SURVEYS ON ANTIMICROBIAL DRUG RESIDUES IN SLAUGHTERED CATTLE AND CHICKEN IN NIGERIA

BY

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DECLARATION

I declare that this thesis is the result of the work I carried out at the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, under the supervision of Prof. J.U. Umoh and Dr. V.J. Umoh. The work of other investigators is acknowledged and referred to accordingly. I solemnly declare that no part of this thesis has been submitted elsewhere for a degree.

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CERTIFICATION

The project thesis entitled **Surveys on Antimicrobial Drug Residues in Slaughtered Cattle and Chickens in Nigeria** meet the regulations governing the award of Master of Science(Veterinary Public Health) of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to the memory of my late father, Alhaji Muhammadu Kabir Wali, for his love and respect for knowledge. And to all those that would not have the opportunity to scavenge for knowledge.

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This work has involved the energy, resources and time of several people, who I wish to acknowledge but cannot mention all by name.

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ABSTRACT

The study comprised two distinct surveys. In the first a questionnaire survey was carried out using two separate structured questionnaires, to determine the nature of veterinary drug use, extent of abuse and misuse of these drugs among animal health workers, livestock and poultry producers and to assess awareness and concern for veterinary drug residues in meat, among livestock and poultry producers, meat sellers/butchers, and the consuming public. The second survey determined the prevalence of antimicrobial drug residues in 876 routinely slaughtered animals consisting of 500 cattle in Zaria and 376 chicken in Kaduna, using a microbial inhibition test(MIT); *Micrococcus luteus* ATCC 9341, was used to test urine and faeces of cattle and chicken respectively at the time of slaughter for the presence of antimicrobial substances.

One hundred and forty four, responses were obtained from 130 veterinarians and 14 other veterinary staff, One hundred and thirty eight (95.8% of respondents) believed that there is gross abuse and misuse of veterinary drugs in Nigeria. These practices were attributed to both veterinary staff and owners of the animals(51.6% of respondents). Antibacterial drugs and antihelminthics were considered to be the most commonly misused drugs (93.0% and 56.3% of respondents respectively), particularly in intensive poultry production and free range ruminant production. The misuse was believed to be capable of causing residues particularly in meat largely due to over dosage, use of long acting preparations ana failure to observe withdrawal times; this is especially so when animals are treated by owners. There was

ignorance about laws regarding use of veterinary drugs in food animals, and this was not influenced by nature of respondents' employment ($\times^2$,p>0.05). Public health education and the provision of drug use legislation were the most advocated strategies for control of residues in Nigeria (97% and 89% of responses respectively).

Three hundred and ninety eight responses were obtained from 220 consumers(53.3%), 59 meat sellers/butchers(14.8%), and 119 livestock **and poultry** producers (29.2%) . Animals at the point, of slaughter showed the risk of producing residue-containing meat. Two hundred and twenty **two**(55.8%) respondents were concerned about residues in meat. This concern was related to age, sex, location and literacy level($\times^2$, p<0.05). The public favours the introduction of legislation for residue prevention including condemnation of contaminated meat.

Thirty seven (7.4%) of the urine samples and **82(2**1. .8%) of the faecal samples were positive for antimicrobial substances. Positive cases were not distributed according to breed(Fisher's exact Lest, P>0.05) bur occurred significantly more in males (X^2, p<0.0l), cattle aged 3-9 years and in those with good body condition (Fisher' s exact **test, P<0.05). Thirty** eight cases, in the form of 16 different disease or pathological conditions, were seen from visual inspection of **the** carcasses. Lymphadenopathy was seen in 10(23.7%) carcasses including 1 carcass positive for antimicrobial substances. Dermatophilosis and emaciation constituted 15.8% and 13.2% of conditions observed. Broilers had the highest class specific prevalence tate(33.1%) for poultry, followed by pullets (23.6%) while local birds had the least (4.8%) . There was a high prevalence of antimicrobial Subtance in slaughtered animals particularly

intensively raised chicken. It is essential to fully restore the meat inspection process including screening of animals for drug residues before slaughter.

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CHAPTER ONE

1.0 INTRODUCTION

The use of chemical substances in agriculture, both in arable and livestock production, is accompanied with the concern for the persistence of these chemical substances in the environment(Cremlyn, 1978) and in the human food chain(Heeschen and Bluthgen, 1982; WHO, 1988, Guest and Paige, 1991; Kan, 1991). Such substances in foods introduced accidentally or intentionally during production at effective or sub effective concentrations are called chemical residues(Booth, 1977b). Veterinary and animal production practices employ chemicals in the form of insecticides, pesticides, herbicides, acaricides, fumigants, disinfectants, preservatives and various drug preparations, in order to enhance one or more factors, such as weight gain, and feed conversion efficiency(Beeson, 1969), for the purposes of prevention, control and treatment of several infectious and non infectious diseases of animals(Meyer Jones *et al.*, 1977; Blood *et al.*, 1983); or in preservation or enhancement of product appeal(Gracey, 1986). When these substances persist in animal tissues, particularly edible animal tissues, mainly meat, milk, and eggs, as drug residues, they exist as the parent drug or its metabolite, freely or bound to tissue components such as proteins or other macromolecules(Gillete, 1974) in biologically active or inactive forms(WHO, 1988).

The presence of residues in meat, milk, or eggs is a significant public health problem. In addition it poses a number of problems pertaining to marketing and subsequent production of livestock. The Food and Agricultural Organisation(FAO) and the World Health

Organisation(WHO) have set up a Joint Expert Committee to look into the problems of food additives; amongst its functions is the evaluation of certain veterinary drug residues in foods. The work of the committee highlights the significance of residues to agricultural practices, its social and economic implications and ways to ensure safe residue levels(WHO, 1988; 1989).

Most of the studies of veterinary drugs in animal food products are reported from countries where intensive commercial livestock production is the primary source of animal products.

Drug residues may have a direct biological or toxicological consequence on the consumer. Chloramphenicol at doses considered residual has shown a direct toxic and even fatal outcomes(Settepani, 1984; WHO, 1988), warranting its prohibition for use in food animals and restricted use in human medicine. However, there are many drug preparations containing chloramphenicol for use in food animals in the Nigerian market. Chloramphenicol is commonly used for the treatment of human and animal salmonellosis(Asherkoff and Bennett, 1969; Huber, 1977b). Nitrofuran drugs are now banned for use in food animals in many countries including Nigeria(NAFDAC, 1996), because of mutagenic effects. It is a drug commonly incorporated into livestock feed.

Consumption of these drugs in foods have been associated with specific problems. Antibiotics have been implicated in causing allergic reactions.

Because of their antibacterial nature residues of these drugs, when found in milk, can inhibit starter cultures used in the production of cultured milk products(Tamime and Deeth, 1980). Antibacterial drug residues can also cause alteration of gut microbial

flora and promote the spread of antibiotic resistant bacteria(Baldwin, 1970). There is also the concern for promoting the phenomena of transferable drug resistance and colonization of the gut and environment by drug resistant bacteria(Baldwin, 1970; Smith, 1974; Rollins *et al.*, 1974; Linton *et al.*, 1977). Many epidemics of emerging bacterial diseases are being attributed to multiple antibiotic resistant bacterial strains including *Salmonella* species and *Escherichia coli*(Neu *et al.*, 1975; Lyons *et al.*, 1980; Nair *et al.*, 1995)

Nigeria like most sub-Saharan African countries is faced with the problems of many enzootic livestock and poultry diseases. Control measures to a large extent require the use of drugs, either for prevention and treatment of active cases. Poultry antibiotic preparations are popular among farmers both literate and illiterate. Many of such preparations are recommended for use in feed or drinking water; very commonly they are fed to birds up to the time of slaughter without respecting the withdrawal time.

The practice of treating animals by lay owners is common among Fulani and semi-literate animal owners. When veterinary services are sought, the quality may be poor. Veterinary drugs are easily obtainable over the counter and used by unqualified persons. The quality control of the drugs is hardly carried out. It is common to see drugs without recommendations or instructions on issues like withdrawal period. Where available, these recommendations may not be followed or are not understood by the lay user. The Fulani herdsmen are particularly susceptible to this problem and have a greater exposure since they feed normally on the milk of their animals even

when such animals are undergoing treatment with a veterinary drug. Use of higher than normal(overdosage), use of drugs in the wrong species and concurrent disease conditions commonly occur in the course of treatment. These factors have been associated with residue occurrence(Booth, 1977b). Often animals with poor prognosis may be slaughtered while undergoing treatment with a veterinary drug.

Eventhough a draft meat(public health) decree has listed meat with antibiotic residues as adulterated(Federal Ministry of Agriculture, 1992), the law has not come into effect and the meat inspection procedure is rarely carried out even in urban abattoirs. The lack of good hygiene and husbandry practices makes animal products emanating from countries like Nigeria suspect for residues.

Increase in the incidence of antimicrobial drug resistant bacteria will further limit the range of effective antibacterial drugs for use in treating human infections, to more potent, and perhaps more expensive options(Cohen, 1992). The increase in the incidence of resistance to commonly used antimicrobial drugs in the population will be another set back to the primary health care delivery and the much desired health for all by the year 2000 and beyond. So also will it badly affect the drive towards improving agricultural exports since many countries of the world, in particular EEC countries, judge animal products by the amount of residues contained in them(Council, 1986; Kan, 1991). The WHO is encouraging countries to determine the level of this problem in their animal products.

This project, therefore, is intended to study the problem of veterinary drug residues in Nigerian slaughter animals, with the following specific objectives:

1. Determining the pattern of veterinary drug use, nature and extent of abuse and misuse of veterinary drugs, and the potentials of these and other related practices, to cause residues in animal food products.

2. Assessing the awareness of veterinary professionals, livestock owners and producers, and the public about the drug residue problem, and the level of concern about drug residues as a public health problem.

3. Screening the urine and faeces of cattle and chicken respectively to determine the incidence of antimicrobial substances in routinely slaughtered cattle and chicken.

4. Determining the factors that may be associated with the occurrence of antibacterial drug residues in Nigerian slaughter animals.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Non Medical Drug Usage In Animal Production

2.1.1 Feed Additives:

Increased food production, in particular commercial animal production, is largely due to the use of non - nutritive feed additives (Shillam, 1974). Before the banning of Diethyl-stilbestrol (DES), close to 80% of all meat and eggs in the United States was derived from animals fed the medicated feed (Van Houweling and Kingma, 1969; Shillam, 1974).

Feed additives are employed for a variety of purposes.Commonly, they are used to enhance one or more factors of production, such as weight gain , feed conversion efficiency (Eyssen and De Somer 1963; Nelson *et al*, 1963; Beeson, 1969; Kilkenny and Suntherland, 1970; Huber ,1971a; Shillam, 1974; March *et al*, 1978; Visek, 1978; Pensack *et al*, 1982; Foster and Stevenson, 1983; Stutz and Lawton, 1984; for the control and prevention of diseases (Beeson, 1969; Huber, 1971b; Dafwang, *et al*, 1987, Braunius, 1982; 1985), preservation (Huber, 1971b), control of reproductive cycle and breeding performance(Shillam, 1974; Langlois *et al*, 1978a; 1978b), and the enhancement of acceptability of feed or end product by consumers(Shillam, 1974).

Cravens and Holck (1970) highlighted the economic benefits of using feed additives to the livestock producer in terms of decrease in feed consumption due to improved efficiency and faster rate of gain

therefore reducing time per unit weight, decrease labour requirement, and to the consumer in terms of the lowered cost of these products, and increase in the volume of products in the market. In the USA for example up to $215 million was spent on animal health pharmaceuticals. More than half the antibiotic production in the US was used as feed additives(Huber , 1971a).

Antibacterial feed additives: A broad range of compounds active against bacteria are used . These include :

Antibiotics: These are mainly the tetracyclines(chlortetracycline, oxytetracycline); the aminoglycoside group (streptomycin, dihydro-streptomycin, oleandomycin, spiramycin, tylosin); polypeptide antibiotics (bacitracin, polymyxin B, colistin), synthetic antibiotics(chloramphenicol, hygromycin B) (WHO, 1969; Huber, 1971a; 1971b; Huber, 1977b; Choi and Ryu, 1978) and more recently ionophore antibiotics(lasalocid, monensin, salinomycin) (Van Maanen *et al*, 1978; Braunius, 1982; 1985; Weiss and Mac Donald, 1985). Others include virginiamycin and flavomycin (Braude, 1978; Solomons, 1978).

Non - antibiotic antibacterials: There are mainly different classes of sulfonamides and some of the nitrofuran drugs (furaltadone, furazolidone, and nitrofurazone) (WHO, 1969; Huber, 1971a; 1971b; 1977b).

Non - antibacterial feed additives : In addition to antibacterial agents, substances with hormonal actions such as melengeserol acetate, stilbesterol (Beeson, 1969), diethylstilbesterol (Beeson, 1969; Huisintveld *et al*, 1970; Kilkenny and Suntherland, 1970),

testosterone, progesterone, estradiol 17*B* (WHO, 1988; Schimedaly *et al*, 1992), trenbolone acetate (Best, 1972; WHO, 1988; Schimedaly *et al*, 1992), zeranol (WHO, 1988; Nold *et al*, 1992) and resorcyclic acid lactone (Beeson , 1969) have been used as feed additive.

Similarly, tranquilizers have been used to enhance weight gain in restless animals (Beeson, 1969; Booth, 1977a). Those tried include hydroxyzine, reserpine , rauwolfia, triflupromazine and perphenazine . Catropam an analog of librium hydrochloride, has shown the greatest promise in improving the performance of feedlot cattle (Beeson, 1969). Different metallic compounds have been incorporated into feed e.g. compounds of copper and arsenic (Wallace, 1970; Visek, 1978), although they are now rarely used. Attempts have also been made to improve feed utilization in cattle by adding certain enzymes to feed (Burroughs *et al*, 1960; Nelson and Catron, 1960; Yang *et al*, 1962; Beeson, 1969).

2.1.2 Antimicrobial Feed Additives:

Use pattern: There are up to 280 use levels and combinations of chlortetracycline and other feed additive drugs for purposes of growth promotion and control of diseases in livestock and poultry production (Huber, 1971b; Jenkins and Friedlander,1982). Chlortetracycline has been used as a feed additive at concentrations ranging from 10 - 500g per ton of feed (Huber, 1971b). It is used for the control of calfhood diseases in dairy cattle production (Wallace, 1970). There are reports of improved conception rates, farrowing rates, and litter sizes following feeding of medicated feed (Jordan *et al*, 1961; Johnson *et al*, 1964; Ruiz *et al*, 1968). Feeding 55mg

chlortetracycline per kilogram continuously in diets of swine herds improved reproductive performance . Litter size at birth and at weaning were compared to those herds fed non medicated feed (Langlois *et al*, 1978a). In ten trials involving 340 sows conducted to determine the effect of antibiotic feeding in breeding, reproductive performance and lactation length , no significant difference was found between treatment and control (Sosa *et al*, 1963; Mayrose *et al*, 1964; Soma and Spencer, 1974). Assessment of monensin fed to steers showed that these steers were heavier , tended to be fatter and had more marbling and higher yield grades than those of control steers (Wadestean *et al*, 1978) and in both growing and finishing steers fed different protein levels (Perry *et al*, 1983). Wallace (1970) also reported the use of chlortetracycline for fattening lambs. Combinations of chlortetracycline , oxytetracycline and zinc bacitracin have been described as a valuable and effective antibacterial feed additive for cattle. At a daily dose 70 - 80 mg per head of either chlortetracycline or oxytetracycline up to 9% daily gain and 8% feed efficiency were obtained (Beeson, 1969). Chlortetracycline(CTC) 350mg combined with sulfamethazine(SMZ) 350mg have been fed per head per day to control shipping fever (Beeson , 1969) or to enhance productivity (Beeson , 1969; Zinn, 1993). Cattle fed this combination gained weight 74% more rapidly than those fed CTC only. Sulfamethazine alone was not very effective (Beeson, 1969), although SMZ on its own is certainly effective in pigs. It was employed in 75% of the hogs marketed in the USA (Ausburg , 1989).

Penicillin is used at 100g per ton of feed. Usually it is employed in combination with streptomycin in feed for livestock and

poultry production (Huber, 1971b). The broad spectrum action demonstrated by the combination has made it very valuable (Huber, 1977a). A combination of penicillin with several antibiotics such as tylosin, chloramphenicol, oxytetracycline, streptomycin, or other antibiotic combinations have been used to control bloat over longer periods (Johnson *et al*, 1960; Van Horn , 1961; 1963; Heinemann and Fanelli, 1963).

Neomycin has particular potentials for use as a feed additive because of its local gastrointestinal effects in feed or drinking water (Huber, 1977b; Jenkins and Friedlander, 1982). It was used at 35 - 100ppm (g/ton), in milk replacers at 100 - 400mg/gallon to prevent neonatal bacterial enteritis, alone or in combinations with different classes of tetracycline (Beeson, 1969; Blood *et al*, 1983).

Following the Swann Committee report , bacitracin became a suitable and popular feed additive or feed antibiotic because of its little patronage in human medicine (WHO, 1969; Huber, 1971b; Brander and Pugh, 1977; Huber, 1977b). There was an increase in weight gain (g) , per unit increase (mg/kg) of zinc bacitracin in the diet when fed to broilers (Choi and Ryu, 1987). Feeding zinc bacitracin significantly improved growth rate , feed conversion, and shank pigmentation (Marusich and Ogrinz, 1978; Stutz *et al*, 1983a; 1983b; Choi and Ryu, 1987).

