due to the fact that the farms were not in production at the time of the questionnaire. Other reasons included the absence of the farm owner or manager or refusal to participate. The validity of the questionnaire was assessed by comparing answers with reliable data i.e. 95% of the respondent said that they don't know about any rules and regulations of antibiotic usage, which actually doesn't exist. Furthermore, all questions related to ARs implications were almost answered in the same way. For example, 89% of the farmers don't believe that drugs in eggs affect human, 85%don't believe that drugs pass from chicken body to eggs and 98% sell eggs during and after using drugs.

2.1 Demographic data:

As shown in Table 14, layer farms in 17 different production areas were surveyed. Eleven areas (64.7%) were in Bahry Locality, 35.3% in Khartoum Locality, while no farms were surveyed in Omdurman Locality. The reason for the absence of Omdurman Locality there were no farms in production in this locality while the survey was conducted. In addition to that, as shown in the census above Omdurman has a small number of farms compared to Bahry and Khartoum Localities. About 59% of the farms that were surveyed were in Bahry Locality while 41% were in Khartoum Locality. A high proportion of farms were surveyed in El-Kalakla North (100%), El-Kalakla & Dekhinat (94%) and Soba (82%) areas. In contrast, farms in Tyba Hasanab were the least surveyed (13%) because the area was occupied by companies serving as competitors for the company of the survey conductor and were not willing to participate in the survey. Few farms were questioned in El-samrab and El-shegailab because the total number of farms in these areas was few and houses were found to be used as layer rearing houses at the time of the survey.

Only one closed system out of twelve closed systems in Khartoum State was prepared to take part in the questionnaire. The fact that the investigator was employed by a rival company was perceived to be a threat. Therefore, essentially only farms with traditional open houses participated in the questionnaire. Most of the farmers were small scale producers that had a maximum of 1,000 birds (95%) that were mainly distributed into one or two houses (83%).
The most common breed in the survey was Hi-sex (51%) as it is considered to be the most tolerant of the breeds to high ambient temperatures. In addition to that, Hi-sex was introduced to Sudan over three decades ago and the local supplier had built a good business relationship with the farmers. Nevertheless, Lohman (13%) and Hyline (5%) both only introduced to the Sudanese market in the last seven years by foreign companies were starting to take a good share of the market. Bovan breed (Dutch breed introduced to Sudan in the 1980s) shared 10% of the total breeds found in the state, while the unknown breeds, not including indigenous breeds, were 14%.

In 68% of the surveyed farms the age of the flocks varied from 4-12 months of age. Thirty seven percent of the farms surveyed had multiple ages on the same farm. These

were farms with more than one house. It must be reported that the distance between layer houses within a farm or between farms was less than 30 meters, indicating that a true all-in-all-out system was not practiced.

2.2 Antimicrobial use Patterns

It was clear from the survey that traditional farming systems relied heavily on antimicrobial medication to control disease, as 48.9% of farms surveyed were treating their flocks with antimicrobials, while a further 9.1% had used antimicrobials within 3 months prior to this survey. The fisher exact result showed that there was no association between antimicrobial use and the presence of antimicrobial residues in eggs ($P<0.44$).

The main purpose of using antibiotics was to treat (61%) a variety of diseases including salmonellosis (29.5%) and chronic respiratory disease (25%). In fact there was a significant association between antimicrobial therapy ($P<0.01$) and disease, with the odds that farms that had disease would be treating with antibiotics being 21 times more than farms currently free from disease and 18 times more on those that had diseases in the last three month which had been treated with antibiotics. This high level of disease is believed to be as a result of the type of housing, poor environmental sanitation, poor biosecurty, close grouping of farms and poor management.

Although, almost all the antibiotic classes were found in the Sudanese market for purchase either as separate products or as products with a combination with multivitamins and minerals, the highly competitive price of oxytetracycline, its broad-spectrum coverage and its combination with multi-vitamins led to it being the most commonly used antibiotic; 24.5% in current use and 22.9% having used it in the last three months. These findings agree with Babiker *et al*., (2009), who classified Salmonellosis and respiratory disease as highly prevalent in layer flocks in Khartoum State. Oxytetracycline appears to be widely used on poultry farms in Africa as Mitema *et al*., (2001), Kabir *et al*., (2004) and Nonga *et al.,* (2009) found that it was the most used antibiotic in Kenya, Nigeria and Tanzania respectively. Other commonly used antibiotics were tylosin (18.7%) which is used to treat infectious coryza and *Mycoplasma* infections

in birds, the broad-spectrum enrofloxacin (14.3%) and colistin (14.3%) which is used to treat diarrhoea (Reinhardt *et al.,* 2005).

Prophylactic antimicrobial therapy was less common (13%) and tended not to be associated with disease ($p<0.15$). This finding was expected because small scale farmers may not be able to treat prophylacticaly due to their limited resources.
The two variables: antibiotic use (outcome variable) and disease on farm (exposure variable) were stratified with the variable (uses of antibiotics), which has four strata (therapeutic, prophylactic, therapeutic and prophylactic and others). The adjusted odds ratios differed from the crude odds ratio, which means there is a confounding effect on both variables (outcome and exposure) controlled by stratification.

As is the trend in all poultry production systems globally, drinking water medication is frequently used (Vermeulen *et al.,* 2002). The preferred method of treatment by 97% of the farms was by the mass medication of drinking water. Feed was not used as a route of administration because the feed mills used for food preparation don't provide a high quality mixture (non-homogenized) of small quantities of drugs in feed resulting in slow absorption and uneven distribution of drugs in feed. Furthermore, sick birds will continue to drink, but will not eat.

2.3 Regulatory and public health awareness

There was a significant association between those (85% of respondents) who believed that drugs don't pass from the hen's body to her eggs and those (89% of respondents) who don't believe drugs in eggs can affect humans ($P<0.02$). Furthermore 75% of the farmers did not understand the concept of a drug withdrawal period in eggs. An overwhelming majority of respondents (95%) were not aware of any government regulations pertaining to the sale of eggs during the withdrawl period of antimicrobials. Therefore it was not surprising that 98% of the farms questioned continued selling eggs while their hens were on antibiotic treatment. The lack of knowledge of the withdrawal periods are greater than for farmers from Tanzania (Nonga *el al*., 2009) where 80% knew about the withdrawal period, but still sold eggs during this period. Like the Sudanese poultry farmers, the Tanzanian farmers were unaware that antimicrobials in eggs have any detrimental effect on humans.. It is difficult to farm without antibiotics

especially in a situation like Sudan, but simple regulations to avoid certain antibiotics and follow withdrawal periods are needed.

Quality control measures applied to egg products such as cracked eggs, grading of eggs, cleaning of dirty eggs or fumigation of eggs was not a common procedure for farmers.

3. Laboratory analysis for validation of new in-house method for antimicrobial screening

The main reason of conducting a susceptibility test for the three bacteria to the several antibiotics using the Kirby-Bauer test was to evaluate the practicality and performance of *B. megatarium* as a screening organism. *S. aureus* and *E. coli* were used as a quality control for the test to assure that they were working for all antibiotics test, as well as to compare the relative susceptibility of *B. megatarium.*

The MIC results of *B. megatarium* when eggs were used and without eggs were not affected by inhibitory substances in the eggs, probably because of the low concentration of egg material used or the fact that the egg used had low levels of lysozyme. It was found that the bacterium could be used to detect antibiotic residues of ampicillin, gentamicin, enrofloxacin, tylosin, erythromycin and tiamulin at or below the MRL, while colistin, doxycycline, fosfomycin, lincomycin, trimethoprim, spectinomycin and sulphadimidine were not detectable below the maximum residue limit.

S.*aureus* was not affected by inhibitors in egg samples but it was able to detect antimicrobial residues only for ampicillin, neomycin, erythromycin, trimethoprim and lincomycin. Since *B. megatarium* was not considered broadspectrum enough to act as screen, to was decided to test *G. stearothermophilus.* This paid off as *G. stearothermophilus* was proven to be more sensitive than *B. megatarium* could detect more antibiotic classes. On the other hand, *G. stearothermophilus* was able to detect all the antibiotics screened at or below the MRL excluding sulfadiazine, trimethoprim, spectinomycin and fosfomycin. The effective inactivation of lysozyme and other bacterial inhibitors in the samples, by heating the samples to 80℃ for ten minutes and the use of the thermophilic bacteria *G. stearothermophilus*, resulted in a test with high sensitivity and able to detect 11 antibiotics (Table 16) and a test capable of detecting levels at or

below the MRLs and The Premi®test values. In contrast, *B. megatarium* was able to detect six of the antibiotics tested. (Table 22).

When antimicrobials are administrated to chickens at the therapeutic dose for a relatively short period (7 days), residues can be detected in eggs within the period of administration. This was expected, because the level of the antibiotic in the plasma of the hens is high as the birds were individually given the antimicrobials by oral gavage. As stated by Kan & Petz (2000), residues of antimicrobials are first seen in the albumin as a reflection of the plasma levels and that generally happens 2-3 days after administration. This was different to what was noted in the study where eggs were only positive as long as the antibiotic was administrated. This indicates the possible reason why antimicrobial residues were not detected in eggs shortly after the stoppage of administration may be due to their short withdrawal periods such as erythromycin, tiamulin and oxytetracycline, with the exception of trimethoprim which has a relatively long withdrawal period of 10 days.

Antimicrobial residues found in the yolk are a result of the plasma levels accumulating for the 10 days of the yolk's formation phase; these residue levels in yolk can fluctuate depending on the period of the drug exposure. Furthermore, chickens need to be exposed to antimicrobials for approximately 8 to 10 days for the residues to reach a constant level. It also takes 8-10 days thereafter for residues to be absent. These periods of time are a reflection of the rapid growth phase of the follicles which takes approximately 10 days (Kan & Petz, 2000). A single exposure to a drug might be enough to detect the drug in yolk or albumen depending on the type of the drug and the test used. In addition to that, drug clearance from the yolk and albumen relies on the plasma levels of the drug tested; thus, the higher the plasma level, the longer the clearance time.

4. Antimicrobial residues surveillance

The total number of farms sampled was 175 farms in the three periods of collection. Forty three farms (24.6%) were sampled in April, 79 farms (45.1%) and 53 farms (30.3%) were sampled in June and August 2008 respectively. The layer houses (sampling unit) sampled were 335 houses in the three periods of collection. Sixty eight

layer houses (20.3%) were sampled in April, 154 houses (46%) in June and 113 houses (33.7%) in August 2008.

In total 933 egg samples were analyzed, 197 samples (21.1%) from the April sampling, 427 (45.8%) from June and 309 samples (33.1%) from the August sampling. Eggs were collected randomly from each layer house and all productive layer houses in the state were sampled. Selection bias was unlikely to occur, except for the last point where bias may occur, farms were only excluded in the following situation:

- Non egg-producing farms (rearing period).
- The farm location is unknown.
- A company very strict with biosecurity.
- Unwillingness of the owner to participate in the study.

The variation of the sampled houses had the same causes as given for the farm sampling. It was clear that June sampling had the highest number of sampled farms, but April sampling had almost the same number of farms as June when the 37 farms of which eggs were damaged are added. The August sampled farms were the least because of the increased feed prices, which forced small producers to leave the business by either selling their flocks as spent hens or depopulating their flocks. In addition to that, August is the rainy season in The Sudan, which results in damage to the inadequate poultry housing causing farmers to avoid having laying flocks in this season.

4.1 Antimicrobial residue surveillance in Khartoum State, April 2008

The overall proportion of layer farms where antimicrobial residues were detected was 61.1%, while the total layer houses where antimicrobial residues were detected was 56%. The farm was considered positive if any at least one of the houses showed positive results for antimicrobial residues. It is worth mentioning that there were some farms with only one house with antimicrobial residues, while the rest are negative, resulting in less houses with antimicrobial resides as compared to farms with antimicrobial residues. In addition to that, it was observed within the layer house, one egg sample may be positive and the other two samples negative. With the assumption that all hens were treated at the same time this situation may be explained according to the following hypothesis:

- Eggs collected in the last day of the drug treatment or the day after that resulting in very low concentration in some eggs of the same farm.

- Individual variation of hens in secretion and absorption of antibiotics and residues.
- The dose administered was not optimized across the house, nor was the dosage correct i.e. under dosing may have occurred.
- Birds may have variable intake of medicated water.
- Poor quality or the use of expired or degraded antibiotics.
- Although highly repeatable (100%), the test used was not sensitivity enough to detect some classes of antibiotics in eggs.

A high prevalence of antimicrobial residues in April was detected in farms in Shambat area (88%), Gerief Garb (89%), El-Kalakla (75%), El-Tibna and Zakiab (67%) and El-selait (67%). These areas are characterized by high density of farms, increasing the risk of spread of diseases between farms. The other major problem was the large number of broiler farms among the layer farms and birds of different ages on the same farm. El-mazalat had no antibiotic residues. However, only one farm from a total of three farms was sampled in this area. The results of antimicrobial residues detected in farms in the areas mentioned above, when observed from "house sampling" point of view were also higher than the rest of the areas. The proportion was slightly lower than the proportion of layer farms sampling in the same area for the reasons mentioned earlier.

4.2 Antimicrobial residue surveillance in Khartoum State, June 2008

Farms with antimicrobial residues represented 60.2% of the total farms screened in June 2006, while 54% of all layer houses sampled had antimicrobial residues; this was less than in August. Several areas sampled showed 100% prevalence such as Hilat Kuku, El-Halfaya, El-Haj Usif & El-Shigla, Botry, Soba, Tyba Hasanab and El-Shigailab. During this sampling period a small number of farms were sampled in each area due to the dramatic increase in feed prices, which resulted in the depopulation of many flocks. Eid Babiker, El-Salama and Abrof had the lowest prevalence (0%). However, the sample size was not large enough to declare these areas as free from antimicrobial residues (only one house was sampled). Almost 40% of the areas screened in June had prevalence of 80% or above. The climatic change in June (the beginning of the rainy season) may serve as one of the factors for the significant increase of antimicrobial usage in June compared to April. This finding was evidenced by the total number of

areas with low prevalence (≤ 50%), which were only six areas out of 23 areas, while in April they were three out of nine areas.. In total 22% of the all layer houses in the state were sampled in June 2008. The remaining houses were not productive during the time of the survey. Few farms (9.4%) were sampled in areas of highest layer house densities (El-Halfaya, El-sagai and El-Tibna & Zakiab), which did not reflect the true picture of the prevalence in these areas.