Tylosin is also used , in particular , for the control of poultry mycoplasmosis given in drinking water (Huber, 1971b; Huber,1977b), also in combination with monensin to increase feed efficiency in finishing steers at 12mg/kg tylosin and 31mg/kg monensin (Gaylean *et al*, 1992). Antibiotic supplemented diets containing virginiamycin or

tylosin improved pig weight gains higher than diets containing CTC or bacitracin (Langlois *et al*, 1978b). Virginiamycin enhanced growth rate and conversion in poultry(March *et al.*, 1978). Oleandomycin, spiramycin , novobiocin, and polymyxin are less commonly used (Buber , 1971b). Two compounds, namely carbadox and olaquinox are being evaluated by the WHO for use as feed additives . They are antimicrobial agents shown to promote growth in pigs and to control swine dysentery and bacterial enteritis . Thrasher et *al* (1969) demonstrated increased gains when carbadox is fed to young pigs. Carbadox was given in feed at 50g/ton (Froe, 1970). A 70-day withdrawal is recommended for and a zero tolerance limit (Booth, 1977b). The commercial product is intended for use in finisher rations. It is administered in feed at 50mg/kg carbadox and 20 - 150mg/kg olaquinox and may be given to pigs for up to 4 months of age. The two drugs are yet to be cleared (WHO, 1990; WHO, 1994).

Newer ionophore antibiotics have been approved for use in the US as feed additives for poultry, sheep and cattle. These are monensin , lasalocid, and salinomycin (Weiss and Mac Donald, 1985). Monensin and lasalocid have been approved in the EEC as anticoccidial drugs at a dosage rate of 100 - 125ppm and 75 -125ppm respectively and withdrawal period of 3 to 5days (Braunius, 1982). They are very effective feed additive drugs. They have been given to feedlot cattle and sheep to improve efficiency (Van Maanen *et al*, 1978; Weiss and Mac Donald, 1985; Gaylean *et al*, 1992; Kung *et al*, 1992) and for control of coccidiosis (Braunius, 1982; 1985; Weiss and Mac Donald , 1985). Salinomycin is used at 11 - 33ppm to control coccidiosis in birds (Weiss and Mac Donald, 1985).

Ilieva *et al*(1985), studied the growth promotion effect of grisin (kormogrisin) in feed of pigs and found an average daily gain of 17% in different weights of pigs (8 - 70kg), feed consumption also decreased by 13.2%.

Risks *versus* Benefits: A significant part of drug usage in animal production may have public health implications. Huber (1971a) listed the conditions under which antibiotic use could be beneficial, such as when: poor quality rations are fed ; poor sanitation procedures are employed and the premises are unclean ; disease (clinical or sub clinical) is present; when there are "runts" or undersized animals in the herd ; and or the animals are in a state of rapid growth. These stipulations are supported widely in literature. Antibiotics prevented the growth depressing effects of high stocking densities(Dafwang *at al.*, 1987). Under high stocking densities, birds may have improper development of the bursa and thymus (Dafwang *et al*, 1987). Such birds may be immunocompromised (Siegel, 1960; Stanley and Krueger, 1981; Vo and Fauguy, 1982).

The probability of gain and feed efficiency benefits is decreased if the animals are healthy, fed good quality rations and are past the rapid growth stage (Wallace, 1970; Huber, 1971a; Bartov, 1992). Numerous factors influence the gross measurements of body weight and feed efficiency . Among these are the variances in subclinical diseases, parasite load , genetic make up and nutrient in-take (Wallace, 1970; Visek, 1978).

Sometimes the poor performance is a side effect of the drug. For example, nitrofurazone and furazolidone lowered egg production in a

dose dependant manner when fed at doses of 7.5 - 30mg/kg orally to laying turkeys (Ali *et al*, 1987). Nitrofurans have been shown to adversely affect fertility and reproductive activity of both male and female humans and animals (Cooper and Skulski, 1956; Albert *et al*, 1974). Nearly all anticoccidial drugs cause (slight) growth depression even at the recommended dose levels in the absence of coccidial exposure (Braunius, 1985). Wadesteen *et al* (1978) used the anticoccidial drug monensin in steers and got better carcass traits. Other studies found no difference at all between treated and controls (Sosa *et al*, 1963; Mayrose *et al*, 1964; Soma and Spear, 1974).

The use of antibiotics for preventive purposes have increased the morbidity of certain diseases e.g. bacterial enteritis in swine (Huber, 1971a). Continuos use may result in residues in tissues (WHO, 1969; Loftsgaard *et al*, 1968; Righter *et al*, 1970; Huber, 1971b; Jones *et al*, 1977; Ilieva, *et al*, 1985) that could lead to drug toxicity or allergy (WHO, 1969; Huber, 1971b) or drug resistant bacterial pathogens (Baldwin, 1970; Smith, 1974). Antibiotics in feed may also modify the composition of gut flora and the metabolism of nutrients and non nutrients (Walker, 1974). Huber (1971a), screened different classes of slaughter animals and found residues of antibacterial substances in 309 of 1,224 hogs (25%) , 10% of the 309 were positive for penicillin . Many of the residues are as a result of oral administration, 2% of mature cattle, 7% of veal calves and 6% of chicken had penicillin residues. Up to 17% of chickens sampled had antibacterial drug residues, out of which 6% were penicillin residues.

Some reports are not in favour of using antibacterial feed additives while others argued that the phenomena like build up of

resistant bacteria is of little consequence to practical livestock production (Wallace , 1970). In related studies to determine the possible effects of non therapeutic antibiotic use in animal production. Solomons (1978), concluded that the total animal reservoir of *Salmonella* is reduced by sub therapeutic use of tetracycline or combinations of drugs. There was no evidence of increased incidence of disease in man exposed to animal *Escherichia coli* (Kemp and Kiser, 1970; Solomons, 1978), and no evidence was found for antibiotic feed compromising therapy for animal diseases (Solomons, 1978; Fagerberg *et al*, 1978). Other studies found slight increases in shedding of resistant bacteria but this had no relationship with growth performance, and shedding of resistant organisms declined with withdrawal of the drug (Kemp and Kiser, 1970; Langlois *et al*, 1978a; 1978b). Braude (1978) presented an elaborate argument in favour of the use of antibiotics as feed additives, severely criticising the provisions of the Swann report (Swann, 1969). Similar justifications for use of antibiotic feed additives because of economic benefits to producer and consumer (Cravens and Holck, 1970), and the implication of regulating the practice on the feed industry (Williamson and Cravens, 1972) and on the pharmaceutical industry (Kemp, 1972) have been highlighted. Concurrent information has strongly challenged this view , in particular the reports of multiple resistant *E. coli* from pigs (Smith , 1974), *Salmonella* from calves associated with human infections (Anderson and Lewis, 1965) and similar organisms of varied animal sources (Benzanson, 1981; St. Louis *et al*, 1988; Lee *et al*, 1993; Whipp *et al*, 1994). Sequel to above debate , regulatory agencies have intervened with guidelines for

assessing drugs used as feed additives based on human health safety, animal health safety and anti-bacterial effectiveness criteria (Lehmann, 1972). The WHO has given general guidelines for the use of antibacterial agents , in particular, as additives (WHO, 1969).

2.1.3. Characteristics of antibiotic and other antibacterial feed additives:

When antibiotics are used in combination, they are synergistic in their actions (Beeson , 1969). Wallace (1970) and Visek (1978) reviewed the mode of action of antibacterial feed additives and noted that these additives have one common characteristic *ie* they suppress or inhibit the growth of microrganisms. Three main mechanisms of action were identified. a). Metabolic effect, more logically explained as an indirect effect on the metabolism of digestive tract microflora. b). Nutrient sparing effect - changes do occur in the quantity of certain nutrients available to the host when additives are fed (through one or all of the following ways: by stimulation of certa... microrganisms e.g, yeast cells increasing the availability of certain nutrients; by suppressing organisms that compete for critical nutrients; or by improving nutrient absorption resulting from thinner healthier intestinal walls due to suppression of organisms that cause damage or thickening). c). Disease control effect. By far the disease suppression effect is the most important (Beeson , 1969; Wallace, 1970; Visek, 1978; Fuller *et al*, 1983) and this has formed the basis for use of many additives (Beeson, 1969; Wallace, 1970; Visek, 1978; Braunuis ,1982; Fuller, 1983; Stutz *et al*, 1983a; Stutz and Lawton, 1984; Dafwang *et al*, 1987). Healthy animals fed

well, living in carefully cleaned quarters do not respond well to drug additives; response to antibiotic feeding is greater in a contaminated environment (Bowland, 1959 ; Wallace, 1970). In one study chlortetracycline induced a 14.3% improvement in gain in pigs fed in an old dirty barn, while an increase of only 7.5% was obtained in the newer cleaner barn (Bowland, 1956). In a similar study a greater response to spiramycin was noted, supposedly due to prophylactic effect when there is a build up of infection in an uncleaned environment(Hays and Speer, 1960).

Overall these factors overlap, inter relate and compound each other. However, the greatest responses are obtained when the additives are fed to animals during their early growth phase (Beeson, 1969; Wallace, 1970; Visek, 1978).

2.2 Antimicrobial Drug Residues

2.2.1 Drug Types And Incidence Of Antibacterial Drug Residues

The presence of a drug residue renders a product adulterated and may constitute a violation of the law (Guest and Paige , 1991). Studies conducted earlier in the US to ascertain the status of slaughter animals , found a large number of slaughter animals with residues using a *Bacillus subtilis* microbial assay. Huber *et al* (1969), screened the urine of slaughtered swine and found 188(35%) out of 536 to be positive for antimicrobial substances, 53 of which were due to penicillin. Out of 634 veal calves screened 125(20%) were positive and 45 (7%) were identified to be penicillin residues, 17% of the 235 sheep had antimicrobial substances, 4% due to penicillin,

while mature beef cattle showed the least prevalence with 6% of the 360 screened positive , 2% as a result of penicillin. In the same study, 25% of 214 chicken sampled indicated positive for antimicrobial substances, 13 were identified as penicillin residues. Therapeutic doses of parenteral preparations of penicillin produced residues below 4 days withdrawal for procaine penicillin and below 11 days for benthazine penicillin (Loftsgaard *et al*, 1968). In a wider study, Huber (1971a) used urine , faeces and or tissues of slaughter animals to screen for the presence of antimicrobial substances. A total of 1,224 swine were tested; 25% of urine samples were positive, 418 beef cattle , 756 veal calves , 328 fat lambs, and 798 chicken were tested and positive urine samples were detected from 6% of beef cattle and 21% of fat lambs. Out of 231 chicken livers tested 5 were positive for penicillin, 2 for dihydrostreptomycin , 3 tylosin and 2 tetracycline; 15 antimicrobial substances could not be identified. Use of long acting preparations such as benzathine penicillin and dihydrostreptomycin given to calves parenterally (particularly intramuscularly) has left residues at injection sites and in kidneys for greater than 30 days (Mercer *at al.*, 1971). Streptomycin concentrate in the kidneys and leave long lasting residues (Mercer *et al*, 1970).

In an overview of field investigations in the US, drugs most commonly involved in residues included streptomycin (20.2%), penicillin (31.1%), SMZ (15.2%), and Oxytetracycline (9.1%) (Van Dresser and Wilcke, 1989). In 1992, 2.8% of the 33,305 cows slaughtered in California were positive for antimicrobial residues , majority of cases were due to penicillin and oxytetracycline (Fajo *et*

al, 1995). A monitoring study in 1988 across the US using the Calf antibiotic sulfa test (CAST), to detect residues in veal calves , screened 3,095 calves, 967 of the CAST positives were analysed for specific antibiotics using the kidney, liver, and muscle. Neomycin was the most reported from the kidneys with detection rates of between 24.6% to 46.8% from eastern to western regions of the US.; gentamicin 3.1 - 24.6%. Sulfamethazine was least frequently detected (Wilson *et al*, 1991a). Experimental studies in calves with neomycin sulphate showed high concentrations in the kidneys even after 24 hrs. post injection (Black and Claxton, 1982). Sulfamethazine is a residue of concern; because of its occurrence the sulfa - on - site (SOS) test was developed (Guest and Paige, 1991). In an experiment in swine fed with 3-fold and 5-fold of chlortetracycline, penicillin, and sulfamethazine in feed to simulate conditions of overdosage in the course of production, results obtained did not show any harmful effects when they were fed continuously for 14 weeks based on their performance, heamatological examinations, urinalysis or gross histopathological examination. Residues of SMZ in edible tissues were found to be 4 ppm on day 0 and fell to 0.1 ppm on day 7. A 0.1 ppm tolerance has been established for SMZ (Ausburg ,1989; Cordle, 1989; Norcross and Post, 1990). SMZ residues have been identified in as many as 13% of slaughtered hogs (Bevill, 1984; Rosenberg, 1985). The incidence of violative residues of sulfanamides in liver samples of slaughtered swine in 1985 was about 6%, with significant variation between geographical locations; an estimated 2 - 5% of marketed hogs had violative SMZ residues in muscle tissue (Cordle, 1989). In a study involving 5 commercial swine producers , over one year period,

using different feeding procedures , pigs were given SMZ at approved concentrations and the recommended withdrawal period observed before slaughter. Sulfamethazine residues exceeding legal tolerances were detected in 118 out of 600(19.7%) swine urine samples in 26 of 60(43.3%) farm visits using thin layer chromatography(TLC), incidence of SMZ residues in the herds ranged from 17 - 92% (Bane *et al*, 1989). When sulfathiazole is fed orally to feeder pigs at therapeutic levels of 330 mg/kg for 3 days at 3 day withdrawal period, residues were below 0.1 ppm in the kidney and liver (Righter *et al*, 1971b). A withdrawal period in excess of 7 days is necessary in order to reduce residues to below 0.1 ppm when sulfaquinoxaline is fed orally to chicken (Righter *et al.*, 1970). Feeding broilers and laying hens with prophylactic and therapeutic dosages left residues in tissues. Within 3 days residues were depleted from muscle and fat but persisted in renal tissues and egg yolk (Righter *et al*, 1970). Residues persisted at a concentration greater than 0.1 ppm 10 days after withdrawal period when sulfamethazine was administered to breeder chicken in feed at 0.4 % or 0.1% in water (Righter *et al*, 1971a).

Samples obtained in Canada over a 5-month period from suspected slaughter hogs showed residue problem due to tetracycline residues at between 0.05 - 2.07 ppm and chlortetracycline at between 0.1 - 1.3 ppm (Salisbury, 1990). Detectable levels of tetracycline have been found in osseous tissues of pigs, calves, and chickens fed 5 - 8 ppm in the diet. Up to 1.79 ppm was found in calves fed 60mg/day for 56 days and 5.5ppm in chickens fed 9.2 ppm for the same duration of feeding (Booth, 1977b). When 48 pigs were fed feed containing 4 different levels of oxytetracycline (12.5 - 200 ppm) from weaning to slaughter

and tested with microbiological assay method, no evidence of residues were found in blood , urine, muscle, liver, and kidneys at slaughter, but all levels except 12.5 ppm showed urine with antibiotic activity. Bone samples gave positive microbiological results and fluorescence due to oxytetracycline under UV light (Jones *et al*, 1977). Similar results were obtained by Amuda - Giwa (1994) using 72 local Nigerian broiler chicks fed 6 levels of oxytetracycline (between 0 to 300ppm) and sacrificed at different ages. Residues were tested for from tissues and eggs of mature layers fed the same diet . At 2 days withdrawal birds tested negative at 50, 100, and 150 ppm but positive for 200 ppm oxytetracycline in feed. By 3 days no residues were found. Oboegbulem and Fidelis (1996), found residues in chichen fed antibiotic supplemented diet and in tissues of slughtered cattle in Nigeria. Oxytetracycline residues have been reported from commercial eggs and chicken meat in Kenya(Mitema and Omija, 1992).

Chloramphenicol (CAP) has also enjoyed wide usage both orally and parenterally (Clark, 1978a; 1978c). Chloramphenicol appears in all tissues 1 hour after oral administration and concentrates in kidneys , liver, and bile (Huber, 1977b; Clark, 1978c). Feeding 40 ppm in drinking water to birds for five days produced residues in the yolk for up to 4.5 days, up to 0.33 ppm CAP was detected in yolk while the birds were on the feed (Sissodia and Dunlop, 1972b). Chloramphenicol 40 ppm given to broilers in drinking water for a similar duration resulted in residues of about 0.2ppm in various tissues; up to 0.6ppm in the kidney and persisted for over 72hours after the medicated water had been withdrawn (Sissodia and Dunlop, 1972a). Survey for chloramphenicol in the US found 4 out of 585 calves positive for CAP

in 1981, 4 out of 1406 in 1982, and 5 out of 1.029 in 1983, residues ranged from 0.02 - 12,671 ppm (Settepani, 1984). Critical assessment of CAP usage in man and animals (Huber, 1977b; Clark,1978a; 1978b; 1978c; Anadon *et al*, 1990), established no acceptable tolerance level for CAP (WHO, 1969; Settepani, 1984; WHO, 1988), yet CAP residues were found in normal meat and milk supply(Martenuik *et al*, 1988; Salisbury, 1990).

After feeding calves with milk containing procaine penicillin, residues were found in their urine (Prange *et al*, 1984). Contaminated milk for long has been recognised as source of exposure to high levels of residue for man. Eberhert *et al.* (1963), studied persistence of drugs following intramammary infusion for treatment of clinical mastitis and realised that milk samples from treated quaters were positive for longer times compared with bucket samples. Mastitic cows treated with intravenous tetracycline or sulfanamides or combinations produced no detectable residues beyond the third milking approximately 36 hrs. after treatment (Hokanson *et al*, 1963). Intrauterine infusion of nine antibacterial compounds caused detectable residues in 17 out of 165 cows or in 25 out of 1110 post treatment milkings (Black *et al*, 1979). Heningson *et al* (1963) found no residues when intrauterine infusion of penicillin - streptomycin and furacin are used and when furacin was deposited in the vagina for a period of 12 - 96 hrs. after administration.