4.3 Antimicrobial residue surveillance in Khartoum State, August 2008

August falls in the middle of the rainy season in Sudan (June-October). Although the temperature decreases in August, most of the traditional open-house farms are affected by rains, due to the inadequate housing system and high humidity inside the layer houses. In addition, the poor hygiene measures during this season increase the incidence of diseases in the entire industry. Almost sixty nine percent (68.7%) of the farms sampled showed antimicrobial residues in the eggs which were slightly higher than April and June sampling. The same trend was observed when results were compared by poultry house, rather than by farm. A high prevalence (100%) of AR was seen in Shambat , El-haj Usif & El-shigla, El-faki Hashim, Hilat Kuku, El-selait, El-kabashi and Gerief West. All the above-mentioned areas were located along the Nile, and layer houses were affected by the moist environment. El-samrab was the only area that didn't have antimicrobial residues. Since only two farms were sampled, this result is not statistically significant.

4.4 Comparison of the surveillance results between the three periodic collections

There was no significant difference (p=0.57) in the overall number of farms or layer houses (p=0.88) with antimicrobial residues during the three periods of collection. However, there was a significant difference between individual sampled areas in the three collection periods. "El-selait" and "El-Tibna & Zakiab" showed a significant difference among the three periods of collection (p=0.02) and (p=0.08) respectively. El-sagai (p=0.09), El-kadaro & Droshab (p=0.05) and El-selait (p=0.006) were significantly different between June and August.

The main explanation for not finding any significant difference along the three periods of collection is mainly due to the effect of the factors associated with the presence of

antimicrobial residues during the year in laying farms. The farmers use antibiotics all year round the year in their production. Antibiotics are used to try to counter the factors such as inadequate housing, presence of diseases, effect of the hot environment, stress, low quality of chicks and poor biosecurity measures. Furthermore, the lack of understanding of the effects of antimicrobial residues on human consumers is an associated factor.

4.5 Comparison of Khartoum State antimicrobial residue results with the results of other countries

Published data on antimicrobial residues in eggs or even in chicken meat is scarce. This section highlights some results of surveys carried out in several countries, including results on surveys done on chicken meat as well, for comparative purposes.

Available data suggests that ARs may be present in a large proportion of poultry products in developing countries, especially in Africa, the Middle East and South America. For instance almost similar results to our study were published by Al-Ghamdy *et al*., (2000), who reported an AR prevalence of 69.7% in chicken meat and 60% in eggs sampled from the eastern province of Saudi Arabia.

A study conducted at Tehran, Iran in 2006 by Salehzadeh *et al.,* HPLC was used for separating, detecting and analyzing of oxytetracycline residues in samples from 86 farms 95% of the farms showed residues of oxytetracycline above the MRLs.

Nonga *et al*., 2009, curried out a study to assess antimicrobial residues in commercial chicken eggs in Morogoro Municipality in Tanzania. The study showed that all eggs sampled (70 eggs) and analyzed with the Delvotest Kit were positive for antimicrobial residues, but the same samples showed 21.4 % of antimicrobial residues when analyzed with agar diffusion test. The difference in performance of the two tests may be explained by differential sensitivities of the test organisms, that is, *G. stearothermophilus var. calidolactis* for the Delvotest as compared with *B. subtilis* for the agar well diffusion test. The study concluded that the presence of antimicrobial residues in eggs in the municipality could be of public health significance to the egg consumers.

Research done by Adesiyun *et al*. (2005), showed that the prevalence of ARs in eggs in Trinidad was (6.5%) from eggs collected from farms, (16.1%) from malls and (15%) from supermarkets. The results of this study are very low compared to the findings of this research which showed a very high prevalence of above 60% in total.
In a study conducted in Senegal to assess ARs in poultry products revealed that 20% of the poultry farms sampled in 2001-2002 had antimicrobial residues in their meat products, 43% of the meat samples sampled in 2003 had ARs in them. (Alambedji *et al.,* 2008).

Most of the above studies show ARs prevalence similar to or higher than those for the work done in Khartoum State. In contrast a study conducted in Nigeria (Kabir *et al*., 2004) found ARs in 1% of the eggs sampled (200 eggs) and in 21.8% of 378 slaughtered broilers were examined for antimicrobial drug residues using a disc diffusion microbial inhibition test with *Bacillus. cereus* and *Micrococcus luteus*.

In a study done in Kuwait to assess the prevalence of antimicrobial residues in eggs, tissue and feed samples; the results showed that all eggs sampled (222) were negative for antimicrobial residues as well as the tissue sampled (268 samples). However, the sample size in this study is small, the results revealed in this study showed that the surveillance systems used in Kuwait for monitoring and applying the standards of antimicrobial residues in food is well conducted and highly efficient (Alomirah *et al*., 2007).

Examples of studies conducted in Europe include a recent study in Bulgaria, where only two chicken meat samples were positive to ARs from a total of 75 samples analyzed using the microbial inhibition test. The study concluded that chicken meat producers in Bulgaria do not respect regulations about withdrawal period of veterinary products (Pavlov *et al.,* 2008).

In Poland, the overall presence of antimicrobial residues was detected was (0.86%) out of 582 samples of animal feeds such as muscle fibers and other meat particles, cartilage, bones, horn, hair, bristles, blood, feathers, egg shells, fish bones, and scales (Monika and Krzysztof, 2006).

An example of developed countries situation is highlighted by the study of Weiss *et al.,* 2007, who conducted a study in Italian poultry; the result of the study showed a very low contamination level in Italian poultry meat (0.33%) and the overall percentage in the period from 1995-2001 was less than 0.5%, which assured that the monitoring programmes were effective for the protection of consumers.

The results of this study do not vary much from some results of developing countries, even though methods of analysis and sampling schemes are different. From the above mentioned examples, compared to our study's results, African countries and the third world countries has a serious problem of antimicrobial residues in food available for human consumers. Action needs to be taken in these countries to protect human health from antimicrobial residues.

It is much more difficult to measure the use of antibiotics and drugs in developing countries where farmers are often unaware of the drug that was prescribed, purchased, or administrated by a veterinarian. Veterinary and pharmacy records may be unavailable. The task is made even more difficult by the wide range of available products and mixtures, adulteration, and uncontrolled sale of drugs (Bojalil & Calva, 1994). This point is quite clearly observed in Sudan. An independent method is therefore needed to assess antimicrobial drug use. Unlike developed countries, where the use of antimicrobial drugs can be readily determined by measuring sales, prescriptions, and surveys of physician prescribing practices (McCaig & Hughes, 1995).

The control of veterinary drug use to ensure safer animal food products is needed in developing countries. Observation of drug withdrawal periods and extension programmes for farmers will be highly beneficial. Alternative practices such as vaccinations may reduce the use of antibiotics in poultry, the presence of antimicrobial residues and the development of drug resistant bacteria. This study serves as the first scientific evidence of the contamination of eggs by antimicrobial residues in Sudan. Although, the concern of antimicrobial residues is an international issue and public health problem in eggs or food of animal origin in general, the study shows that there is a very high prevalence of ARs in table eggs served for human consumption in Sudan.

These findings revealed that all consumers in Sudan are probably at risk and urgent attention is needed. The misuse of antibiotics in the local poultry industry poses a serious health risk to the public and may have an impact on humans in terms of antibiotic treatment. The study serves as a baseline for the Sudanese authorities to consider the health impact of residues in food from animal origins and actions to be taken according to that. Regulatory agencies around the world adopt a multi-stakeholder involvement to deal with food safety matters. The food safety authority examines all the aspects of chemical/ microbiological contaminant, conducts total diet surveys, carrys out risk analyses, formulates standards and suggests appropriate actions, including policies. At present in Sudan, there are no programmes for monitoring antimicrobial residues in poultry products or in animal derived food in general.

CHAPTER VI-CONCLUSION

In conclusion, this study showed that there is a significant relationship between reported disease prevalence, antibiotic use and ARs. A high prevalence of disease in poultry farms was mirrored by the high proportion (±60%) of residues in eggs in The Sudan compared to most developing countries. This was related to the fact that the type of open-house farming system common in The Sudan resulted in salmonellosis, respiratory and enteric diseases, necessitating antimicrobial therapy.

Although not perfect, the in-house screening test was found to be cheap, sensitive and repeatable making it ideal for resource-poor countries (Jambalang, 2009).
The main problem concluded was the lack of knowledge about antimicrobial residues, and the risk possessed by the consumption of these residues among farmers and producers. Most people in the Sudanese poultry industry don't understand the danger of giving antibiotics to chicken to human health. In addition to that, most farmers don't understand the concept of withdrawal period and did not follow it.

The absence of governmental supervision and control on the use of drugs was one of the factors contributing to the high proportion of residues in eggs. In addition to that, the lack of disease control programmes resulting in massive use of antibiotic to control endemic diseases.

Furthermore, farmers were not compelled by regulations in The Sudan to limit the use of antibiotics nor were they aware of any detrimental effects that antibiotic in eggs may have on human health. This lead to the unrestricted administration of antibiotics in water of layer birds.

It is very important that antimicrobials are used in a responsible and appropriate way, otherwise due to the increasing trend and the dramatic development of antimicrobial resistance we may lose the efficiency of these drugs for the treatment of several diseases in human.

CHAPTER VII-RECOMMENDATIONS

1. The author recommends that the Sudanese government conduct a complete and intensive census with data base of farms in Khartoum State. Since the author has done the layer farms, it can form part of the census. This census may serve as a main source of data for researchers and other parties involved in the poultry industry. In addition to that, the database to be created from this census will help in surveillance and monitoring programmes and control strategies in the event of disease outbreaks.
2. The Ministry of Animal Resources and Fisheries and The National Standardization and Metrology Organization, who are responsible for the antimicrobial residues aspect in The Sudan ought to construct comprehensive and well designed regulations for antibiotic use in animals, set standards and limits for residues, monitor and survey products and enforce compliance to ensure that only safe food is marketed and that consumers are protected. The broad objectives of the regulations must have but not limited:

    - Ensure that only safe and wholesome foods are marketed in Sudan.
    - Take decisions based on science.
    - Empower authorities to detect the source of contamination and take the necessary actions to prevent contaminated food from reaching consumers.
    - Enforce compliance by farmers, manufacturers, distributors, importers and stakeholders.
    - Be transparent and promote public confidence.

    To reach this aim, data must be collected through intensive research programmes and surveillance. The Indian surveillance and monitoring system for food safety (ILSI, 2007) may be a good model for Sudan as it:

    - Set standards and limits for residues, even though these standards are internationally available.
    - Prescribe labeling requirements.
    - Indicate methods for analysis

- Set out guidelines for accreditation of laboratories
- Conduct surveys.
- Maintain data.
- Organize training programmes.

3. Building a good collaboration network with reference laboratories in developed countries, beside upgrading and building the capacity of national laboratories to handle the task of residues in food is an important issue.

4. The lack of rules and regulations in the country is behind the lack of awareness among the public with regard to antimicrobial residues in food. An intensive and urgent extension and educational programme about the use and misuse of antibiotics in animals and the public health impact of residues is needed for all public sectors, including farmers, manufacturers, distributors, importers and other stake holders. In addition, the extension programme can inform farmers about disease, biosecurity and the potential dangers of antibiotic residues. Consumers must also be informed so that they can reject any products that can harm their health.

5. Rules and regulations for the use of antimicrobials in veterinary practice need to be structured and enforced, for example construction of a food safety authority which may mainly deal with:
    - Food control management.
    - Food legislations.
    - Food inspection (Risk based inspection system).
    - Establish central food control laboratory.
    - Food safety and quality information, education and communications enhancing co-ordination and information sharing.
6. Figure 22 shows a model designed for integrated food control system in Kuwait developed by Alomirah *et al.,* (2009), this model can give be used as a basis for a food control system in Sudan.

7. It is highly recommended to publish and present this work in local media and address the target audience through direct seminars and extension programmes, beside the international publication of the work in peer reviewed journals.

8. In addition to the well known public health concerns about antimicrobial residues, the presence of these compounds affects international trade and the export of poultry product, even though Sudan doesn't export any poultry products at present, it is highly recommended to meet the standards for future plans to access the regional market.

9. Studies on the structure of the Sudanese poultry industry including housing and environmental management are needed and traditional poultry keeping needs to be improved to limit dependence on antibiotics, even though the closed houses operators also use antibiotics.

10. Biosecurity measures and improvement of farming systems in the poultry industry in the Sudan is needed. In addition to that, strategic disease control *progra*mmes such as, reducing the prevalence of endemic diseases by useful risk assessments, surveys, monitor of disease prevalence, safe and effective treatment or vaccination is highly important.