Murnane(1962) compiled reports of the incidence of penicillin residues in milk in Australia. In New South Wales 25% of 225 individual farmers supplied milk with penicillin content greater than 0.2 units/ml. In Victoria region, 0.04 - 5 units/ml penicillin was

recorded in 9% of over 1,200 deliveries to 5 factories within the same period . Similar picture existed in Britain and the US prior to concerted efforts that brought down the incidence of antibiotic residues in milk from 11% to less than 0.5% as a result of strict monitoring . In Britain for example in 1964 41,700 milk samples examined showed that 11% had antibiotic residues (Huber *et al*, 1971b). The increase in the frequency of so called inhibitory substances in bulk samples occurred after the introduction of more specific and sensitive test methods capable of detecting small concentrations when withdrawal period is not observed (Heeschen and Bluthgen, 1982). Seymour *et al*(1988) studied the presence of antibiotics in milk using 122 samples from 58 lactating cows receiving single antibiotic therapy with 7 different kinds of antibiotics, and found that 21% of milk samples were positive for residues beyond recommended withdrawal period. The residues were due to penicillin and cephapirin, most often due to overdosage. Bishop *et al* (1984) found no residues following the withdrawal period in milk. A study of computerised individual cow records from January 1981 to January 1985 involving 23 dairy herds revealed that 877 cows were culled, 33 cows from 12 herds violated drug withholding time and that the 15 drugs used in the violation most commonly included oxytetracycline, gentamicin, chloramphenicol and a combination containing lincomycin and streptomycin (Martenuik *et al*, 1988). Violation are commonly encountered following dry cow therapy *e.g.* using procaine penicillin - dihydrostreptomycin, novobiocin , cloxacillin, cephapirin (Oliver *et al*, 1984). The problem of contamination of milk is extremely complex due to the number of possible drug preparations, use of multiple

routes of administration and variability in detection methods (Archimbault, 1982).

2.2.2 Factors That Influence The Occurrence Of Antimicrobial Drug Residues

Determinants of antibacterial residues occurrence have been studied, particularly in the dairy industry (Murnane, 1962; Siddique *et al*, 1965; Johnson *et al*, 1977; Archimbault *et al*, 1982; Kaneane and Alwynelle, 1987; Mc Ewen *et al*, 1991). Factors that favour residue occurrence are diverse and compound each other.

Management factors: This is perhaps the most important factor. Van Dresser and Wilcke(1989) attributed residue occurrence to the type of operation and animal class. A higher incidence (33.3%) was noted in dairy cows from a total of 292 field investigative reports of drug residues in food animals from 1983-1988, followed by veal calves. Sulfamethazine residues were highest in market hogs (Ausburg, 1989; Van Dresser and Wilcke, 1989; Norcross and Post, 1990; Guest and Paige, 1991). Geographical distribution *i.e.* variation from region to region and seasonal patterns have been noted, these variations are due to differences in weather and climate(Guest and Paige, 1991; Wilson *et al*,1991), dairy herd sizes (Kaneane and Alwynelle, 1987; Mc Ewen *et al*, 1991; Wilson *et al*, 1991), predominant breed, availability of labour (Wilson *et al*, 1991) and animal slaughter classes (Ausburg, 1989; Van Dresser and Wilcke, 1989; Guest and Paige, 1991). High levels of residues were reported in bob veal calves (967 out of 3,095) using CAST in 1988(Wilson *et al*, 1991a). Special management items

have been implicated such as: failure to maintain(keep) adequate records regarding administration, dosage, weight *e.t.c.* (Van Dresser and Wilcke, 1989; Mc Ewen *et al*, 1989). The FDA in the USA attributed 14.5% of residues to be due to this factor(Van Dresser and Wilcke, 1989). Use of over the counter(OTC) drugs is another factor; the same study found 81.8% of the drugs involved to be OTC drugs(Van Dresser and Wilcke, 1989). Where feed is mixed, residues have been found due to feed mill mixing(Bevill, 1984; Van Dresser and Wilcke, 1989), contamination from improperly cleaned mixing equipment(Booth, 1977b; Bevill, 1984; Ausburg, 1989), error in delivery or accidental feeding of medicated feed soon before slaughter(Heeschen and Bluthgen, 1982; Bevill, 1984; Juszkiewicz, 1982; Guest and Paige, 1991; Kan, 1991). The possible cause of high and prolonged persistence of the coccidiostat nicarbazin in both eggs and broiler tissues was due to recirculation via litter. For metaclorpindol found in tissues of broilers not fed the drug while raised with other birds fed metaclorpindol, this was due to contamination by air either by evaporation or metaclorpindol containing dust particles (Kan, 1991). In 94% of cases investigated, the drug was administered by the owner of the animal or someone responsible to the owner(Van Dresser and Wilcke, 1989), these practice greatly predispose to residue occurrence.

Feeding milk or colostrum to calves from treated cows was responsible for up to 9% of a total of 490 residues(Guest and Paige, 1991). Use of common equipment to milk treated and untreated cows in milking parlours(Mc Ewen *et al*, 1991), use of hired labour or part time assistance(Marteniuk *et al*, 1988; Mc Ewen *et al*, 1991),

increased frequency of use of antibiotics(Kaneane *et al*, 1987; Mc Ewen *et al.*, 1991). Failure to vary withdrawal times for different drugs, marking treated animals, and use of on farm screening tests(Mc Ewen *et al*, 1991) are important factors that determine residue occurrence in dairy herds. The type of on-site screening test used in any farm to a large extent could determine how frequent residues may be found . Bane *et al*(1989) evaluated 3 SMZ residue detection methods in swine farms and showed deficiencies in all the 3 methods in terms of sensitivities in routine residue screening when compared with Thin layer chromatography(TLC).

Culling of cows due to retained placenta and milk fever was found to be an important but rare determinant of residue occurrence(Martenuik et at., 1988). Production levels of dairy farms(Kaneane *et al*, 1987; Van Dresser and Wilcke, 1989; Mc Ewen *et al*, 1991) and mammary quater(Katz *et al*, 1974) may be contributory. Siddique *et al*(1965) reported that rate of disappearance of drugs from milk is slower in dairy cows with lowered milk production(volume), like wise levels of penicillin residues detectable in milk on farm and in market milk depends on extent of dilution of contaminated milk with bulk milk(Murnane, 1962). Milk samples obtained from treated quaters generally contained detectable concentrations for longer periods than did bucket samples(Emberhart *et al*, 1963).

Drug usage: The presence of antibiotic residues results from misuse of drug preparations(Van Dresser and Wilcke, 1989), and these are a function of sales volume of the drug(Van Dresser and Wilcke, 1989). Feeding or use of unapproved drugs was responsible for 10% of 460

residues(Guest and Paige, 1991). Written orders by veterinarians ensures compliance and reduces residue occurrence. FDA investigations of records of drug sources in the USA showed that 44 were veterinarians and 59 lay, such as stores, cooperative societies(Van Dresser and Wilcke, 1989). The veterinarian's knowledge of drug pharmacokinetics, factors like age , pathophysiologic state, withdrawal periods and related variables are important determinant of residue occurrence(Riviere, 1991).

Pharmacokinetic factors: In a study to determine the persistence of residues in milk in dairy cattle, the antibiotic administered is related to drug persistence(Seymour *et al*, 1988). The drugs commonly found as residues are streptomycin, penicillin, oxytetracycline, gentamicin, neomycin, and SMZ(Guest and Paige, 1991). This is influence by the following pharmacokinetic factors.

1. **Dosage form**: Use of boluses caused 30% of residues in a 5 year retrospective study(Van Dresser and Wilcke, 1989). Slow release preparation have been incriminated in residue causation. Residues of penicillin are associated with sustained release preparations (Emberhart *et al*, 1963; Huber, 1977a; Archimbault, 1982). Similarly, chemical nature of the drug is involved, intramuscular procaine penicillin is withdrawn faster than intramuscular benthazine penicillin(Loftsgaard *et al*, 1968; Huber,1977a; Van Dresser and Wilcke, 1989). Violations due to SMZ are due to the use of soluble powder solutions of the drug(Ausburg, 1989).

2. **Route of administration:** Van Dresser and Wilcke (1989)proposed that certain routes of administration are associated with higher than average risk of drug residues. While others are less likely to lead to residues due to poor bioavailability. Chloramphenicol and penicillin for example given orally attain little or no plasma concentration due to first pass elimination by acid hydrolysis or microbial degradation in the rumen (Huber, 1977a; Clark, 1978b; de Backer and Bogart, 1982; Koritz, 1982), therefore are given systemically.

Injectable drugs are more likely to cause residues as determined by FDA in the USA in 51.3% of cases compared to those in feed additives(21.4%) (Van Dresser and Wilcke, 1989). Intravenous and intramuscular routes of administration showed considerably greater potential to cause oxytetracycline residues in milk than intrauterine route(Anderson *et al*, 1995). Intramammary infusion produced 9% of cases in culled dairy cows(Guest and Paige, 1991).

Large molecular size drugs such as the aminoglycosides or the gut acting sulphonamides are poorly absorbed and are thus less likely to cause residues(Baggot, 1977; Booth,1977b; Black and Claxton, 1982; Jenkins and Friedlander, 1982; Van Dresser and Wilcke, 1989).

Oral administration of penicillin left no residues at 24 hours while no rsidues were found by 4 days (Loftsgaard *et al*, 1968). In a rare circumstance the oral route resulted into residues in calves after consumption of milk containing penicillin(Prange *et al*, 1984). Righter *et al*(1970) found a higher concentration of sulfaquinoxaline in the tissues of water medicated birds, perhaps due to greater solubility of the drug. Lipophilic drugs accumulated more in cream

part of milk when the drugs were given intramuscularly compared to intramammary infusion(Ziv and Rasmussen, 1975). Residues were not found in milk following intrauterine treatment with furacin(Heningson *et al*, 1963), however, residues were detected when penicillin-streptomycin combination was infused intrauterine(Bishop *et al*, 1984). Subcutaneous administration produced effective plasma concentration of penicillin and eliminated the problem of residues at injection site when the intramuscular route is used(Mercer *et al*, 1971).

3. **Dosage and dose rates**: Exceeding recommended dose(extra label administration) has often accounted for residues in tissues(Kaneane *et al*, 1987; Van Dresser and Wilcke, 1989; Guest and Paige, 1991; Mc Ewen *et al*, 1991; Krainock, 1991). For example, the higher the dose of the penicillin the longer the persistence of penicillin levels in milk(Murnane, 1962; Krainock, 1991). Doubling the dose of a drug extends the withdrawal period by one biological half-life of the drug(Baggot, 1977b; Booth, 1977b; Riviere, 1991). When procaine penicillin G was administered at low dose levels no residues were found in tissues at 24 hrs. post administration(Loftsgaard *et al*, 1968).

4. **Tissue sequestration and protein binding**: Persistence of drugs in tissues is a factor of the extent of its tissue binding, covalent binding to tissue macromolecules, proteins and other biological components(Mercer *et al*, 1971; Booth, 1977b; Delatour, 1982). Initially this depends on the ability of the drug to get into systemic circulation(Huber, 1971b; Huber, 1977b; Baggot, 1977), and

subsequently its sequestration into tissues. Aminoglycoside antibiotics are known to sequester and bind covalently to kidney tissues and persist for long periods after exposure to even very low concentrations(Huber, 1971b; Huber, 1977b; Booth, 1977b; Van Dresser and Wilcke, 1989; Black and Claxton, 1982; Wilson *et al*, 1991a).

Excretory patterns and tissue affinities of sulfanamides are different; sulfanamides bind to plasma proteins and could persist in the blood due to renal process of resorption which recycled filtered drug back into the blood(Bevill and Huber, 1977). When sulfathiazole was administered orally to feeder pigs at therapeutic dose levels for three days, residues were not detectable in liver, kidney and fat at 10 days post treatment but urinary levels were found (Righter *et al*, 1971). Sulfathiazole is not reabsorbed by the kidneys which explains its higher urinary levels compared to tissue levels (Righter *et al*, 1971; Bevill and Huber, 1977). Residues of sulfaquinoxaline in poultry were more rapidly eliminated from liver, muscle, and fat but residues were higher in the urine and kidney at the initial and at the end of withdrawal. Concentrations in the skin approximated that of kidney(excretory function)(Righter et *al*, 1970). Residues of sulfaquinoxaline also concentrated more in egg yolk and decreased more slowly(Righter et *al*, 1970). Similarly, chloramphenicol has been shown to accumulate at higher concentrations in tissues than plasma(Clark, 1978b) requiring repeated injections to sustain effective plasma level(Huber, 1977b; Clark, 1978a; 1978b). It readily diffuses into egg and milk(Clark, 1978a).

Drugs administered systemically, in plasma may sequester into milk, leaving appreciable residues(Baggot, 1977; Huber, 1977a; Bishop *et al*, 1984; Heeschen and Bluthegen, 1982; Juszkiewicz, 1982; Krainock, 1991). Drugs that are weak bases have a greater tendency to cross the blood mammary epithelium boundary and concentrate in milk(Baggot, 1977). Antibiotics have been found to have transferred from treated to untreated quaters(Ziv *et al*, 1974). Intrauterine infusion using 9 different antibiotics caused detectable residues in milk(Black and Claxton, 1982). However, intrammamary infusion may result in complete elimination(Achimbault, 1982). Following intramammary infusion and intramuscular injection of benzyl penicillin, spiramycin, dihydrostreptomycin, and tetracycline, the ultimate concentration of their residues in milk by-products depended on drug concentration in whole milk. Lipophilic drugs like chloramphenicol, tetracycline compared to benzyl penicillin concentrate in the cream part, while casein concentrates more dihydrostreptomycin and tetracycline and less benzyl penicillin(Ziv and Rasmussen, 1975). The pH differential between the blood plasma also favours the concentration of chloramphenicol into the rumen following systemic administration(Baggot, 1977; Koritz, 1982).

5. **Failure to observe withdrawal period**: This has been established to be the leading cause of illegal residues(Booth, 1977; Bevill, 1984; Van Dresser and Wilcke, 1989; Guest and Paige, 1991).

When withdrawal period was observed no residue was found in milk from cows treated late in the dry period (Johnson *et al*, 1977), in colostrum following antibiotic dry cow therapy(Oliver *et al*. 1984).

Violative residues were not found when rations containing variable concentrations of chlortetracycline, sulfamethazine and penicillin were fed to swine(Messersmith *et al*., 1967) for various feed additives when good management practices are employed(Huber *et al*, 1971b; WHO, 1969). Residues may still persist even after observing the withdrawal period when this it happens it may be use of either due to improvement an assay techique that is more sensitive than the one used in establishing the withdrawal period (Bane *et al*, 1989; Van Dresser and Wilcke, 1989); use of extra-label dosage(Anderson *et al*, 1995) or as a result of some biological variability in individual animals (Baggot 1977, Riviere, 1991; Nouws, 1992).

Age of the animal: This is related to differences in pharmacokinetics(Nouws, 1992). In neonates for example there is increased absorption of orally administered drugs including macromolecules such as aminoglycosides, because of their greater intestinal permeability(de Backer *et al*, 1982; Jenkins and Friedlander, 1982; Van Dresser and Wilcke, 1989; Nouws, 1992), enhanced absorption from intramuscular injection site due to spongy nature of tissues of young animals(Nouws *et al*, 1986; Nouws, 1992), larger volume of distribution in younger animals due to a larger extracellular fluid volume(Svenden, 1976; Righter *et al*, 1979; Nouws, 1992). There is an age difference in ability to metabolise and excrete drugs in animals, due to immaturity of kidney and liver functions in young animals(de Backer *et al*, 1982; Nouws *et al*, 1986; Nouws *et al*, 1988; Miert *et al*, 1988; Nouws, 1992), and impaired function of the organs in aged animals(Baggot, 1977) therefore

changing the elimination half-life and subsequently the withdrawal period(Baggot, 1977; Riviere, 1991; Nouws, 1992).

Pathophysiologic state or disease conditions: Disease conditions can prolong the elimination half-life of drugs particularly those with long half-life(Van Dresser and Wilcke, 1989; Riviere, 1991). Oxytetracycline residues were found in 18.1% of animals that did not pass slaughter due to disease conditions(Oka *et al*, 1991). Disease conditions such as diarrheae(Ahrens, 1978; Jenkins and Friedlander, 1989), renal disease(Bywater, 1976), endotoxeamia, ruminal atony or pneumonia(Young, 1973) could markedly alter bioavailability as a result of decreased clearance, altered volume of distribution due to increased extracellular fluid volume(Baggot, 1977; Riviere, 1991). Elimination of penicillin residues is slower in animals suffering from mastitis compared to healthy ones(Siddique *et al*, 1965; Achimbault, 1982). Ziv *et al*(1974) reported that chronic mastitis can promote sequestration of drugs like chloramphenicol into milk, probably due to compromise of mucosal integrity. Antemortem characteristics may suggest high risk. Cows with less "udder fill", those deviating from normal posture or disposition, and cows soiled with faeces were more likely to contain residues than normal cows in each category compared to normal cows(Norman *et al*, 1995 .

Post harvest handling: Treatment of edible animal products could alter the content or eliminate the presence of residues in tissues. Ultra heat treatment of milk containing residues of antibiotics at 150°C for 7.9 seconds inactivated tetracyclines and

erythromycins(Yingprayoon, 1989). Pasteurising milk samples in the laboratory at 62.5 to 65°C for 30 minutes had no observable effect on penicillin content of milk, nor did momentary heating to 94°C by immersion in boiling water for 2 minutes(Berridge, 1956).

2.3. Regulatory Aspects

2.3.1 Regulations And Regulatory Agencies:

Regulatory agencies developed to check the increasing threat of chemical contaminants and residues in individual countries, atleast for public health reasons. The Swann Committee was appointed in the United Kingdom in 1969 to look into the problems posed by the use of antibiotics in animal husbandry. In a report(Swann, 1969) cited by several authors covering this subject(Huber, 1971b; Brander and Pugh, 1977; Gracey, 1986), the committee recommended that only those drugs that have shown little chance of inducing resistance in bacteria, and those that have little or no application in human medicine should be used as feed additives in food producing animals. More regulations followed: The injection of calves up to the age of 6 months with DES was prohibited in 1973 in the UK; in Northern Ireland the administration of estrogens and arsenic compounds to animals and to poultry was prohibited except under veterinary inspection. The Food and Drug Administration(FDA) in the USA ordered an end to the use of DES in feed or as implants as from 1973(Gracey, 1986). These pioneer legislations evolved into a comprehensive public health policy in several countries involving defined units of government or agencies with legislative powers. In Canada for example the first line of defense is contained in the Food and Drug Regulations. Safety of

drugs is determined by the Bureau for Veterinary Medicine that assess the efficacy and safety of veterinary drugs and their residues (Campbell, 1978). The bureau undertakes residue monitoring programs and enforce regulations via the Health Protection Branch and through provincial authorities at packing plants, farms, e.t.c.(Campbell, 1978; Fitzpatrick, 1990). In Sweden the use of antibiotics for growth promotion purposes in livestock was banned in 1985(Gracey, 1986).

The USA has the broadest and most comprehensive regulatory infrastructure and coverage from available literature. Two separate agencies namely the FDA's Centre for Veterinary Medicine(CVM) and the United States Department of Agriculture(USDA)-Food Safety Inspection Service(FSIS), form the basis for major regulatory functions. The CVM regulates and inspect foods other than meat, animal feeds and drugs(Norcross and Brown, 1991) to protect the public's health by regulating animal drugs, food additives and devices, and establish tolerance levels for veterinary drugs in tissues(Settepani, 1984; Cordle, 1989; Benson, 1991; Fitzpatrick, 1990; Guest and Paige, 1991).

Another agency, the Environmental Protection Agency(EPA) regulates pesticides that could be used in production and other industrial chemicals that have potential for contaminating food, and sets tolerance limits for pesticides and other chemicals(Forgleman, 1972; Norcross and Brown, 1991). Meanwhile the FSIS is responsible for ensuring the safety and wholesomeness of the nations meat and poultry. It ensures minimal consumer exposure to drug residues in edible tissues from food animals(Guest and Paige, 1989; Norcross and Brown,

1991). In 1987 alone FSIS inspectors examined 5 billion birds(Norcross and Post, 1990). In 1988 FSIS detected 3,095 antimicrobial violations in bob veal calves(Wilson *et al*, 1991b).

With advent of the European Economic Community(EEC), European Community(EC) countries came up with moves aimed at harmonising legislations including those regarding trade of food animals and animal products. Some of these legislations cover national markets of member states, intracommunity markets and importations from other countries(Council, 1964; 1981; 1986). Generally, EC regulations do not allow residues in meat(Ferrando, 1984; Kan, 1991), and there is a specific directive 89/437/EEC for toxic substances in eggs(Kan, 1991). All additives unless licensed remain strictly prohibited(Ferrando, 1984).

Earlier, strategies towards residue prevention have been outlined: Antibiotics given as feed supplements are subject to regulations and may be given without the interference of a veterinarian; Medicated feeds(containing >20ppm of antibiotics) only under veterinary prescription; Substances with thyrostatic, oestrogenic, androgenic, or gestagenic action(Council, 1981) and parenteral antibiotics could only be administered by a qualified veterinarian. Only those antibiotics that do not increase resistant bacteria are allowed, those that are not used in human medicine are preferred(Van Schothorst *et al*, 1978). Tolerances should be established based on microbiological and toxicological evaluation. Provisions were made for inspection of emergency slaughtered animals or otherwise suspected animals. Suspected carcasses are examined, when residues are found, carcasses are condemned(Van Schothorst *et al*, 1978; Council, 1986). Under the

National Residue Survey(NRS) program, Australia's Quarantine and Inspection Service carries out monitoring for residues in abattoir and other inspention establishment with standard comparable to that of the EU(Corrigan, 1992).

The World Health Organisation(WHO): There is a joint FAO/WHO expert committee on food additives which is a scientific advisory committee to the *Codex Alimentarius* on matters relating to food additives and contaminants, and residues of veterinary drugs in foods. Since 1969, the committee has established Acceptable Daily Intake(ADI) and other end points for well over 700 additives, chemical contaminants and veterinary drug residues in food(WHO, 1994). The committee's work has so far covered antimicrobial agents, antiprotozoals, anthelmintics, hormones, production aids, B-adrenoceptor blocking agents and tranquilizers(WHO, 1988; 1989; 1990; 1991; 1993; 1994).

Regulations and Regulatory Agencies in Nigeria: The skeleton of regulations regarding the use of veterinary drugs and veterinary drug residues in animal tissues in Nigeria is provided by the Food and Drugs Decree(1974) and the draft Veterinary Public Health(Meat Hygiene) Decree(1992).

Under the Food and Drug Decree, it is provided in Section 1, sub-section 1, that : " No person shall sell any article of food which: a) has in it or upon it any poisonous or harmful substances not being a food additive or contaminant of a type, within the level permitted by regulations made under the decree; b) is unfit for human consumption". Sub-section 2 continued as: " No person shall sell any

article of food or any drug which is adulterated". In the same decree, Section 16 provided for regulations mentioned in section 1: " The commissioner(i.e. the Minister of Health) may make regulations determining what constitutes the adulteration of any food or class of food or drug ....and prescribing the type and level of food additive or contaminant that may be present in any food offered for sale".

Meanwhile, the Meat Hygiene Decree provided under section 7, sub-section 1, that : " Except in the case of an emergency , all animals to be slaughtered shall be subjected to ante-mortem inspection ....and any food animal believed to be or diagnosed to be pregnant or affected by any of the diseases or afflictions listed under the second schedule to this Decree during such ante-mortem inspection shall be excluded from being slaughtered". The eighty first item listed under the second schedule is: Residues of antibiotics, chemicals, biologicals, and radioactive substances.

Under the Food and Drug Decree(1974), "drug" includes any substance or mixture of substances manufactured, sold, advertised for use in: (i) diagnosis, treatment, mitigation, or prevention of any disease, disorder, abnormal physical state, or symptoms thereof in man or in animals; (ii) restoring, correcting or modifying organic function in man or animals; (iii) disinfection or the control of vermin, insect or pests; or (iv) contraception. This definition therefore covers all those drugs evaluated by the FAO/WHO expert committee(WHO, 1994). "Food" includes: "any article manufactured, sold, or advertised for use as food or drink for man,....and any ingredients that may be mixed with food for any purpose whatever, but does not include: (i) Water, or live animals, birds or fish, fodder or feeding stuffs for animals,

birds or fish, other than supplements produced for addition to animal and poultry foodstuffs, or articles or substances used only as drugs. This therefore includes edible animal products, namely meat, milk, and eggs, and fish produced by food animals as provided in the first schedule of the Meat Decree. The Food and Drug Decree has provisions for the control of importation of drugs(section 3) and food(section 8,sub-section, 2 and 3).

At the same time the Meat Hygiene Decree(1992), has made provisions for the importation and exportation of meat, carcass, and animal by-products(section 12). The difference between the two provisions however is that each decree has empowered a different person or authority -commissioner of health(Minister) in the Food and Drug Decree(1974) and the Minister of Agriculture in the Meat Hygiene Decree(1992)- to issue permit. The importation of live animals, hatching eggs and poultry is controlled by the Animal Disease(Control) Decree(1988).

Existing regulations under the Food and Drug Decree have provided for the monitoring and surveillance, through inspection, sampling, analysis(section 10 and 13), prohibition from sale(consumer protection) and setting tolerances(section 4 and 5) for various substances in foods.

Under section 1 and 2 a new decree, National Agency for Food and Drug Administration and Control(NAFDAC), the FDA and all its powers(including regulatory roles) have been taken over by the new agency(NAFDAC)(Federal Republic of Nigeria, 1993). Despite these provisions there are no specific regulations for residues of veterinary drugs in animal products, nor are there provisions that

coordinate regulatory functions between the two agencies under the decrees(NAFDAC and Federal Department of Livestock and and Pest Control Services) as practised in other coutries like the USA(Norcross and Brown, 1991). However the NAFDAC has recently started implementing WHO recommendations; it banned the use of Nitrofuran drugs in livestock and poultry feed(NAFDAC, 1996).

2.4 Methods Of Qualitative And Quantitative Determination Of Residues Of Antibacterial Drugs

2.4.1 Microbiological Methods:

Microbiological methods of determining of antibacterial residues have been found to be suitable for screening purposes. Screening of slaughter animals relies on the use of urine(Huber *et al*, 1969; Van Schothorst and Peelen-Knol, 1970), kidney swabs(Johnson *et al*., 1981), kidney tissue(Takacs snd Kovaes, 1969) and tissue extracts(Meredith, 1965; Loftsgaard *et al*, 1967; Van Schothorst and Poelen-knol, 1970; Moreno, 1980; Singer and Katz, 1985), to estimate the presence and load of antibacterial residues.

Microbial inhibition tests(MIT): The greatest usage is in screening of milk for residual antimicrobial agents. Silverman and Kosikowski(1952) used the disc assay method to detect penicillin and other antimicrobial substances in milk. Numerous modifications of this technique followed. Three modifications of the MIT currently in use include: Calf antibiotic sulfa test(CAST) using *Bacillus megaterium* on Mueller Hinton agar at 44^{0}C(Korsrud *et al*, 1987), and two MITs one using swab sticks, Swab tests on premises(STOP), and

another using filter paper discs as sample applicator, are being used(Korsrud and Macneil, 1988).

The test organisms commonly used include: *Sarcina lutea*(*Micrococcus luteus*) ATCC 9341 using a cylinder mono layer technique(Loftsgaard at al., 1967), in disc plate assay for penicillin(Huber *et al*, 1969; Van Schothorst and Peelen-knol, 1970; Katz and Fassbender, 1978; Brady and Katz, 1989), chloramphenicol(Singer and Katz, 1985; Brady and Katz, 1989), and other antibiotics(Huber *et al*, 1969; Van Schothorst and Peelen-knol, 1970); *Bacillus subtilis* ATCC 6633 in disc assay for screening of slaughter animals for routinely used drugs, using filter paper discs in urine(Huber *et al*, 1969), Kidney tissue(Van Schothorst and Peelen-knol, 1970; Johnson *et al*, 1981) and for screening milk(Silverman and Kosikowski, 1952). Also, in the assay of penicillin(Kaufmann, 1977; Macaulay and Packard, 1981), streptomycin(Brady and Katz, 1989) and for detecting other antibiotics in milk(Macaulay and Packard, 1981); *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 was employed to screen urine of normal slaughter animals on microtiter plates containing agar with 0.9% of brilliant black indicator to show acid production(Van Schothorst and Peelen-knol, 1970). The sensitivity of this organism has made it very popular for use in different methods of screening milk (Igarashi *et al*, 1961; Van Os *et al*, 1975; Van Os and Benkers, 180; Kelly, 1982). *B. stearothermophilus* var. *calidolactis* on PMI agar detected less than 0.0025 units of penicillin per ml of milk and requires less time than the *B. subtilis* penicillin assay(Kaufmann, 1977).

A common method involves using about 10^6 spores of the thermophilic bacterium mixed with 0.3ml plain agar in an ampoule enriched with a nutrient tablet and incubated at 64^0C for 2.5 hours(Van Os *et al*, 1975) or for 2.75 hours in the Delvo-P^R test for very large number of samples(multisystem) at a time (Van Os and Beukers, 1980). Positive results are indicated by color change due to acid production from purple to yellow. The Delvo-P^R test was employed to screen contaminated reconstituted milk and detected 0.01 units of penicillin per ml (Huhtanen *at al.*, 1977).

Factors that influence microbial inhibition tests: Bielecka *et al*(1981) evaluated parameters affecting sensitivity, accuracy and simplicity of plate diffusion method for determining antibiotics in meat with *B. stearothermophilus*, and found that sensitivity was most affected by concentrations of innoculum(optimum concentrations 2 x 10^5 spores/ml.); quantity of assay medium on plate(6ml/10cm diameter plate is optimum),single layer plates were more reliable; sample size of 100 ul on a 12.7mm diameter disc most ideal(Read *et al*, 1971; Bielecka *et al*, 1981; Messer *et al*, 1982; Peeler *et al*, 1983). The larger volume of sample conveyed by cotton swabs in STOP test could account for its greater sensitivity over MIT with filter paper discs(Korsrud and Macneil, 1988).

Bacillus megaterium has been reported to be most ideal for screening of animal tissues, urine, or milk for residues of sulfonamides(Read *et al.*, 1971; Gudding, 1976). *Bacillus cereus* var. *mycoides* ATCC 11778 is best suited for assay of the tetracycline antibiotics, it was employed on assay agar medium A for

oxytetracycline, and on assay agar medium D for chlortetracycline in milk(Brady and Katz, 1989), in meat(Van Schothorst and Peelen-knol, 1970) and in determination of OTC in feeds using cylinder plate method. Average recovery rate from feed was up to 92.4%(Billman and Clark, 1968). The target drug for analysis determines the test organism for the analysis, for example *B. subtilis* produces bacitracin so is unsuitable for its assay, *B. cereus* produces penicillinase enzyme thus will destroy the penicillin in sample and would not be inhibited(Read *et al.*, 1971). For routine practical work *M. luteus* and *B. stearothermophilus* variety *calidolactis* have been found to be most suitable(Gudding, 1976).

Temperature and duration of incubation are important factors in MIT (Berridge, 1956; Van Schothorst and Peelen-knol, 1970; Read *et al.*, 1971; Kaufmann, 1977; Johnson *et al.*, 1981; Peeler *et al.*, 1983; Brady and Katz, 1987; 1989). Kabay(1971) using *B. stearothermophilus* ATCC 12980 compared disc assay tests for 10 different antibiotics using 4hrs and 18hrs incubation period. No significant differences on the sensitivity of the test was noted between the two periods of incubation. Temperature of incubation at 29°C for 16-18hrs(Johnson et al, 1981), 37°C for 18-20hrs(Huber *et al.*, 1969: Van Schorthorst and Peelen-knol, 1970), and higher temperatures of up to 55-60°C(Kaufmann, 1977; Brady and Katz, 1987) or even 64°C for 2.5-4.5hrs(Vilim *et al.*, 1979; Van Os *et al.*, 1975) have been reported for thermophilic bacteria. Read *et al*(1971) found 37°C to be the most ideal temperature for incubation. Similarly, sensitivity of the MIT is affected by size of innoculum(Berridge, 1956; Loftsgaard *et al.*, 1967; Billman and Clark, 1968; Huber *et al.*, 1969; Huhtanen *et al.*, 1977; Rajkowski

et al., 1986); and the type and content of culture media(Kaufmann, 1977; Katz and Fassbender, 1978; Messer *et al.*, 1982; Peeler *et al.*, 1983; Rajkowski *et al.*, 1986; Bielecka *et al.*, 1987; Brady and Katz, 1989). Antibiotic medium No. 1 employed in standard methods for antibacterial drug analysis is generally insensitive to sulfa drugs because it contains p-aminobenzoic acid or folic acid(Read *et al.*, 1971). Mueller Hinton agar was demonstrated to be suitable for the assay of most antibacterial drugs(Read *et al.*, 1971; Korsrud and Macneil, 1988). Other factors that influence the sensitivity of the MIT include: concentration of the test organism(Davis, 1971; Kaufmann, 1977; Bielecka *et al.*, 1981; Singer and Katz, 1985) and pH. pH plays a role in extraction of drug from tissues and the sensitivity of the test(Katz and Fassbender, 1978; Korsrud and Macneil, 1988), pH is also very important to both the antibiotic potency and test organism's growth. Streptomycin and erythromycin are most sensitive to pH(Bielecka *et al.*, 1981). Katz and Fassbender(1978) used *S. lutea* to assay penicillin and noticed increased sensitivity when pH is lowered to 4.5 to precipitate proteins then adjusted back to 6.0. Assay specificity could also be enhanced using different pH(Brady and Katz, 1989): pH 6.0 for penicillin(Katz and Fassbender, 1978); 4.5 for chlortetracycline, 8.0 for streptomycin, and 6.0 for chloramphenicol(Brady and Katz, 1989). Moreno *et al.*(1980) reported the occurrence of non specific zones of inhibition in concentrated chicken muscle extracts. Other workers have identified this source of error in microbial assay tests, using concentrated extracts of porcine liver and kidney(Loftsgaard *et al.*, 1967), and muscle extracts(Loftsgaard *et al.*, 1967; Singer and Katz,

1985). Buffering of the extraction solvents with phosphate buffer pH 7.0(Moreno *et al.*, 1980) and 6.0(Lofstgaard *et al.*, 1967; Singer and Katz, 1985) greatly reduced the appearance of the non-specific inhibition zones. Contrary to these reports Yoshimura *et al.*(1982) found that muscle and liver extracts did not inhibit *M. luteus* or *B. subtilis*. The size and intensity of this zones are dependant on the duration after slaughter, highest after 24 hours(Loftsgaard *et al.*, 1967; Moreno *et al.*, 1980) and the extraction solvent or solvent mixtures(Loftsgaard *et al.*, 1967; Singer and Katz, 1985).

The pH of the normal porcine, bovine and ovine urine were found not to be sufficiently acidic or basic tc inhibit the growth of test organism *B. subtilis* when urine was employed to screen routine slaughter animals(Huber *et al.*, 1969; Van Schothorst and Peelen-knol, 1970). Eventhough specific pH values may produce better results for specific drugs. Singer and Katz (1985) obtained sharper edges of zone of inhibition due to chloramphenicol at pH 6.0. Differences in pKa values cause differences in concentrations of active drug at any given pH(Baggot, 1977). TerHune and Upson(1969) related incorrect live animal swab test for oxytetracycline in urine of animals to urine osmolarity and pH. Urine samples with false positive results had significantly higher osmolarity and pH.