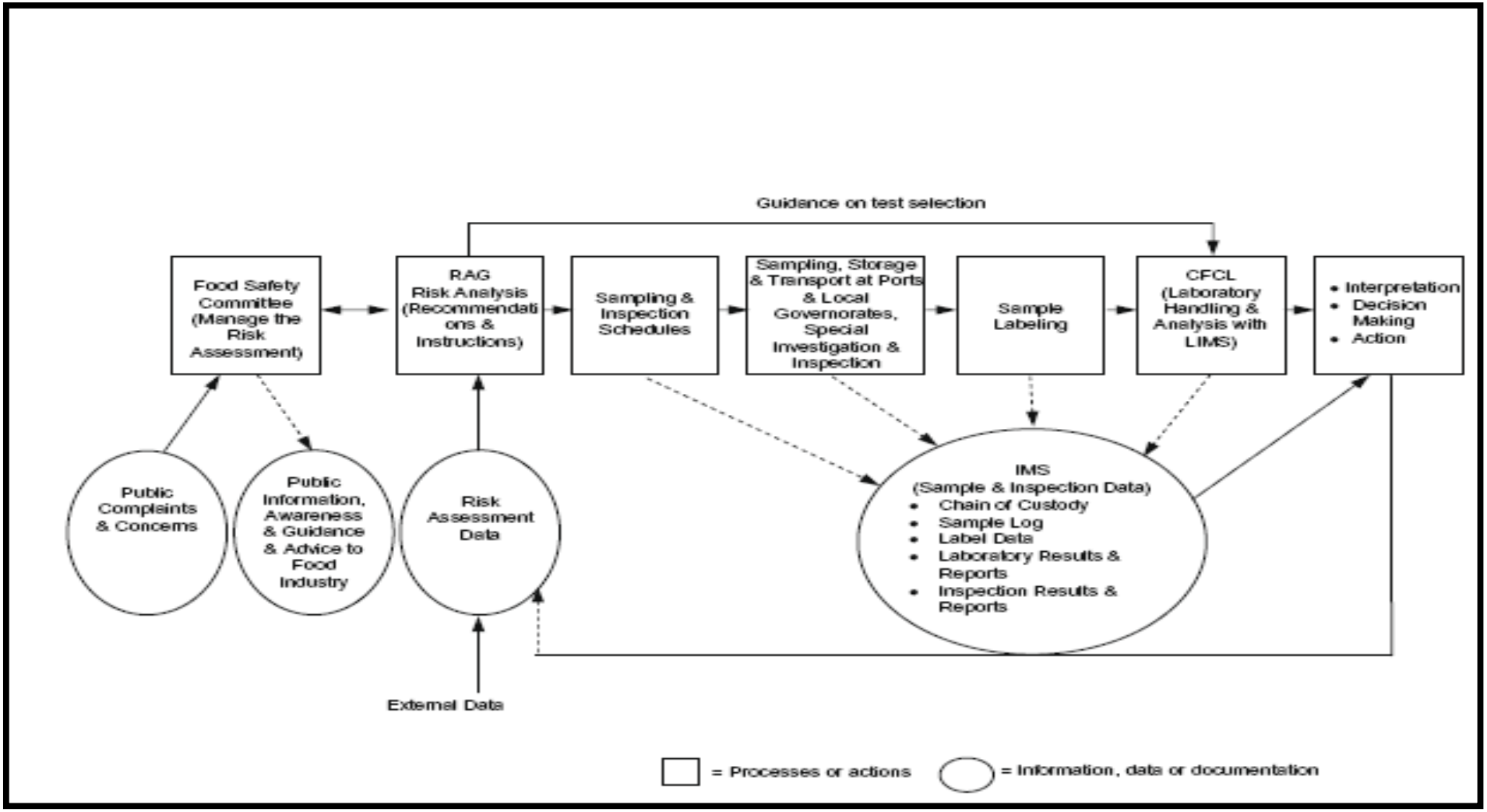


Figure 22: A model for an integrated Food Control System in Kuwait (Alomirah *et al.*, 2009)

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APPENDICES

APPENDIX I - QUESTIONNAIRE:

No:	Date	Area:
		GPS coordinates:
		Region:
Farming system:	Chicken breed:	Breeding system:
No. of Chickens/ house:	No. of house / farm:	Current age of flock:

1. What antibiotics are your birds on now?
2. What antibiotics have you used in the past 3 months (collect labels)?.....
3. For which reason did you use these antibiotics? Therapeutic…. Prophylactic……...Other……..
4. Which route of administration did you use? Water…….. Feed…….. injection…….. others…….
5. What dose did you use for each antibiotic given above?.....
6. What withdrawal period did you use for each drug listed above? …….
7. Do you continue selling the eggs during administration of the drug? Yes… No…
8. Do you believe that the drug passes from the body of the chicken to the egg when administrated? Yes…….. No……..
9. Do you believe that drug in the eggs can affect human consumers? Yes..… No…….
10. Where do you keep the drug in the farm? Store room…… Fridge…… chicken house…….. others……
11. What form of quality control do you use? ……
12. Do you know of any rules and regulations for the usage of antibiotics in poultry production? Yes…… No……..
13. If yes, what is the governmental body responsible for enforcing these rules?.....
    ..
14. What diseases do you have on the farm now?
15. What diseases have you had on the farm in the last 3 months?

APPENDIX II-IMPORT PERMIT:

agriculture
Department:
Agriculture
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF AGRICULTURE
REPUBLIC OF SOUTH AFRICA

DEPARTEMENT VAN LANDBOU
DIREKTORAAT VEEARTSENY DIENSTE
PRIVATE BAG/PRIVAATSAK X138
2008 -08- 04
PRETORIA 0001
DIRECTORATE VETERINARY SERVICES
DEPARTMENT OF AGRICULTURE

Directorate of Veterinary Services
Import-Export Policy Unit
Private Bag X138
Pretoria, 0001
Republic of South Africa

Tel: (27)-012-3197514
Fax: (27)-012-3298292

PERMIT NO: 13/1/1/30/2/9/13-67
Valid from: 2008-08-04
Expiry date: 2008-11-04

IMPORTER:
DR. J.A. PICARD
DEPT. OF TROPICAL DISEASES
FACULTY OF VETERINARY SCIENCE
UNIVERSITY OF PRETORIA
PRETORIA
0001

VETERINARY IMPORT PERMIT FOR SAMPLES TO BE IRRADIATED
(Issued in terms of the Animal Diseases Act, 1984)

Authority is hereby granted for you to import 1800 FROZEN CHICKEN EGGS into Republic of South Africa:

from: DR. MUHAMMED SIDAR, AGRICULTURE AND ANIMAL PRODUCTION, POULTRY SECTION, SUDAN
subject to the following conditions:

1. the consignment must be accompanied by this original permit;
2. the CHICKEN EGGS must be securely packed and transported in leakproof containers, sealed by an authorised official of the Veterinary Authorities of the exporting country;
3. the consignment to be airfreighted through port of entry **O.R.TAMBO INTERNATIONAL AIRPORT**
4. the CHICKEN EGG samples must be transported directly to an approved facility for irradiation under cover of a red-cross permit. Once a certificate of irradiation has been issued, the consignment may be forwarded to DEPT. OF TROPICAL DISEASES, FACULTY OF VETERINARY SCIENCE, UNIV. OF PRETORIA .
5. the CHICKEN EGGS must be kept and used for purposes of testing/research at the laboratories of DEPT. OF TROPICAL DISEASES, FACULTY OF VETERINARY SCIENCE, UNIV. OF PRETORIA under the personal supervision of DR. J.A. PICARD
6. on completion of tests/research the CHICKEN EGGS must be destroyed by incineration;
7. The State Veterinarian: KEMPTON PARK Tel: 011-973 2827 must be advised timeously of the arrival of the consignment. The samples may under no circumstances be off-loaded without the written permission of the State Veterinarian or his/her representative.
8. This permit is subject to amendment or cancellation by the Director Veterinary Services at any time and without prior notice being given.
9. This permit is valid for three (3) months from date of issue and FOR ONE CONSIGNMENT ONLY