Other factors like centrifugation of milk samples(Katz *et al.*, 1974) and the use of more than one disc tc innoculate sample(Huber *et al.*, 1969) may affect sensitivity. Singer and Katz(1985) used tetrazolium dye to enhance the appearance and measurement of the zone of inhibition, and addition of sub inhibitory concentrations of OTC to enhance detection of low level OTC. Use of kidney tissue directly on

agar media has shown increased sensitivity when screening animals(Takacs and Kovaes, 1969). The type of tissue which the sample was sourced is an important source of variation. The recovery rate of unbound chloramphenicol from bovine urine and serum was 88.5%, milk 79.3%, and from muscle 61.0%(Singer and Katz, 1985). Variability in sensitivity rates could be due to errors as a result of the presence of natural inhibitory substances in tissues such as lysozymes(Skanes and Watson, 1957), high contents of heavy metals such as cadmium from the kidney of a horse inhibited *S. lutea*(Pekkanen *et al.*, 1974), or products of post mortem changes(Loftsgaard *et al.*, 1967; Moreno *et al.*, 1980). Sischo and Burns(1993) studied 4 different cowside antibiotic residue screening tests and found that depending on the test, 5-22% of the samples were inappropriately identified as having residues. Thus no one test has perfect specificity. The annual losses due to this false positive results are enormous: From 1993-94, 30.9 million Kg of milk was dumped because of positive assay results(Gardner *et al.*, 1996). Romond *et al.*(1971) noted that potential source of error could be from the presence of antibiotic producing bacteria in milk, especially in extended incubation. Read *et al.*(1971) supported heating milk to about 82^{0}C for 2-5minutes to distinguish residues from natural inhibitors in milk. So also other workers(Messer *et al.*, 1977; Macauley and Packard, 1981). Microbial inhibition tests are limited to detection of antibacterial substances, their abilities to identify specific agents is limited. A number of workers have managed to identify penicillin residues in urine(Huber *et al.*, 1969; Singer and Katz, 1985) and in milk using penicillin discs(Berridge, 1956; Read *et al.*, 1971; Singer and Katz, 1985) and

liquid penicillinase(Kelley, 1982; Rajkowski, 1986). Read *et al.*(1971) proposed the use of p-aminobenzoic acid discs to identify sulfonamides residues from other antibiotics. Addition of low concentrations of trimethoprim to Mueller Hinton agar was found to improve the sensitivity of test bacteria towards sulfonamides by 20-50 times(Gudding, 1976) based on their synergistic effects. The increased sensitivity was not exhibited by other antibiotics.

2.4.2 Physico-Chemical Methods:

Chromatographic Determination: Combined biological and physical methods have been employed mainly in the form of thin layer chromatograpy(TLC)-bioautography for general identification of antibiotic residues. Neidert *et al.*(1987) reported a method that successfully identified 14 different antibiotics that were positive by the STOP test. Routinely TLC bioautography is employed for determination of antibiotic residues in Canadian slaughter animals(Salisbury *et al.*, 1989). Earlier Yoshimura *et al.*(1982) reported a TLC-bioautographic determination of aminoglycosides in urine and tissues of slaughter animal.

Penicillins: Bossuyt et *al.*(1976) evaluated 14 different antibiotics used to treat mastitis apart from penicillins, and detected a minimum of 1, 0.1, and 0.5ug/ml of milk for cloxacillin, ampicillin, and oxacillin respectively, using *Sarcina lutea* bioautography. Yoshimura *et al.*(1981) described a TLC procedure for ampicillin and penicillin G in animal tissues on silica gel with bioautography. Herbst(1982) determined 4 *B*-lactam antibiotics in milk using reverse phase plates

with bioautography. Moats(1983a) developed a more rapid and specific method for determining penicillin G and cloxacillin in milk. Samples were extracted with acetonitrile and concentrated in methylene chloride and run on silica gel with chloroform-acetone-glacial acetic acid mixture as mobile phase. Detection was with 1N Hcl spray followed by 0.5% soluble starch and exposure to iodine vapour. Limit of sensitivity for penicillin is 0.01 ug/ml.

Liquid chromatographic techniques have also been applied for detection of penicillins using anion exchange columns(Tsuji and Robertson, 1975). Detection from biological materials have been tried, such as Moats(1983b) from milk involving multiple analysis for 3 penicillins using reverse phase column and UV detection. Munns *et al.*(1985) described a method for multi residue determination of 8 neutral β-lactam penicillins in milk by fluorscence-liquid chromatography. Samples were extracted with acetonitrile, converted to a fluorescing derivative with dansyl hydrazine and separated on a C18 column, acetonitrile-water as mobile phase and detected by UV. Recovery from milk for benzyl penicillin is up to 79.4%.

Chloramphenicol: Bossuyt *et al.*(1976) separated CAP on silica gel G in ethanol-water-ammonia, methanol-chloroform and methanol-acetone, a minimum detectable concentration 1ug/ml of milk was reported. Detection was by bioautography with *B. subtilis* (Betina, 1964; Bossuyt *et al.*, 1976), *Staphylococcus aureus* and *Escherichia coli*(Aszalos *et al.*, 1968), *Micrococcus flavus* and *Sarcina lutea*(Bossuyt *et al.*, 1976).

A method for determination of CAP was described on silica gel G using chloroform-methanol-ammonium hydroxide as mobile phase that could achieve up to 100% recovery and could detect 10ppb CAP in milk(Karkocha, 1977), beef(Karkocha, 1978) and eggs(Karkocha, 1979). Chemical detection was employed using Tin chloride spray followed by p-amino benzaldehyde producing a yellow spot. Allen(1985) reviewed other methods of TLC for determination of CAP from pig and hen kidney or muscle using Kieselgel 60F 254 stationary phase and dichloromethane ethyl acetate as solvents. Less than 50ug could be detected by this method with UV-fluorescence at 366nm.

Schwartz and Mc Donough(1984) determined CAP from skimmed milk on silica gel G at equal to or greater than 60ug CAP following chromosorb column separation. Allen(1985) compiled Gas Chromatographic(GC) methods reported from 7 countries. GC methods with electron capture were found to be most sensitive with limits of detection as low as 1ppb or less. Similar reports detected CAP from muscle, liver, and kidney(Nelson *et al.*, 1983), using nitrogen(Wal *et al.*, 1979; Nelson *et al.*, 1983) or argon as gas phases. Most of the detection methods employed electron capture detector(Wal *et al.*, 1979; Nelson *et al.*, 1983); others used flame ionization detector and thermionic detector(Allen, 1985). Various column types have been described. Gas-chrom.Q(Allen, 1985), chromosorb-W with 3% methyl silica DC 200(Allen, 1985), Gas Chrom. Q with 3% OV-1(Nelson *et al.*, 1983; Allen, 1985) and capillary columns(55m) coated with Se-30 or Se-52(Allen, 1985).

Liquid chromatographic techniques described for detection of CAP in muscle meat(Van der Lee *et al.*, 1982), from calf and hen

muscle(Bories *et al.*, 1983) using acetonitrile(Allen, 1985), ethyl acetate(Wal *et al.*, 1980; Bories *et al.*, 1983; Allen, 1985) and buffer(Van der Lee *et al.*, 1982) as extraction solvents. Water-methanol mixture(Wal *et al.*, 1980; Bories *et al.*, 1983; Allen, 1985), buffered acetonitrile, buffered methanol, diisopropyl ether-methanol were most commonly used mobile phases. Nucleosil 10-NO_2, Nucleosil C_{18}(Allen, 1985), 5um Spherisorb ODS in addition to reverse columns have been reported. LC methods utilise UV detectors at 254nm, 280nm(Wal *et al.*, 1980; Bories *et al.*, 1983), 278/350nm(Van der Lee *et al.*, 1982). Detection of as low as 2ppb have been reported(Allen, 1985).

Tetracyclines: Blakely *et al.*(1969) developed a paper chromatography for identification of tetracycline in the tissues of fish that could detect the 3 common tetracyclines using nitromethane, chloroform and pyridine mixture. Tissues were extracted with n-butanol the test is sensitive at 0.1ppm CTC.

TLC procedure for determining TC, OTC, and CTC in milk has been reported(Bossuyt *et al.*, 1976) using Kieselguhr as stationary phase. Rf values of 0.35, 0.20, 0.60 for TC, OTC, and CTC were obtained by running plates in methanol-chloroform(9:1). A minimum detectable concentration of 0.3ug/ml of milk was reported.

TLC methods rely on the use of boiautography with *Bacillus cereus* var. *mycoides*(Mayers and Smith, 1964; Blakely *et al.*, 1969; Bossuyt *et al.*, 1976) and *Staphylococcus*(Aszalos *et al.*, 1968); Chemical detection with 3% ferric chloride in methanol gives a light brown spot for tetracycline(Betina, 1964); and UV detection(Britsh

Pharmacopea, 1988). Tetracyclines were also determined on acid washed diatomaceous earth treated with buffer consisting of EDTA at pH 7.0, glycerin, and polyethylene glycol 400, and developed with ethyl acetate mobile phase. The technique is suitable for tetracyclines and their metabolites(Ascione *et al.*, 1967). Ashworth(1985) reported a procedure based on conversion of tetracycline to anhydro forms in the presence of 1N acid and heat. Methylene chloride or chloroform as extraction and developing solvents, on plates devoid of gypseum are employed. The procedure is accompanied with a bioassay and could achieve determination of tetracycline concentration equal to or greater than 0.1ppm.

Liquid chromatographic procedures for tetracyclines from tissues have been difficult. Ashworth(1985) developed a unique solvent system for tetracyclines using 0.05M diethanolamine(buffered to pH 7.3 with phosphoric acid) and tetraammonium EDTA. Good responses were obtained with silica of pore diameter 100-150A° pre-treated with nitric acid wash and EDTA to remove residual ions. C_{18} bonded materials were used with UV detection. Average recovery of tetracyclines is higher from muscles compared to liver and kidneys(Ashworth, 1985). A new contineous separation method of 5 different tetracyclines have been described(Tsai and Kondo, 1994), using initial bioassay on Minimum Medium with *B. subtilis*. Clear zones of inhibition are then extracted for HPLC using a *u*-Bondapack C_{18} column and a mobile phase consisting of 0.01M oxalic acid: acetonitrile: N,N-dimethyl formamide.

Sulphonamides: Bane *et al.*(1989) demonstrated the superiority of TLC in terms of specificity(94%) over other methods of detection of

SMZ residues in swine. TLC methods have been described on silica gel GF_{254}(Sigel *et al.*, 1974) for the detection of trimethoprim sulfamethoxazole in plasma and from tissues -milk, muscle, liver, and kidney using fluorescamine derivatization on silica gel(Sigel *et al.*, 1975) with quantitative detection using spectro densitometry at 290nm. The method could detect 0.049-0.051ug of sulfadiazine. A similar method has been described for determining SMZ in swine plasma(Bevill *et al.*, 1978). TLC methods have been found to be more specific. Phillips and Trafton(1975) separated colorimetric products following a Bratton Marshall reaction for detection of sulfonamides in chicken tissues because of possible interferences on silica gel G. A miture of acetone-heptane-methanol-ammonium hydroxide-butanol was used as mobile phase. Thomas *et al.*(1981) described a TLC technique that was able to separate, identify and quantitate 5 of the commonly used sulfonamidess: sulfathiazole, sulfadimethoxine, sulfaquinoxaline, sulfamethazine and sulfabromomethazine from swine tissues. Samples were extracted with ethyl acetate and spotted on Whatmann LK6D stationary phase, tert-butanol - chloroform as mobile phase. Detection of spots followed dipping of plates in fluoroscamine derivatives. A slightly modified procedure with glycerine buffer(pH 12.25) recovered more sulfonamide types from beef, turkey, and duck muscle and liver(Thomas *et al.*, 1983). Slight variation in analysis of tissues was reported between red meat and poultry samples due to differences in fat content.

Goodspeed *et al.*(1978) reported the use of Gas liquid chromatography for the determination of free and conjugated sulfonamides extracted from edible animal tissues with acetone.

Samples were screened with Brantton Marshall reaction derivatized with diazomethane before detection with Ni-electron capture detector. Manuel and Stellar(1981) extracted SMZ residues from tissues with acetone-chloroform; residues were methylated with diazomethane and separated through a glass column packed with OV-101 on 60-80 mesh Gas Chrom. Q, with argon-methane as carrier gas and Ni-electron capture detector. Residues were quantitated using an external standard, the procedure can detect 0.10ppm SMZ in cattle and swine tissues and resolve methylated derivatives of related sulfonamides. Suhre *et al.*(1981) detected diazotized derivatives using mass spectrometry with a stable isotope(C13) labelled SMZ. Malonowski *et al.*(1981) compared 3 methods for the determination of SMZ residues in swine liver and muscle using the method of Tishler *et al.*(1968), a diazomethane derivative of SMZ detected by electron impact mass spectrometry, and GLC techniques with electron capture detector. GC-MS was found to be most reliable with recovery rates between 90-100%. Paulson *et al*(1985) developed a GC-MS method for identification of C18 labelled SMZ and its metabolites in swine tissues.

Colorimetric Determination: Colorimetric determination rely on the production of chemical products of the drug that could be detected under UV. Fellig and Weistheimer(1968) described a technique that could measure as little as 1.0ug of sulfadimethoxine in tissues following a chloroform extraction and Bratton Marshall reaction. Recovery rate ranged from 70-90% for different tissues. Tishler *et al.*(1968) improved the procedure for determination of sulfonamides in milk and tissues based on a diazotization coupling reaction with N(1-

napthyl)ethylene diamine dihydrochloride. The method recovered 10 sulfonamides at the rate of from 81-100% at 100ppb level in milk, and from kidney, liver, muscle and fat at the rate of 76-100% at 100ppb level.

Herrett and Buzard(1960) reported a method for the determination of furazolidone and nitrofurazone in chicken tissues, with a prior column chromatography to separate non-specific reactants in liver extracts from the phenyl hydrazone derivative of the nitrofurans. As low as 0.5ppm could be demonstrated visually and quantitated at 1 to 5ppm. The same technique, without the initial chromatography detected 1 to 5ppm of the drug in fat and muscle. Cox and Koetis(1963) determined as low as 0.01ppm of furaltadone in milk using chloroform and 0.1N Hcl. There is a report of an initial TLC procedure and colorimetric determination of antibiotic residues in animal tissues(Fernandez *et al.*, 1969).

2.4.3. Immunoassay Techniques

Immunoassay techniques have been employed for the assay of hormones and drugs in serum and plasma. These macro molecules called haptens are coupled to proteins to make them immunogenic. Monoclonal antibodies induced against them are harnessed and used in the assay; Hefle(1995) reviewed the principles behind the immuoassays, production of antibodies, enzyme substrate systems, assay formats, and solid phase choices. Pestka *et al.*(1995) reviewed the use of immunoassays for the detection of mycotoxins in food, and another for the detection of pesticides, herbicides, growth regulators, fumigants, and antibiotics(Bushway and Fan, 1995).

Enzyme immunoassay(EIA): Enzyme immunoassay offer quickness, accuracy, reproducibility, and sensitivity in analysing chemicals in foods(Bushway and Fan, 1995). Bane *et al.*(1989) found EIA to be very rapid, convenient, and inexpensive, but reported it to lack specificity(59%) and sensitivity(58%) in analysis of SMZ in tissues, particularly when compared to TLC. Several improved EIA techniques have been described and have been found to be suitable for screening purposes(Haagsma and Van de Water, 1992). Enzyme immunosorbent assay(ELISA) for drug residues is either competitive direct ELISA or competitive indirect ELISA(Golden, 1991; Dixon-Holland, 1992) using enzyme labelled antibody or enzyme labelled drug(Haagsma and Van de Water, 1992).

Several commercial kits are available for antibiotics and SMZ in milk, urine, serum, tissues and feed(Dixon-Holland, 1992; Bushway and Fan, 1995). Detection rates of SMZ is high(Medina *et al.*, 1992). The assay may be quantitative or qualitative. Quantitative assays employ a spectrum of color reaction, the intensity of which depends on the concentration of the drug in the sample(Golden, 1991). ELISA test kits have shown lowest detection limits of 2-10ppb by visual interpretation, and 1ppb by photometer reading(Bushway and Fan, 1995; Helfe, 1995). A typical dose-response curve is usaully plotted(Singh *et al.*, 1989; Golden, 1991) Deviations from the standard curve may be due to non-specific binding, low affinity and non optimal reaction temperature and pH(Golden, 1991). A simple assay using test strip immunoassay(Quick-card^{R}) has been applied for the detection of chloramphenicol in milk(Nouws *et al.*, 1988), and in urine and eggs(Haagsma and Van de Water, 1992). Campbell *et al.*(1984)

described a competitive ELISA for detection of chloramphenicol. Using the commercial immunoassay card test, Keukens *et al.*(1992) detected chloramphenicol residues in meat at 2ppb and at 0.5ppb in milk. As low as 0.2ppb in meat was also detected(Jackman, 1993) and streptomycin from tissues at 2.5ppb. Reports of determination of other antibiotics with immunoassay include Dixon-Holland(1992) for the determination of chlortetracycline, oxytetracycline, and tetracycline in milk and other tissues; penicillin and its derivatives in cow's milk and serum at concentrations as low as 1ppb(Rohner *et al.*, 1985). Berkowitz and Webert(1986) used ELISA to monitor for gentamicin in the serum of slaughter animals. Up to 200-400 animals could be processed per analyst per day and could detect 2-3ng/ml. There was no cross reaction between gentamicin and any other drug used for agricultural purposes. Ram *et al.*(1991) developed a quick test for SMZ in plasma; as many as 2,400 samples could be processed in 8 hours. ELISA developed for swine plasma recovered over 97.6% SMZ in spiked plasma. The test can analyse 80 plasma samples in 2 hours(Singh *et al.*, 1989). Another report showed a limit of detection of 13ppb in swine serum; the test was highly specific for SMZ, but could not recognise other related sulfonamides, chlortetracycline or penicillin(Dixon-Holland, 1992). Sometimes the test could be adjusted to lower limits. Haagsma and Van de Water(1992) showed that the detection of chloramphenicol from swine muscle could be lowered by introduction of streptavidin-biotin system in the assay. Immunaffinity clean up procedure is used to increase specificity by using affinity column with specific antibody coated solid support. Matrix and interferring substances are

washed through the column while the analyte is retained and later determined using HPLC(Haagsma and Van de Water, 1992).

Radioimmunoassay (RIA): In other instances RIA is used with antibodies of high affinity, the ligands are labelled with ^{14}C, ^{3}H, or ^{125}I(Steiner and Harris, 1992). It requires extraction and separation of interferring matrices using a column chromatography. RIA has been employed for trace analyses of chloramphenicol residues in eggs, milk, meat with a sensitivity of 1ppb for milk and 5ppb for meat(Arnold and Somogyi, 1985). The precision depended on the type of the tissue and the concentration of the drug. Residues were confirmed by Gas chromatography. A radio-receptor assay was also reported. It uses bacteria like *Bacillus stearothermophilus* bearing receptor for different antibiotics instead of an antibody to detect the presence of drugs(Bishop and White, 1984).

2.5 Effects Of Antibacterial Drug Residues On Human Health

2.5.1 Antibacterial Drug Resistance:

Mechanisms of antibacterial drug resistance: Mechanisms of resistance to antibiotics by bacteria generally involves one or more of the following processes: a). The cell providing an alternative to the metabolic step blocked by the drug; b). Production of metabolites that can antagonise the effect of the drug on the bactria; c). Increasing the amount of an enzyme blocked by the drug; d). Decreasing the cells dependance on the pathway blocked by the drug; e). Inactivation of the drug; f). Changing of the target site; and or blocking transport of the drug into the cell(Ogarawa, 1981; Young and Amyes, 1986).

These phenotypic expressions are chromosomally based or extra-chromosomally encoded on what is referred to as R-factor, extrachromosomal genetic material, DNA, which resembles the sex factor(F) of *E. coli* and other episomes or plasmids of bacteria(Datta, 1965). Genetic information can be shared between bacteria of different genera horizontally by transformation, trasduction or transposition(Watanabe, 1967; Guiney, 1984). Since the initial report the phenomena has been reported world wide particularly amongst the members of the family *Enterobacteriaceae*(Gill and Hook, 1966; Kabins and Cohen, 1966; Smith and Halls, 1966; Walton, 1968), and other bacteria(Yagi *et al.*, 1976; Clewell, 1981). Cultures infected with R-factors appear almost unaltered in their phenotyphic characteristics other than drug resistance. Curing removal of the plasmid restores previous antibiotic sensitivity. The plasmid may code purely for survival in the presence of antibiotics(Datta, 1965). Resistance may exist in nature, like resistance to B-lactams demonstrated by both producing and pathogenic strains of bacteria(Ogarawa, 1981), or as a result of artificial introduction of an antibiotic, like in the case of a plasmid encoded trimethoprim resistance(Young and Amyes, 1986; Singh *et al.*, 1992). Antibiotic resistance genes have also been exchanged between bacteria via transposable elements or mobile genes(Rubens *et al.*, 1979; Clewell, 1981), and by transducing bacterial phages particularly in Gram positive cocci(Malke, 1974; Clewell, 1981). Trimethoprim inhibits the enzyme dihydrofolate reductase; there is no known substance in nature that mimics the action of trimethoprim(Koch, 1981).

Selection pressure and propagation of resistance: Selection pressure from animal husbandry is promoting the carriage of small numbers of *E. coli* in the gut of normal people(Hartley and Richmond, 1975; Levy *et al*, 1976b). The ability to persist under selection pressure is essential for the propagation of resistant genes(Koch, 1981). Cases of multiple resistant bacteria are increasingly being reported. Multiple resistant faecal bacteria have been reported in 68.5% of persons not exposed to antibiotics in the recent past(Ismaeel, 1993). Often therapy in man promotes survival and shedding of resistant bacteria(Asherkoff and Bennett, 1969). It is difficult to partition blame according to antibiotic usage in human and veterinary medicine. The possibility of bacteria in man developing resistance to antimicrobial agents by continued exposure to antibiotic residues in foods have been implied(Baldwin, 1970), since under natural conditions the occurrence of bacteria carrying R factor appear to increase in the presence of low antibiotic concentrations(Clewell, 1981). More recently Brady and Katz(1992) reported that additive or synergy effect resulted from exposure to residual levels of 3 antibiotics, apparently raising their individual minimum inhibitory concentrations. As low as 2-10ppm of antibiotics have been shown to increase the number of multiple resistant *Salmonella typhimurium* from animals(Baldwin, 1970). Selection pressure exerted by feeding livestock with small doses of antibiotics has been responsible for multiple resistant *E. coli* isolated from farms and abattoirs(Rollins *et al.*, 1974; Linton *et al.*, 1977), and from the gut of normal people(Hartley and Richmond, 1975). These bacteria are more prevalent in herds exposed to continuous feeding of antimicrobial agents(Mercer *et al.*, 1971; Hirsh

and Wiger, 1978; Langlois *et al.*, 1978a; Dawson *et al.*, 1983). Similarly isolation of multiresistant *Salmonella* from several animal species(Sojka *et al.*, 1974), *S. heidelberg* serotype *newport* in cattle(Homberg *et al.*, 1984) and *S. typhimurium* in swine(Williams *et al.*, 1978) potrays a large reservoir of multiresistant *Salmonella* for man and animals.

Environmental contamination: Environmental pollution with resistant organisms by both man and animals is common. Analysis of coliforms from raw sewage and effluents showed R-factor carriage in 8.91% of total coliforms and 10.8% of faecal coliforms, up to 7 R-determinants were shown to be simulteneously carried by the same organism(Bell *et al.*, 1981). Antibiotic resistant bacteria have been found in drinking water(Amstrong *et al.*, 1981) collected from distribution system and from sewage(Dhillon and Dhillon, 1981) and dairy farm environment(Rollins *et al.*, 1974; Smith *et al.*, 1994). Data from environmental sources suggested that antibiotic resistant coliforms may survive better than sensitive ones in surface waters, but the same generalisation could not be extended to other Gram negative genera. However, antibiotic resistance pattern of bacteria of different genera correlated well, perhaps indicating that bacteria share a common mode for developing antimicrobial drug resistance(Kelch and Lee, 1978).

Resistant bacteria from animals to man: The greater concern is that of transfer of resistant bacteria from animals to man. Close relationship have been demonstrated between the incidence of antibiotic resistant strains isolated from humans and animals(Smith,

1974; Bezanson *et al.*, 1981). A higher incidence of R+ *Escherichia coli* was found in animal attendants and kitchen personel compared with the general population(Smith, 1974). Handling of meats in packing plants is a major source of *E. coli*(Stiles and Lai-King, 1981), and *Salmonella*(Bryan, 1981). Bacteria resistant to more than one antibiotic were isolated from retail market meat(Hankin *et al.*, 1976) and healthy broiler chicken at slaughter(Lee *et al.*, 1993). The primary sources of these bacteria is the gastrointestinal tract of the animals most probably contaminating the carcass. *E. coli* O-serotypes isolated from rectal contents have been demonstrated on the carcass of the same or related animals at slaughter(Howe and Linton, 1976). Resistance exhibited by isolates of *Salmonella typhimurium, S. saintpaul*, and *S. heidelberg* from animals was similar to the pattern shown by human isolates(Neu *et al.*, 1975; Nair *et al.*, 1995).

Escherichia coli with plasmid encoding multiple antibiotic resistance was spread from chicken to chicken and from chicken to man in a poutry farm(Levy *et al.*, 1976a). Similar cross infections with resistant *Salmonella* were reported. An *S. heidelberg* epidemic accurred in a hospital nursery (Lyons *et al.*, 1980), the bacterium was resistant to chloramphenicol, sulfomethoxazole, and tetracycline, and was traced to a dairy herd as the source.

Colonization of the human gut by antibiotic resistant bacteria from animals depends on duration of contact between the animal source and man. However, short time handling of raw chicken carcass has demonstrated transfer and establishment of resistant *E. coli* in the human gut(Linton *et al.*, 1977). After the establishment the period of carriage of the acquired bacterium is variable and relative.

Colicines were suggested to be helpful in establishing infection with resistant strains in the gut(Cooke *et al.*, 1971). R-factors were found to be less wide spread than they might be in those *E. coli* O-serotypes that are to persist well in the human gut. If R-factor carriage in *E. coli* O1 and O2 - the strains that persist well in the human gut - increases, then an increase in the proportion of resistant *E. coli* should be expected(Hartley and Richmond, 1975). There are specie variation in duration of shedding of multiple resistant bacteria, *S. typhimurium* were shed for shorter period than multiple resistant *E. coli*, eventhough they harboured a common plasmid(Linton *et al.*, 1981). Certain host factors like age, use of antacids, previous exposure to antibiotics have been suggested to favour disease occurrence with resistant bacteria(Riley *et al.*, 1984). Meanwhile barriers exist that limit R-factor transfer between bacteria due to suitability or otherwise of cell surface proteins and lipopolysaccharides essential for physical contact, presence of appropriate restriction enzymes(Guiney, 1984), and due to plasmid incompatibility(Corliss *et al.*, 1981). Also, in-vitro plasmid transfer could be determined by culture media type and conditions employed in the laboratory(George *et al.*, 1981; Hill and Carlisle, 1981).

2.5.2. **Direct Toxic Effects**:

When residues occur they occur as free residues of parent drug or its metabolites, conjugated or covalently bound to tissue components(WHO, 1988). Residues may have a direct toxic effect. Certain toxicities are mediated by metabolites of otherwise inert

substances that covlently bind to tissue macromolecules like DNA, lipids, and enzymes(Gillete, 1974). Thus in assessing toxicity, metabolites of the drug may be used(WHO, 1988), unfortunately, metabolites of importance may not be isolated from blood or urine, therefore tests to determine effects *in vitro* are handicapped(Gillete, 1974).

Commonly, toxic effects associated with drug residues are limited to allergic reactions in man and other effects that are observed in laboratory animals or from adverse reactions during therapy; Weston(1970) has cautioned on interpretation of experimental results in animals and their extrapolation to human subjects.

Allergic reactions: Results of toxicity studies of 10 commonly used antibiotics, using mice, showed that penicillin is the least toxic at different doses using different routes of administration(Bacharach et *al.*, 1959). But penicillin is a major cause of allergic reactions. In a survey of 1000 hospital patients in the USA, 7.2% were hypersensitive to penicillin, 1.6% to sulfonamides(Huber, 1971a). The WHO has estimated overall prevalence of penicillin allergy in different populations to be between 3-10%(WHO, 1990). Usually the initial sensitization must have occurred following exposure to large doses parenterally. Subsequent exposure to minute amounts of penicillin can provoke these reactions. The amount of penicillin found as residues is sufficient to cause allergic reactions in sensitized subjects. One individual was reported to have collapsed after drinking a glass of milk that contained penicillin(Siegel, 1959). Milk containing 0.16 units/ml caused allergic reactions. Oral

administration of 0.4units of penicillin can induce reaction(Siegel, 1959; WHO, 1990). Reaction following consumption of penicillin in pork(WHO, 1990) and chicken(Teh and Rigg, 1992) have been reported. Sometimes penicillin in foods may not be of veterinary origin, but may be added accidentally or deliberately to foods, like a case of penicillin in soft drink(Wicker and Reisman, 1980) and another report that found penicillin in steak(Shwartz and Sher, 1980).

Adverse reactions to tetracyclines following consumption of food believed to have been contaminated with tetracycline residues (Anthony, 1977) and streptomycin residues(Tenkelman and Bock, 1984) have been reported. Skin allergies in hypersensitive subjects to sulfonamides could occur following consumption of foods like eggs containing high concentrations of sulfonamide residues(WHO, 1989). Allergy to sulfonamides is known in humans undergoing therapy with the drug, eventhough the likelihood of residue induced allergy is low, residues of the drug must be kept as low as possible(WHO, 1989). Similarly photo-allergy and phototoxicity are known to occur in people undergoing therapy with chlortetracycline. Aminoglycoside(like streptomycin and neomycin) and bacitracin can cause skin reactions(WHO, 1969). Meanwhile evidence implicating these drugs is unavailable, their concentrations in edible animal tissues must be kept at permissible levels.

Other toxic effects: These are effects noted as adverse drug reactions following therapy in man or observed from carcinogenicity and teratogenicity studies in mice, rats, rabbits or dogs, and mutagenicity studies using bacteria(WHO, 1990). In laboratory animal

feeding experiments, with the most sensitive animal species, no observed effect levels(NOEL), are determined. The NOEL is the maximum concentration of the drug in feed that produced no observable pathophysiologic change in the laboratory animal expressed as drug per unit body weight. These on weight basis are extrapolated to man. From the NOEL, acceptable daily intake(ADI) is established for man(WHO, 1990). The recommendation of ADI in man uses a safety factor, a fraction of the levels in animals in its computation. Also, a target tissue, a marker(test) residue and an analytical method to quantitate the residue are required to establish ADI(WHO, 1990; Fitzpatrick et *al.*, 1995). Consumption factors vary. Generally it is assumed that an average person consumes 300g for meat, 100g liver, 50g kidney, 50g tissue fat, 100g eggs, and 1.5 litres of milk(WHO, 1988; 1990). The variation in consumption factors vary the ADI from one country to another (Fitzpatrick *et al.*, 1995). Maximum residue levels(MRL) determination in tissues of food animals assumes that animals are raised under good management practices(WHO, 1988).

No ADI is allocated for chloramphenicol(WHO, 1988). Chloramphenicol(CAP) causes bone marrow depression in people resulting from mitochondrial injury, depressed erythropoeisis(Brest, 1967), immunodepression and behavioural changes that are reversible. It is also associated with an irreversible aplastic anemia; affected people may develop leukemia, this response is dose independant(Settapani, 1984). There are reports of people that have developed aplastic anemia following the use of CAP occular preparations 32-82mg divided over several days. A rancher was reported to have developed aplastic anemia and died after feeding his cattle with CAP(Settepani, 1984).

This dose independant sensitivity to CAP appears to be genetically mediated(Settepani, 1984; WHO, 1988). The toxicity is due to a metabolic product: nitrosochloramphenicol(Yunis *et al.*, 1980). Studies of CAP in chicken showed similar toxic effects, CAP showed a higher half life in chicken due to slower metabolism; metabolic products are still detectable in appreciable quantities after the withdrawal period(Anadon *et al.*, 1994), residues of CAP have been reported in poultry products(Sissodia and Dunlop. 1972a; 1972b). No ADI is allocated because the effect caused by CAP can be dose independant, there is no dose-response relationship, no suitable animal models since the idiosyncracy is reported only in people, the mechanism of pathogenesis is unknown, therefore no acceptable residue level(WHO, 1988). The WHO has prohibited the use of CAP in food animals(WHO, 1988). A CAP related drug, thiamphenical (TAP) was found to be an effective replacement for CAP (Abdenebi *et al.*, 1994). It has been used successfully in human therapy and is free from the side effects of CAP. In animals TAP has been found to be a suitable replacement for CAP. Thiamphenical is rapidly absorbed from intramuscular injection site. It is well distributed in body tissues including the mammary gland (Abdenebi *et al.*, 1994).

Tetracycline have also been associated with a genetically determined disorder characterised by defects in renal tubular function reportedly due to ingestion of degraded tetracycline(Gross, 1963). Tetracyclines are also known to sequester in to bones and cause a reversible cessation of bone growth and discoloration(WHO, 1969). Residues of metabolites of tetracycline drugs have not demonstrated this effect(WHO, 1969; 1990).

Sulfonamides have demonstrated antithyroid activity in feeding experiments using laboratory animls(WHO, 1989), and in human therapy(Mandell and Sande, 1985). Sulfonamides caused thyroid cancer in laboratory animals. SMZ is a major cause of residues in pork(Ausburg, 1989; Cordle, 1989; Norcross and Post, 1990); residues of sulfonamides should be kept as low as 0.1ppm(Ausburg, 1989). The nitrofuran drugs have caused a dose-dependant decrease in luteinizing hormone production in chicken fed 15-30mg/kg, drop in egg production(Ali *et al.*, 1987) and interference with spermotagenesis in man(Nelson and Burge, 1957). Nitrofurans are mutagenic in laboratory animals(WHO, 1993). A recent notice by NAFDAC has described nitrofurans as human mutagens and carcinogens and has therefore banned its use in poultry and livestock feeds in Nigeria(NAFDAC, 1996). Olaquinox, an antimicrobial drug used for growth promotion in pigs is considered to be genotoxic and is a germ-line mutagen from animal experiments. No MRL is established for olaquinox(WHO, 1990). Lasalocid is routinely used as an anticoccodial drug in poultry. Safran *et al.*(1993) reported cases of severe neuromuscular deficits involving 10 dogs that consumed dog food containing about 10-15mg of lasalocid per kg of food. It is feared that human exposure to such amount may be toxic. The drug is known to have cumulative effect upon persistent exposure to small quantities(Galitzeer and Oehme, 1984).

CHAPTER THREE

3.0 MATERIALS AND METHODS

Two separate surveys were conducted. The first one studied the nature of drug use in veterinary practice and animal production, and assessed the level of awareness and concern about veterinary drug residues in meat in Nigeria. The second survey determined the occurrence of antibacterial drug residues in slaughtered cattle and chicken using a microbiological screening test.