FOR **DIRECTOR: VETERINARY SERVICES**

NOTE:

APPENDIX III-PERMIT TO MOVE ANIMAL PRODUCTS:

176
0825
9031
05 BOXES

G.P.-S. 013-0116 AGR 06/053

Permit in	Permit uit/out
R/P	1367

Verwysing / Reference: A 186/09

REPUBLIC OF SOUTH AFRICA • REPUBLIEK VAN SUID-AFRIKA

DEPARTEMENT VAN LANDBOU
DEPARTMENT OF AGRICULTURE

PERMIT VIR VERVOER VAN DIERE/DIERLIKE PRODUKTE
PERMIT TO MOVE ANIMALS/ANIMAL PRODUCTS

Kragtens die Wet op Dieresiektes, 1984 (Wet 35 van 1984), soos gewysig, en onderworpe aan die voorwaardes hieronder gestel, word toestemming hiermee verleen aan—

In terms of the Animal Diseases Act, 1984 (Act 35 of 1984), as amended, and subject to the conditions specified below, permission is hereby granted to—

Naam / Name: DR. J.A. PICARD

Adres / Address: DEPT. OF TROPICAL DISEASES, FACULTY OF VETERINARY SCIENCE, UNIVERSITY OF PRETORIA, 0001

Om te beweeg met/Vir die vervoer van / To move with/To transport: 1080 EGGS

Van die plaas/plek / From the farm/place: OR TAMBO INT'L AIRPORT **in die distrik / in the district of:** KEMPTON PARK

Na die plaas/abattoir/plek / To the farm/abattoir/place: DEPT. OF TROPICAL DISEASES, UNIVERSITY OF PRETORIA **in die distrik / in the district of:** PRETORIA

EX, SUDAN

VOORWAARDES

1. Hierdie permit— CHICKEN EGGS

the CHICKEN EGG samples must be transported directly to an approved facility for irradiation under cover of a red-cross permit. Once a certificate of irradiation has been issued, the consignment may be forwarded to DEPT. OF TROPICAL DISEASES, FACULTY OF VETERINARY SCIENCE, UNIV. OF PRETORIA .

the CHICKEN EGGS must be kept and used for purposes of testing/research at the laboratories of DEPT. OF TROPICAL DISEASES, FACULTY OF VETERINARY SCIENCE, UNIV. OF PRETORIA under the personal supervision of DR. J.A. PICARD

on completion of tests/research the CHICKEN EGGS must be destroyed by incineration;

vermoedelik besmet is met 'n siekte wat sodanige diere aantas, vermy word.

4. Ander voorwaardes.....

CONDITIONS

1. This permit—

infected or suspected of being infected with any disease to which the animals are susceptible.

4. Other conditions BY ROAD DIRECT

DEPARTEMENT VAN LANDBOU
KWARANTYNSTASIE
QUARANTINE STATION
POSBUS/P.O. BOX 2098
KEMPTON PARK 1620
DEPARTMENT OF AGRICULTURE

Plek / Place: KEMPTON PARK **Datum / Date:** 2008/09/18

Staatsveearts/State Veterinarian

VERSPREIDING • DISTRIBUTION

Hiermee word gesertifiseer dat ek die vervoermiddel verseël het.
I hereby certify that I have sealed the conveyance.

Die / The: OWNER / CONVEYOR

Die / The: S.V. PRETORIA

Die / The: BOOK / FILE

DATUMSTEMPEL
DATE STAMP

Staatsveearts/Veeinspekteur
State Veterinarian/Stock Inspector

APPENDIX IV-CERTIFICATE OF GAMMA IRRADIATION:

(Reg. No. 1998/024219/07)
5 Waterpas Street
Isando Extension 3
P.O. Box 3219
Kempton Park 1620
Tel: (011) 974-8851
Fax: (011) 974-8986
Email: info@isotron.co.za

Gamma Radiation Processing

Certificate of Gamma Irradiation

Certificate Number
68055

This is to certify that
ISOTRON SOUTH AFRICA (PTY) LTD

Has given an irradiation treatment in accordance with:

- The national and international quality assurance system standards: ISO 9001:2000
- The precision and accuracy of the dosimetry system used is traceable to International Standards.

to the following goods (as described by the Manufacturer):

Customer	DR. J.A. PICARD (COD)	Customer reference no.	Irradiation completion date
Isotron control number (Internal order number)	73284 / 1	0001367	19/09/2008
Isotron stock code	000 001 TR01	Conforms to the Specified Irradiation Dose of:	
Product description	TRIAL/SAMPLE FOR 1kG	Minimum:	1.0 kGy
		Maximum:	kGy
Quantity of containers	Mass of containers Kg	Routine Dose Reading:	2.4 kGy
5	5.00	Ref. Dose Mapping:	

Date 19/09/2008 Product Released By Quality Department

RIO Consumables (012) 653 6638 Re-Order BS0054

ISO-9. REV: 08

APPENDIX V-EXPORT PERMISSION

REPUBLIC OF SUDAN
MINISTRY OF ANIMAL RESOURCES AND FISHERIES
DEPARTMENT OF QUARANTINES AND MEAT HYAGINE

Date: 3/6/2008

TO WHOM IT MAY CONCERN

SUB.: ANALYSIS OF EGG SAMPLES IN SOUTH AFRICA

Referring to the above mentioned subject we convey that the Undersecretary of the Ministry of Animal Resources and Fisheries has no objections for sending egg samples from Sudan to the University of Pretoria for analysis of antibiotic residues for the master study of Dr. Mohamed Sirdar.
The sample details are:

1. 600 eggs (freezed in plastic bags) to be sent on 15/6/2008.
2. 600 eggs (freezed in plastic bags) to be sent on 15/7/2008.
3. 600 eggs (freezed in plastic bags) to be sent on 30/8/2008.

Kind regards

3/6/8

DR. A.A. BAIYOMI
FOR/ UNDERSECRETARY OF
ANIMAL RESOURCES AND FISHERIES

Republic of the sudan
Ministry of Animal Resources and Fisheries
Undersecretary Office

APPENDIX VI- Khartoum State layer farms census 2007-2008

No	Locality	Area	Farms	Houses	Houses Capacity	Total Capacity/ birds	Housing system
	Bahry	**Soba East**	1	1	(1*3 000)	3 000	Open
			2	4	(4*5 000)	20 000	Open
			3	1	(1*1 000)	1 000	Open
			4	1	(1*1 000)	1 000	Open
	Total		**4**	**7**		**25 000**	

No	Locality	Area	Farms	Houses	Houses Capacity	Total Capacity/ birds	Housing system
	Bahry	**El-Ezba**	1	4	(4*1 000)	4 000	Open
			2	2	(2*1 000)	2 000	Open
			3	1	(1*1 000)	1 000	Open
			4	2	(2*2 000)	4 000	Open
			5	1	(1*3 000)	3 000	Open
			6	1	(1*1 000)	1 000	Open
	Total		**6**	**11**		**15 000**	