3.1 Drug Use, Abuse/Misuse Pattern And Occurrence Of Residues:

A close ended questionnaire(Appendix I) was administered to 200 animal health personnel and workers responsible for drug usage in animal production. The study subjects in this category included veterinarians, veterinary assistants and livestock superintendents. Respondents were in the employment of government and private veterinary establishments that carry out academic, administrative, clinical, pharmaceutical, legislative, and or other responsibilities in animal health and production.

Questions were aimed at determining the nature of drug use, extent of drug abuse and misuse, perception on the potentials of residue occurrence, factors that favour residue occurrence, distribution of the residue problem according to animal production and management systems, animal by-products, and possible residue prevention strategies in Nigeria. The pattern of drug use by animal owners and producers was also studied using another questionnaire(Appendix II). Responses were assigned number codes and entered into a computer coding sheet(Microsoft Excel^{R}). Relationships

and associations between factors were assessed statistically using Chi square and Fisher's exact test.

3.2 Awareness And Concern About Drug Residues In Meat:

A close ended questionnaire(Appendix II) was administered to 500 persons involved with animal production, marketing of animals, slaughter, processing and marketing of meat, and to the consuming public.

Questions were asked to obtain demographic information, type and source of animal or meat, preslaughter evaluation and condition. Similarly, concern about residues in meat, possible reasons for concern or lack of concern about consuming meat of animals treated with veterinary drugs before slaughter, what should be the fate of residue containing meat, level of awareness and other factors that could influence respondents opinion were determined. Responses were assigned number codes and entered into a computer coding sheet(Microsoft Excel®). Relationships and associations between responses and factors were assessed statistically using Chi square and Fisher's exact test.

Prior to the survey the questionnaires were validated by administering to 25 respondents. Using these responses modifications were made accordingly in the questionnaires.

3.3 Screening Of Slaughter Animals For Antibacterial Drug Residues:

3.3.1 Samples Collection

Samples from cattle: A total of 500 cattle slaughtered in Zaria abattoir were screened for the presence of antibacterial substances at slaughter. All cattle were slaughtered using the Halal method. The age, breed, sex, and body condition was determined for each animal. The age was evaluated using dental and cornual ring markers. A body condition score was assigned to each animal or carcass according to a scale adopted from the method of Pullan(1978), ranging from 0 to 1(very poor),2(poor), 3(fair), 4(good), to 5(very good). Carcasses with gross post-mortem pathologies detected by visual examination were noted. Immediately after evisceration of each carcass, 10ml of urine was collected by direct aspiration from the urinary bladder with a sterile 20ml syringe and 18G needle. Urine samples were transferred into 10ml sterile serum vials and transported to the laboratory on ice and processed immediately or frozen at -20^{0}C until the following day, to minimise drug decomposition due to changes in urine pH as a result of bacterial multiplication.

Samples from poultry: Chicken routinely slaughtered at a government approved slaughter facility in Kaduna were sampled. Meat inspection procedure before and after slaughter was not conducted.

Based on type and management system, birds were categorised into: Broilers, Pullets and Local chicken. Improved chicken(Broilers and Pullets) came from intensive and semi-intensive farms in and around Kaduna metropolitan, while local chicken are sold in an

adjoining local market, and were of different origins within and outside Kaduna state.

Ten birds were randomly screened from each batch of broilers or pullets coming from the same source or farm, while local chicken were screened individually for the presence of antimicrobial substances. All birds were bled according to the Halal method of slaughter. Terminal feaces was collected from the cloaca of each bird into a sterile 10ml glass test tube and transported on ice to the laboratory and processed immediately or frozen at -20^{0}C until the following day. A total of 376 birds were screened.

3.3.2. Sample processing

Determination Of Urine pH: Urine pH was determined for the first 81 samples using an electronic pH meter. Frozen samples were allowed to thaw at room temperature before processing. Urine samples were transferred into a wider 50ml test tubes placed firmly in test tube rack. The electrode of the pH meter was then dipped into the urine sample and held while the meter reads the pH. The electrode was wiped with moist cotton wool and rinsed with distilled water then immersed back into a buffer solution pH 7.0 before another sample is read.

Screening for antimicrobial substances: A microbial inhibition test was employed to screen animals for evidence of antibacterial drug residues in urine as previously described (Huber *et al.*, 1969; Van Schorthorst and Peelen-knol, 1970; Granite Diagnostics, 1985). Mueller Hinton agar(GIBCO Limited, Paisley, Scotland) was employed and prepared according to the manufacturers directions in 15cm diameter

plates to a thickness of about 6mm. *Micrococcus luteus* ATCC 9341 was used as test organism. An 18 hour culture of the test organism in 10mls nutrient broth(Oxoid Basingstoke, Hampshire, U.K.) was used to inoculate plates. Broth suspension of the test organism was adjusted to a concentration approximately equal to the turbidity of a $Bacl_2$ Mc Farland standard solution No. 1(equivalent to 3×10^8 cells/ml.), using physiological saline as diluent. Plates were inoculated with the test organism by dipping a sterile cotton swab sticks into the suspension of the test organism until it is saturated with the suspension. The Mueller Hinton agar plates were then gently and thoroughly swabbed uniformly, one quadrant at a time as described by Granite Diagnostics(1985), to achieve a lawn of confluent growth. Inoculated plates were allowed to dry for 5-10 minutes. Urine from cattle samples were tested by dipping a sterile 1.2cm diameter Whatmann filter paper disc into the test urine until the disc is saturated with the urine sample. Excess urine was allowed to drain along the walls of the tube. With a sterile thumb forceps, the impregnated disc was gently placed on the surface of the Mueller Hinton agar plate inoculated with the test organism. The disc was firmly but gently pressed on to the surface to allow for proper diffusion on the agar around the disc. Test discs were then labelled accordingly; 8-12 samples were tested per plate(Plates I, II, and III).

In the case of chicken samples, an initial purification process was undertaken as described by Huber *et al.*(1969). To about 1g of feaces, 5ml of sterile phosphate buffer(pH 7.0) was added and mixed thoroughly by shaking. The suspension was then placed in boiling

water bath for 15 minutes to reduce bacterial interference during the microbial inhibition test. After heating, the sample suspension was then centrifuged at 3000 rounds per minute for 10 minutes(IEC HN-SII Centifuge, Damon International Equipment Company Massachusetts 012194). The supernatant fluid was used to inoculate sterile filter paper disc for the test as described for urine samples from cattle.

Whatmann filter paper discs containing 30ug of chloramphenicol were used as control discs, placed in the centre of the plate with test discs arranged along the periphery of the plate(Plates I, II, III).

After application of the test and control discs, plates were then incubated at 37^{0}C for 18-20 hours. After the incubation period, plates were then viewed for the presence or absence of zones of inhibition of test organism around the test and control discs. The diameter of the zones of inhibition were measured in millimetres from one edge of the zone to the other across the disc. The difference between the diameter of zone of inhibition and that of the disk was calculated. Any disk with a difference of 1mm or more is considered positive (Plate I, II and III).

CHAPTER FOUR

4.0 RESULTS

4.1. Drug Use, Misuse And Abuse Pattern, And Residue Occurrence In Nigerian Animal Food Products. Response Of Veterinarians And Other Veterinary Personel.

A total of 144 responses to the questionnaire, in Appendix I, were obtained from 130 veterinarians(90.3%) and 14 non-veterinarians(livestock superintendents and veterinary assistants), out of 200 questionnaires administered. This represents a 72% response rate.

Most of the respondents, 119(82.6%), were aged 40 years and below while 24(16.7%) were above 40 years. One hundred and twenty five (86.8%) of the respondents were males. Nine(6.3%) have been veterinarians or veterinary personel for over 20 years; 62(43.0%) have been involved in veterinary activity for between 11-20 years, and 73(50.7%) for less than 10 years or less. About 84.7% of the respondents were located in the urban areas.

A large number of respondents, 59(40.9%), work in a veterinary clinic; 22(15.3%) are in government administration or perform legislative duties; 13(9.0%) work in the pharmaceutical industry; 25(17.4%) in an academic institution, and 24(16.7%) are employed in other establishments (Table 1). Out of the 59 that work in veterinary clinics, 31(52.5%) work in a public(government) clinic; 17(28.8%) in a university or research centre clinic and 11(18.7%) are in private practice. Majority of the veterinary personnel, 43(72.9%), handle mainly food animals

in their clinics, while 11(18.6%) are mainly in non-food animal practice(Table 2). Majority of the respondents in mainly food animal practice, 37(86.0%), handle mostly ruminants, 4(9.3%) mostly poultry(avian practice), and only 1(2.5%) handle mostly pigs(Table 2).

A total of 138(95.8%) of veterinarians and other veterinary staff believed that there exist gross abuse and misuse of veterinary drugs in Nigeria. This belief was independent of the respondents age, sex, nature of employment and location(Fisher's exact test, $P>0.05$). Twenty eight(19.4%) were of the opinion that animal owners or clients are mainly to blame for the abuse or misuse. Also 25(17.4%) blame veterinary assistants and livestock superitendents mainly while 8(5.6%) accuse veterinarians. Most, 79(51.6%), however, believed that all the listed categories are responsible(Table 3). Opinion of respondents about who is responsible for the abuse or misuse was significantly influenced by nature of job (Fisher's exact test, $P<0.05$)(Table 4), but not by location of respondent($P>0.05$).

With respect to the drug types, 134(93.0%) and 81(56.3%) responded that antibacterial drugs and anthelminthics respectively are commonly abused and/or misused; 71(49.3%) said that antiprotozoals are uncommonly abused or misused while 40(27.8%) think that abuse and/or misuse of insecticides and acaricides is uncommon(Table 5). The greatest abuse and misuse were attributed to antibacterial drugs(93%). Fifty five(38.2%) and 39(27.1%) believed that the abuse and misuse does not occur

in free range poultry and pig production respectively. These production systems had the highest responses for not abusing or misusing drugs(Table 6). Of the 144 respondents, 56(38.6%), considered the abuse and misuse of veterinary drugs to be uncommon in intensive ruminant production, 70(18.6%) in semi-intensive ruminant production, 62(43.1%) in semi-intensive poultry production, 60(41.7%) in intensive pig production, and 64(44.4%) in semi-intensive pig production (Table 6). Free range or nomadic ruminant producers were blamed by 115(79.9%) respondents to commonly abuse and misuse veterinary drugs, while 58(40.3%) of respondents blamed intensive poultry producers.

One hundred and thirty one(91.0%) of the veterinary personnel believed that the nature and extent of the abuse and misuse could result in the occurrence of residues of these drugs in edible animal tissues. There was no difference between the opinion of younger veterinarians and older veterinarians, and between males and females (Fisher's exact test, $P>0.05$). There was no difference in responses to this question betweeen urban and rural veterinary personnel(Fisher's exact test, $P>0.05$). Six(4.2%) each were unsure and in disagreement respectively. Of the 131 respondents who think residues could occur, 78(59.5%) believed that the residues could most likely occur in meat, 23(17.6%) in milk, and 20(15.3%) in eggs.

On how these residues come about, 70.8% of the respondents believed that use of higher than normal dosage was responsible; 76(52.8%) blamed it on the use of long acting injectable

preparations and 91% on failure to observe withdrawal periods(Table 7). Use of implants and use of drugs in a species in which the drug is not recommended for use in that particular specie was not blamed each by 65(45.1%) of the respondents. Respondents overwhelmingly agreed with the proposed practices and user factors suggested by the questionnaire as capable of leading to residues(Table 8). Insufficient knowledge about the drug was blamed by 86.8% of respondents; 122(84.7%) accused refusal to obey instructions on how to use the drug, 99(68.7%) think that absence of laws generally on use of drugs should be blamed, while 103(71.5%) specifically blamed absence or inadequacy of laws on the use of veterinary drugs. The nature of employment of respondent did not influence this opinion(Fishers exact test, P>0.05), on what may be considered responsible. Similarly, nature of the clinic of respondents did not significantly influence the belief about what are responsible for the occurrence of residues(X^2, Fishers exact test, P>0.05). University or research centre veterinarians were more inclined to blame inadequacy of laws than other veterinarians in private or public clinics(X^2, P<0.05). When residues occur in slaughter animals, the following slaughter practices were suggested to be common and capable of causing residues: Slaughter of animals with a poor prognosis under going treatment with a veterinary drug (86.8% of respondents), slaughter of animals treated by owners (69.4%), and slaughter of birds(poultry) consuming medicated feed or water (77.8%) (Table 9). A lesser proportion

of respondents(4-16%) exonerated these slaughter practices as being incapable of causing residues or as being uncommon. Up to 116(80.5%) believed that slaughter of animals treated by owners is common. In addition, 129(89.6%) and 125(86.8%) think that slaughter of animals with poor prognosis undergoing treatment and slaughter of birds consuming medicated feed or water are common respectively(Table 9).

Five respondents proposed other slaughter practices that are common and could lead to residues: Slaughter of animals within the withdrawal period, slaughter of animals that lack farm and medication records and slaughtering of animals with implants. Regarding judgement on the meat of animal that was slaughtered while undergoing treatment with a veterinary drug, over 70% of respondents judged that such meat should neither be sold nor consumed(Table 10).

Many, 103(71.5%), of the respondents claimed being familiar with legislations locally, nationally or internationally on the use of veterinary drugs and drug residues in animal products. On the whole, only 32(22.2%) admitted lack of of familiarity with legislations on veterinary drugs and drug residues. Totally only 34(23.6%) of respondents knew that chloramphenicol is not suitable for use in food producing animals(Table 11). There was no significant association between this knowledge and type employment of the veterinarian(X^2, P>0.05).

On how to control veterinary drugs, over 70% of respondents agreed with the reasons proposed by the questionnaire(Table 12). The highest agreement was to public education on the dangers of residues in animal products (97.2%), enforcing laws regarding the treatment of animals exclusively to veterinarians(88.9%) and educating veterinary staff on pharmacokinetics of drugs at risk of becoming residues, and enforcing laws regarding the sale and consumption of animal products containing residues(86.1%). The least agreement was with respect to restricting the manufacture and marketing of medicated poultry feed (54.9%). These responses were independent of the nature of employment of the veterinary personnel(Fisher's exact test, $P>0.05$). Other measures proposed by respondents include encouraging more veterinarians to go into private practice(0.7%) and careful supervision of veterinary assistants(0.7%). On the part of the drugs, preventing the sale of expired drugs(0.7%); ensuring good manufacturing practices with greater research into those drugs capable of causing residues(1.4%); and providing withdrawal period on the labels(0.7%) were proposed. In addition, importation of veterinary drugs should be restricted to veterinarians(0.7%). Similarly, some respondents proposed that only veterinarians should carry out meat inspection(0.7%) and that meat inspectors should routinely check for residues in meat(1.4%). Regarding regulations, penalties should be provided for defaulters of laws regulating drug usage in animals(0.7%); the public should be educated on meat(public health) laws(1.4%); and that these laws should be reviewed from time to time(0.7%).

Table 1: Job Description and Duration of Association of Respondents With Food Animals or Meat.

Relationship.	Duration of Association (years) 10 or less	11-20	21-30	>30	N.R.	Total(%)
Working in vet.clinic	33	23	2	0	1	59(40.9)
Govt. Administration /legislations	7	13	2	0	0	22(15.3)
Pharmaceutical industry	7	5	1	0	0	13(9.0)
Academic	12	11	2	0	0	25(17.4)
Others	14	9	1	0	0	24(16.7)
N.R.	0	1	0	0	0	1(0.7)
Total(%)	73(50.7)	62(43.0)	8(5.6)	0	1(0.7)	144

N.R. - No response to either question

Table 2: Range of animals handled in the respondents clinic or job.

Animals	No. of respondents	(%)
Food animals mainly		
Ruminants	37	62.7
Poultry	4	6.8
Pigs	1	1.7
Others	1	1.7
Sub total	43	72.9
Non-food animals mainly	11	18.6
No response	5	8.5
Total	59	100.0

Table 3: Persons considered responsible for the misuse/abuse of veterinary drugs in Nigeria.

Category of persons	No. of respondents	(%)
Owners of the animal/clients	28	19.4
Veterinary assistants/Superintendents	25	17.4
Veterinarians	8	5.6
All of the above	79	54.9
No response	4	2.7
Total	144	100.0

Table 4: Relationship of nature of employment of respondents and their beliefs about who is responsible for drug abuse.

Nature of employment	Person responsible				
	Owners	Vet. assist.	Vets.	All	Total
Veterinarian	21	25	7	75	128
Livestock superintendEnts	5	0	0	3	8
Veterinary assistants	1	0	1	2	4
Total	27	25	8	80	140

Fisher's exact test ($P<0.05$).

Table 5: Pattern of the abuse/misuse of veterinary drugs in Nigeria based on drug type, as perceived by veterinary staff.

Drugs	Commonly (%)	Uncommonly (%)	Not abused/ misused(%)	No Response(%)	Total
Antibiotics/ antibacterials	134(93.0)	6(4.2)	2(1.4)	2(1.4)	144
Anthelminthics	81(56.3)	45(31.3)	12(8.3)	6(4.2)	144
Antiprotozoals	37(25.7)	71(49.3)	25(17.4)	11(7.6)	144
Hormonal preparations	31(21.5)	49(34.0)	57(39.6)	7(4.9)	144
Insecticides and acaricides	39(27.1)	40(27.8)	17(11.8)	48(33.3)	144

Table 6: Responses of veterinary staff on the pattern of the abuse/ misuse of veterinary drugs in Nigeria based on management system.