No	Locality	Area	Farms	Houses	Houses Capacity	Total Capacity/ birds	Housing system
	Bahry	**Hilat Kuku**	1	3	(3*1 500)	4 500	Open
			2	2	(2*2 000)	4 000	Open
			3	2	(2*1 000)	2 000	Open
			4	1	(1*800)	800	Open
			5	1	(1*500)	500	Open
			6	1	(1*2 000)	2 000	Open
			7	3	(3*700)	2 100	Open
			8	2	(2*500)	1 000	Open
	Total		**8**	**15**		**16 900**	

No	Locality	Area	Farms	Houses	Houses Capacity	Total Capacity/ birds	Housing system
	Bahry	**Omdoum**	1	2	(1*6 000) (1*8 000)	14 000	Open
			2	1	(1*6 000)	6 000	Open
			3	2	(2*2 000)	4 000	Open
			4	1	(1*1 000)	1 000	Open
			5	2	(2*2 000)	4 000	Open
	Total		**5**	**8**		**29 000**	

No	Locality	Area	Farms	Houses	Houses Capacity	Total Capacity/ birds	Housing system
	Bahry	**Elkadaro / Droshab**	1	4	(4*5 000)	20 000	Open
			2	2	(2*1 000)	2 000	Open
			3	14	(1*1 600) (13*1 500)	21 100	Open
			4	8	(8*1 500)	12 000	Open
			5	10	(10*1 500)	15 000	Open
			6	2	(2*1 000)	2 000	Open
			7	3	(3*1 000)	3 000	Open
			8	2	(2*1 000)	2 000	Open
			9	1	(1*1 000)	1 000	Open
			10	1	(1*1 000)	1 000	Open
			11	2	(2*1 000)	2 000	Open
			12	3	(3*500)	1 500	Open
			13	5	(5*1 000)	5 000	Open
	Total		**13**	**57**		**87 600**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Bahry	**Haj Usif /Shigla (sharg Niel)**	1	3	(3*5 000)	15000	Semi Closed
			2	5	(5*200)	1 000	Open
			3	3	(3*1 000)	3 000	Open
			4	2	(2*750)	1 500	Open
			5	2	(2*500)	1 000	Open
			6	1	(1*1 000)	1 000	Open
			7	1	(1*500)	500	Open
			8	1	(1*200)	200	Open
			9	1	(1*500)	500	Open
			10	9	(9*1 000)	9 000	Open
Total	**10**		**28**		**32 700**		

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Bahry	**Shambat**	1	2	(2*4 000)	8 000	Open
			2	1	(1*1 000)	1 000	Open
			3	1	(1*1 000)	1 000	Open
			4	2	(2*1 000)	2 000	Open
			5	2	(1*1 200) (1*1 700)	2 900	Open
			6	4	(4*500)	2 000	Open
			7	6	(6*500)	3 000	Open
			8	5	(5*500)	2 500	Open
			9	1	(1*950)	950	Open
			10	3	(3*800)	2 400	Open
			11	5	(5*1 000)	5 000	Open
			12	2	(1*600) (1*250)	850	Open
			13	1	(1*500)	500	Open
			14	2	(2*1 000)	2 000	Open
			15	8	(8*400)	3 200	Open
			16	12	(12*1 000)	12 000	Open
	Total		**16**	**57**		**49 300**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Bahry	**Kabashi**	1	3	(3*10 000)	30 000	Open
			2	6	(3*1 000) (1*1 400) (2*2 000)	8 400	Open
			3	2	(2*95000)	190 000	Semi closed
			4	2	(2*5 000)	10 000	Open
			5	2	(2*800)	1 600	Open
			6	1	(1*1 000)	1 000	Open
			7	1	(1*800)	800	Open
			8	4	(4*1 000)	4 000	Open
			9	1	(1*1 000)	1 000	Open
			10	1	(1*800)	800	Open
			11	1	(1*1 100)	1 100	Open
			12	1	(1*800)	800	Open
	Total		**12**	**25**		**249 500**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Bahry	**El Faki Hashim**	1	1	(1*20 000)	20 000	Open
			2	3	(3*10 000)	30 000	Open
			3	2	(2*2 500)	5 000	Semi
			4	4	(4*2 500)	10 000	Open
			5	16	(16*1 000)	16 000	Open
			6	8	(8*2 000)	16 000	Open
			7	15	(2* 1 200) (13*1 000)	15 400	Open
			8	2	(2*1 000)	2 000	Open
			9	5	(5*3 000)	15 000	Open
			10	2	(2*800)	1 600	Open
			11	3	(3*1 000)	3 000	Open
			12	1	(1*1 000)	1 000	Open
	Total		**12**	**62**		**135 000**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Bahry	**Elsagai**	1	2	(2*10 000)	20 000	Open
			2	4	(4*1 000)	4 000	Open
			3	1	(1*1 000)	1 000	Open
			4	1	(1*1 000)	1 000	Open
			5	2	(2*1 000)	2 000	Open
			6	5	(5*1 000)	5 000	Open
			7	4	(4*600)	2 400	Open
			8	1	(1*2 000)	2 000	Open
			9	2	(2*800)	1 600	Open
			10	1	(1*2 000)	2 000	Open
			11	3	(3*1 000)	3 000	Open
			12	36	(36*2 250)	81 000	Semi/Open
			13	3	(2*20 000) (1*30 000)	70 000	Closed
	Total		**13**	**65**		**195 000**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Bahry	**Tibna & Zakiab**	1	14	(2*2 000) (1*1 500) (11*1 000)	16 500	Open
			2	7	(7*1 000)	7 000	Open
			3	1	(1*800)	800	Open
			4	8	(8*1 000)	8 000	Open
			5	1	(1*1 000)	1 000	Open
			6	1	(1*600)	600	Open
			7	1	(1*800)	800	Open
			8	8	(8*1 500)	12 000	Open
			9	3	(3*600)	1 800	Open
			10	5	(5*800)	4 000	Open
			11	1	(1*800)	800	Open
			12	3	(3*1 000)	3 000	Open
			13	4	(4*1 000)	4 000	Open
			14	1	(1*1 000)	1 000	Open
			15	1	(1*1 000)	1 000	Open
			16	1	(1*1 000)	1 000	Open
			17	2	(2*1 000)	2 000	Open
			18	4	(4*500)	2 000	Closed
	Total		**18**	**66**		**67 300**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Bahry	**Samrab**	1	4	(4*1 400)	5 600	Open
			2	1	(1*3 000)	3 000	Open
			3	6	(6*1 000)	6 000	Open
			4	1	(1*400)	400	Open
			5	5	(5*1 000)	5 000	Open
	Total		**5**	**17**		**20 000**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Bahry	**Halfaya**	1	2	(2*3 000)	6 000	Open
			2	3	(3*5 000)	15 000	Open
			3	2	(2*2 000)	4 000	Open
			4	2	(2*2 500)	5 000	Open
			5	1	(1*3 000)	3 000	Open
			6	4	(4*1 000)	4 000	Open
			7	2	(2*2 000)	4 000	Open
			8	6	(6*800)	4 800	Open
			9	6	(6*500)	3 000	Open
			10	3	(3*700)	2 100	Open
			11	3	(2*2 000) (1*3 000)	7 000	Open
			12	2	(2*500)	1 000	Open
			13	11	(10*800) (1*1 200)	9 200	Open
			14	2	(2*300)	600	Open
			15	3	(2*20 000) (1*16 000)	56 000	Closed
			16	30	(30*2 000)	60 000	Semi
	Total		**16**	**82**		**184 700**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Bahry	**Mazalat**	1	1	(1*1 000)	1 000	Open
			2	4	(4*1 250)	5 000	Open
			3	2	(2*1 000)	2 000	Open
	Total		**3**	**7**		**8 000**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Bahry	**Sababi**	1	1	(1*2 200)	2 200	Open
			2	3	(3*900)	2 700	Open
			3	3	(3*700)	2 100	Open
			4	2	(2*750)	1 500	Open
			5	1	(1*2 000)	2 000	Open
	Total		**5**	**10**		**10 500**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Bahry	**Eid BAbiker**	1	1	(1*1 000)	1 000	Open
			2	1	(1*1 000)	1 000	Open
			3	2	(2*2 000)	4 000	Open
			4	3	(3*500)	1 500	Open
			5	1	(1*800)	800	Open
			6	1	(1*300)	300	Open
			7	2	(2*2 000)	4 000	Open
	Total		**7**	**11**		**12 600**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Khartoum	**Botry**	1	3	(3*5 000)	15 000	Open
			2	4	(4*1 000)	4 000	Open
			3	5	(5*5 000)	25 000	Open
			4	5	(5*2 000)	10 000	Open
			5	1	(1*1 000)	1 000	Open
			6	5	(1*20 000) (4*9 000)	56 000	Closed

	Total		**6**	**23**		**111 000**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Khartoum	**Soba**	1	1	(1*12 000)	12 000	Open
			2	1	(1*1 000)	1 000	Semi
			3	1	(1*1 000)	1 000	Open
			4	1	(1*1 000)	1 000	Open
			5	2	(2*1 000)	2 000	Open
			6	1	(1*1 000)	1 000	Semi
			7	1	(1*1 000)	1 000	Open
			8	1	(1*1 000)	1 000	
			9	1	(1*1 000)	1 000	
			10	3	(3*10 000)	30 000	
			11	1	(1*1 000)	1 000	
	Total		**11**	**14**		**52 000**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Khartoum	**Eid Hussien**	1	1	(1*1 000)	1 000	Open
			2	1	(1*1 000)	1 000	Open
			3	1	(1*1 000)	1 000	Open
			4	1	(1*500)	500	Open
			5	2	(2*1 000)	2 000	Open
			6	1	(1*1 000)	1 000	Open
	Total		**6**	**7**		**6 500**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Khartoum	**Tyba Hassanab (Seleimania/ Traia Biga)**	1	6	(6*37 500)	225 000	Closed
			2	1	(1*6 000)	6 000	Open
			3	6	(6*5 000)	30 000	Open
			4	3	(3*10 000)	30 000	Open
			5	2	(1*12 000) (1*10 000)	22 000	Semi
			6	3	(3*24 000)	72 000	Closed
			7	3	(3*24 000)	72 000	Closed
			8	1	(1*25 000)	25 000	Semi

	Total		**8**	**25**		**482 000**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Khartoum	**Shegailab**	1	1	(1*3 000)	3 000	Open
			2	1	(1*2 000)	2 000	Open
			3	1	(1*1 000)	1 000	Open
			4	2	(2*1 000)	2 000	Open
			5	3	(3*10 000)	30 000	Semi
	Total		**5**	**8**		**38 000**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Khartoum	**Salama**	1	2	(2*10 000)	20 000	Semi
			2	1	(1*1 000)	1 000	Open
			3	1	(1*1 000)	1 000	Open
			4	1	(1*1 000)	1 000	Open
			5	2	(2*1 000)	2 000	Open
	Total		**5**	**7**		**25 000**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Khartoum	**Kalakla & Dikheinat**	1	1	(1*1 000)	1 000	Open
			2	1	(1*1 000)	1 000	Open
			3	2	(2*1 000)	2 000	Open
			4	1	(1*1 000)	1 000	Open
			5	1	(1*1 000)	1 000	Open
			6	1	(1*1 000)	1 000	Open
			7	2	(2*1 000)	2 000	Open
			8	15	(15*2 000)	30 000	Open
			9	1	(1*1 000)	1 000	Open
			10	1	(1*1 000)	1 000	Open
			11	1	(1*1 000)	1 000	Open
			12	1	(1*1 000)	1 000	Open
			13	2	(2*1 000)	2 000	Open
			14	2	(2*1 000)	2 000	Open
			15	1	(1*1 000)	1 000	Open
			16	4	(4*1 000)	4 000	Open
			17	1	(1*1 000)	1 000	Open
	Total		**17**	**38**		**53 000**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Khartoum	**Kalakla North**	1	1	(1*1 000)	1 000	Open
			2	1	(1*1 000)	1 000	Open
			3	1	(1*1 000)	1 000	Open
			4	1	(1*1 000)	1 000	Open
			5	4	(4*1 000)	4 000	Open
	Total		**5**	**8**		**8 000**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Khartoum	**Gerief west**	1	5	(5*2 000)	10 000	Open
			2	3	(3*5 000)	15 000	Open
			3	1	(1*2 000)	2 000	Open
			4	1	(1*1 000)	1 000	Open
			5	2	(2*1 000)	2 000	Open
			6	3	(3*1 000)	3 000	Open
			7	5	(5*1 000)	5 000	Open
			8	1	(1*1 000)	1 000	Open
			9	1	(1*1 000)	1 000	Open
			10	3	(3*1 000)	3 000	Open
			11	2	(2*400)	800	Open
			12	2	(2*500)	1 000	Open
	Total		**12**	**34**		**44 800**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Khartoum	**Gabel Awlia**	1	1	(1*10 000)	10 000	Open
			2	3	(3*5 000)	15 000	Open
			3	4	(4*2 000)	8 000	Open
	Total		**3**	**8**		**33 000**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Omdurman	Aborof	1	3	(3*1 000)	3 000	Open
	Total		**1**	**3**		**3 000**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Omdurman	Garafa	1	1	(1*1 000)	1 000	Open
			2	2	(2*1 500)	3 000	Open
	Total		**2**	**3**		**4 000**	

No	Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
	Omdurman	Elsarha	1	2	(2*2 500)	5 000	Open
			2	5	(5*1 000)	5 000	Open
	Total		**2**	**7**		**10 000**	

Locality	Area	Farms	Houses/ farm	Houses Capacity	Total Capacity/ birds	Housing system
Omdurman	Nefasha	1	3	(2*5 000) (1*15 000)	25 000	Closed
		2	1	(1*5 000)	5 000	Open
		3	2	(2*10 000)	20 000	Closed
Total		**3**	**6**		**50 000**	