Management system	Commonly (%)	Uncommonly (%)	Not abused /misused (%)	No response (%)	Total
Intensive ruminant production	38(26.4)	56(38.9)	29(20.1)	21(14.6)	144
Semi-intensive ruminant production	44(30.6)	70(48.6)	7(4.8)	23(16.0)	144
Free-range(nomadic) ruminant production	115(79.9)	9(6.3)	8(5.6)	12(8.3)	144
Intensive poultry production	58(40.3)	53(36.8)	17(11.8)	16(11.1)	144
Semi-intensive poultry production	51(35.4)	62(43.1)	10(6.9)	21(14.6)	144
Free range poultry production	35(24.3)	30(20.8)	55(38.2)	24(16.7)	144
Intensive pig production	35(24.3)	60(41.7)	18(12.5)	31(21.5)	144
Semi-intensive pig production	29(20.1)	64(44.4)	20(13.9)	31(21.5)	144
Free range pig production	38(26.4)	28(19.4)	39(27.1)	39(27.1)	144

Table 7: Likely explanation(s) of how residues come about: number of respondents among veterinary personnel.

Explanation	No. of respondents			
	YES(%)	NO(%)	N.R.(%)	Total
Use of a particular drug in a species, while the drug in not recommended for use in the particular specie	56(38.9)	65(45.1)	23(16.0)	144
The right drug in the right animal but over dosing the animal	102(70.8)	24(16.7)	18(12.5)	144
Mode of administration such as the use of long acting injectable preparations	76(52.8)	45(31.3)	23(15.9)	144
Mode of administration such as the use of implants or slow release preparations	50(34.7)	65(45.1)	29(20.1)	144
Failure to observe withdrawal time of the drug	131(91.0)	2(1.4)	11(7.6)	144

N.R. - No response.

Table 8: Drug user factors and practices that could lead to residue occurrence as perceived by veterinary personnel.

Slaughter practice	No. of respondents			
	YES(%)	NO(%)	N.R.(%)	Total
Insufficient knowledge about the drug	125(86.6)	7(4.9)	12(8.3)	144
Refusal to obey instructions and recommendations regarding the use of the drug	122(84.7)	10(6.9)	12(8.3)	144
Absence or inadequacy of laws on the use of drugs	99(68.7)	27(18.8)	18(12.5)	144
Absence or inadequacy of laws on the use of veterinary drugs	103(71.5)	25(17.4)	16(11.1)	144

N.R. - No response.

Table 9: Slaughter practices that are considered common and could likely result in residue occurrence.

Slaughter practice	No. of respondents				
	A(%)	B(%)	C(%)	N.R.(%)	Total
Slaughter of animals with poor prognosis undergoing treatment with a drug	125(86.8)	4(2.8)	4(2.8)	11(7.6)	144
Slaughter of animals treated by owners	100(69.4)	16(11.1)	15(10.4)	13(9.7)	144
Slaughter of poultry (birds) consuming medicated feed or water	112(77.8)	13(9.0)	5(3.5)	14(9.7)	144

A - Common would lead to residues
B - Common would not lead to residues
C - Uncommon
NR - No response

Table 10: Judgement of respondents on the meat of an animal slaughtered while undergoing treatment with a veterinary drug.

Judgement	No. of respondents			
	YES(%)	NO(%)	N.R.(%)	Total
Such meat could beconsumed	32(22.2)	101(70.1)	11(7.6)	144
Such meat could be sold to the public	18(12.5)	115(79.9)	11(7.6)	144
Such meat should be condemned	112(77.8)	25(17.4)	7(4.8)	144

N.R. - No Response.

Table 11: Antibacterial drugs considered inapprpriate for use in food producing animals.

Drug	No. of respondents	(%)
Penicillin	20	13.9
Chloramphenicol	34	23.6
Tetracycline	14	9.7
Sulfanamides	16	11.1
None of the above	25	17.4
No response	35	24.3
Total	144	100.0

Table 12: Measure(s) to adopt in order to limit the occurrence of drug residues in Nigerian edible animal products.

Control measure(s)	No. of respondents			
	YES(%)	NO(%)	N.R.(%)	Total
Restricting the sale of veterinary drugs	106(73.6)	24(16.7)	14(9.7)	144
Enforcing laws regarding the treatment of animals exclusively to vets	128(88.9)	13(9.0)	3(2.1)	144
Restricting/limiting the manufacture and marketing	79(54.9)	54(37.5)	11(7.6)	144
Educating veterinary staff about the absorption, distribution, & elimination from the body of those veterinary drugs at the risk of becoming residues	124(86.1)	15(10.4)	5(3.5)	144
Enforcing laws regarding the sale and consumption of residue containing meat	124(86.1)	15(10.4)	5(3.5)	144
Public education on the dangers of residues in animal products	140(97.2)	3(2.1)	1(0.7)	144
Educating animal owners on the use of veterinary drugs	109(75.7)	31(21.5)	4(2.8)	144
Others*				

* Proposed by respondents.

4.2 Drug Usage Pattern, Residue Awareness And Concern Amongst Livestock Producers, Owners, Butchers, Meat Sellers And The Consumers Of Meat.

Three hundred and ninety eight persons responded out of 500 questionnaires administered. This represents a 79.6% response rate. The respondents included 119(29.9%) livestock producers or owners, 59(14.8%) meat sellers and butchers, and 220(55.3%) consumers(Table 13) responded to a questionnaire(Appendix II) administered to study drug use pattern, residue awareness and concern amongst these groups. Most of the respondents(294) had ages between 21 and 40 years(73.9%), 84(21.1%) were between 41 and 60, 7(1.7%) were over 60 years of age. Majority (71.1%) of the respondents were males.

In their relationships with food animals or meat, 117(29.4%) of the respondents have been engaged in the relationship for 11-20 years(Table 13), 120(30.2%) for 21-30 years, while 75(18.8%) for over 30 years. Two hundred and eighty three(71.1%) dwell in urban areas while 112(28.1%) are located in rural areas.

At the time of the survey, 180(45.2%) of the respondents were exposed to meat. Up to 115(63.8%) of the 180 cases were in contact or possession of beef(Table 14), 20(11.1%) mutton, 28(15.5%) goat meat, 13(7.2%) poultry meat, 3(1.7%) pork and 1(0.6%) to camel meat. Out of these, only 80(44.4%) had knowledge of the condition of the animals before slaughter. Twenty eight(35%) of these 80 respondents indicated that the animals had undergone treatment before slaughter. The time of the last treatment before slaughter was less than 1 day in 6(21.4%), between 1-3 days in 5(17.9%), between 4-7 days in

3(10.7%), between 8-14 days in 6(21.4%) and more than 2 weeks in 7(25.0%) of the 28 such cases.

The drug treatment was administered by a veterinarian in 12(42.8%), by a veterinary assistant in 9(32.1%) and by the animal owner in 5(17.9%) of the cases. Majority of the treatment cases, 14(50%), were done to treat infection. Five(17.9%) of the cases were to enhance weight gain. Some, 2(7.1%), did not know what the problem was.

One hundred and nineteen respondents(29.9%) were producers or owners of food animals. Fifty nine(49.6%) produce or own cattle, 11(9.2%) goats, 10(8.4%) sheep, 27(22.7%) poultry. Others included pigs 3(2.5%) and camel in 9(6.7%). The method of production employed was intensive in 21(17.6%) cases(Table 15), semi-intensive in 29(24.3%), and free range in 68(57.1%) out of the 119 responses. Ruminants were kept more under free range while non-ruminants were raised significantly more under intensive system of production(X^2, $P<0.05$)(Table 15). Drugs were used more commonly in ruminants(65.5%) than with non-ruminants(34,4%) for the prevention and treatment of diseases, while in addition to treatment and prevention of diseases they are more commonly used for enhancing growth and production in non-ruminants(69.0%) than in ruminants(31.0%)(X^2, $P<0.01$). Drugs were used for treatment and prevention of diseases significantly more in free range systems(76.1%) than in intensive system(32.6%), while a significant number of respondents used drugs for growth promotion and enhancement of productivity in

intensive(Fisher's exact test, P<0.01). Most of the producers, 66(55.5%), introduce veterinary drugs to their animals in more than one way: 29(24.4%) apply it directly on the animal, 17(14.3%) in medicated commercial feed, 2(1.7%) add to feed and 1(0.8%) add to drinking water(Table 16). Ruminants were more exposed directly(parenterally or topically) to veterinary drugs than non-ruminants, almost equally orally in feed or drinking water, and significantly higher through more than one way in ruminants compared to non-ruminants(X^2, P<0.05). There was no significant difference in the method of introduction of drugs between intensively raised animals and free range(X^2, P<0.05).

The purpose for which the drugs were introduced into the production system was for treatment and prevention of diseases in 67(56.3%) of cases, for enhancing growth and productivity in 4(3.4%), or for both in 45(37.8%) of cases(Table 17). On whether the drugs were always recommended by a veterinarian, 72(60.5%) said yes while 45(37.8%) said no. The drugs were provided by the veterinarian in 48(40.3%) cases, obtained in the market in 35(29.4%) or supplied by a drug company agent in 14(11.8%) of cases(Table 18).

Thirty eight(32.0%) of 119 producers use drugs commonly in their animals while 78(65.5%) described drug use in their animals as uncommon (Table 19). About 48.9% of producers using intensive management system, use drugs more commonly than free range producers(22.0%) while 77.9% of free range producers used drugs uncomonly(X^2, P<0.01). Those that use drugs commonly reported

that in 17(44.4%) of cases, the drugs were administered by a veterinary personnel(Table 20), and in 9(23.7%) cases by self or farm help. In 12(31.6%) of cases both veterinary personnel, the farmer or farm help administered drugs. When the drug was administered by farmer or farm help, the drug was used as recommended by the veterinarian in 9 cases(42.9%), as recommended by manufacturer in 7 cases(33.3%), or as deemed fit in 5 cases(23.8%). Five of the respondents that give the drug as deemed fit had reasons other than lack of instructions for choosing to do so, mainly due to what they call "experience". Producers that use drugs commonly, sell or slaughter the treated animals in less than 7 days after treatment in 8 of cases(21.0%) while 9(23.7%) sell or slaughter in greater than 7 days. Majority, 21(55.3%), retain the animal(Table 21).

Out of the 398 persons that responded to the questionnaire, 271(68.1%) buy or sell their meat in local markets, 70(17.6%) in abattoirs and 32(8.0%) in meat shops. The greater proportion of the respondents, 222(55.8%) were concerned about consuming the meat of an animal exposed to or treated with a veterinary drug soon before slaughter; 138(34.7%) were not concerned,while 33(8.3%) were undecided (Table 22). Among those that were concerned, 188(87.7%) were of the opinion that the animal was probably sick; 192(86.5%), that the meat may still contain some of the drug administered; or 183(82.4%) that the drug may have effect on health(Table 27). Other reasons given for the concern included the alteration of taste of the meat in 1.4%. Being concerned or otherwise was dependent on age, older respondents

>40 years of age were more concerned than younger respondents(X^2, P<0.01). Livestock producers and consumers were more concerned about the possibility of residues in meat than butchers and meat sellers(X^2, P<0.05)(Table 24). But being concerned was not influenced by the duration of relationship of respondent with food animals or meat(X^2, P>0.05). There was a significant difference between the degree of concern by the sexes; females were more concerned than males(X^2, P<0.01)(Table 23). Concern was significantly associated with location, urban people being more concerned(X^2, P<0.05)(Table 25). There was no significant difference between rate of concern between intensive producers and free-range livestock producers(Fisher's exact test, P<0.05). Where people buy and sell meat did not influence their being concerned or not about residues(X^2, P>0.05). Overall, 182(45.7%) of the respondents had a higher education, 56(14.1%) secondary education, 41(10.3%) primary school education and 55(13.8%) had adult and non-formal education. Sixty(15.6%) of the respondents were not literate.

Literacy level was a highly significant determinant of being concerned or not about residues. Respondents that were not literate were more likely to be unconcerned(P<0.01), and those with tertiary education were more likely to be concerned(Table 26).

When all the respondents(398) were asked about their judgement for such meat, 186(47.5%) agreed that the meat could be sold to the public, and 177(44.5%) responded that the meat could be consumed intheir households. One hundred and fourty two(35.7%) of respondents would permit the sale or consumption because failure to sell such meat would cause unnecessary loss to the butcher, meat seller or owner. Similarly, 146(36.7%) of the respondents believed that the meat would not have any consequence on the health of the consumer. Two respondents (0.5%) believed that the drug(if present) may even be useful to the consumer; 2(0.5%) responded that there had not been any case of ill-health due to consumption of meat containing residues. Up to 20(5.0%) of the respondents complained that there was scarcity of meat and therefore such meat should be sold and consumed. There was no significant relationship between agreeing to allow or disallow the sale of suspected residue containing meat with nature of the respondents relationship with the meat. Consumers and butchers had similar response(X^2, P>0.05)(Table 28). There was however, a significant difference between agreeing to allow or disallow the consumption of suspect meat amongst respondents(X^2, P<0.05)(Table 28). About 74.2% of the respondents, 59.0% of producers and 47.5% of consumers agreed to allow consumption(X^2, P<0.05), 52.5% disagreed.

Table 13: Relationship and duration of association of the respondents with food animal or meat.

	Duration of Association (years)					Total(%)
Relationship	< 10	11-20	21-30	>30	N.R.	
Livestock Producer or Owner	46	36	19	18	0	119(29.9)
Meat Seller or Butcher	15	22	8	12	2	59(14.8)
Consumer Only	21	59	93	45	2	220(55.3)
N.R.	0	0	0	0	0	0
Total(%)	82(20.6)	117(29.4)	120(30.2)	75(18.8)	4(1.0)	398(100.0)

Table 14: Type of meat available at the time of survey.

Type of meat	No. of respondents	(%)
Beef	115	(63.8)
Mutton	20	(11.1)
Goat meat	28	(15.5)
Poultry meat	13	(7.2)
Pork	3	(1.7)
Camel meat	1	(0.6)
Others	-	-
No response	-	-
Total	180	(100.0)

Table 15: Distribution of food animals produced by respondents according to management system.

	Management system				
Specie	Intensive	Semi-intensive	Free-range	N.R.	Total
Ruminants:					
Cattle	4	14	41	-	59
Goat	0	3	8	-	11
Sheep	0	1	9	-	10
Camel	1	4	3	-	8
sub total	5	22	61	-	88
Non-ruminants:					
Pig	1	0	2	-	3
Poultry	15	7	5	-	27
sub total	16	7	7		30
No response	-	-	-	1	1
Total	21(17.6)	29(24.3)	68(57.1)	-	118(100)

No response in either question=1; X^2, P<0.05

Table 16: The way veterinary drugs get introduce to respondent's animals.

Method of drug application	No. of respondents	(%)
Applied directly on the animal	29	24.4
Added to feed	2	1.7
Added to drinking water	1	0.8
In medicated commercial feed	17	14.3
Morethan one way	66	55.5
No respone	4	3.4
Total	119	100.0

Table 17: Purpose for introducing drugs into the production system.

Purpose	No.of respondents	(%)
Treatment and prevention of diseases	67	56.3
Enhancing growth and productivity	4	3.4
Both treatment, prevention and to enhance growth and productivity	45	37.8
Others	3	2.5
No response	-	-
Total	119	100.0

Table 18: How the drug(s) was(were) obtained by respondents.

Response	No. of respondents	(%)
Provided by the veterinarian	48	40.3
Obtained in the market	35	29.4
Supplied by a drug company agent	14	11.8
Others	0	0
No response	-	-
Total	119	100.0

Table 19: Frequency of veterinary drug use by respondents.

Response	No. of respondents	(%)
Commonly	38	32.0
Uncommonly	78	65.5
No response	3	2.5
Total	119	100.0

Table 20: Persons that administer drugs to animals COMMONLY exposed to veterinary drugs in (Table 19).

Response	No. of respondents	(%)
Veterinarian or vet assistant	17	44.7
Self or farm help	9	23.7
Both (I) and (ii)	12	31.6
No response		
Total	39	100.0

Table 21: Which of the following describes the fate of the animal after treatment?

Response	No. of respondents	(%)
Sold or slaughtered in less than 7 days	8	21.0
Sold or slaughtered in after 7 days	9	23.7
Retained on farm	21	55.3
No response	1	11.0
Total	39	100.0

Table 22: Respondents feelings about the consumption of the meat of an animal that was exposed to or treated with a veterinary drug soon before slaughter.

Response	No. of respondents	(%)
Concerned	222	55.8
Not concerned	138	34.7
Not decided	33	8.3
No response	5	1.2
Total	398	100.0

Table 23: Relationship of sex of respondents with concern for drug residues in meat.

Sex	Concerned	Not concerned	Not decided	Total
Males	139	112	28	279
Females	83	26	5	114
Total	222	138	33	393

No response in either question= 5; X^2, P<0.01.

Table 24: Relationship of respondents with food animals and concern about consuming meat of an animal given a veterinary drug not long before slaughter.

Relationship	Concerned	Not concerned	Not decided	Total
Livestock producer	73	37	8	118
Butcher/meat seller	16	36	5	57
Consumer	133	65	20	218
Total	222	138	33	393

No response in either question= 5; X^2, P<0.01.

Table 25: Relationship of location of respondents with concern for the meat.

Location	Concerned	Not concerned	Not decided	Total
Urban	167	**91**	21	279
Rural	52	47	12	111
Total	219	138	33	390

No response in either question= 8; X^2, P<0.05.

Table 26: Association of literacy level on concern for consumption of the meat of an animal slaughtered soon before slaughter.

Literacy level	Concerned	Not concerned/ Not decided	Total
Not literate	22	**40**	62
Adult education	12	**13**	25
Primary school education	24	**16**	40
Secondary school education	27	27	54
Higher education	128	**54**	182
Other	8	21	29
Total	221	171	392

No response in either question=6; X^2 P<0.01

Table 27: Reasons for concern about the consumption of the meat of an animal treated soon before slaughter.

Reason(s)	No. of respondents			
	YES(%)	NO(%)	No response (%)	Total
The animal must have been sick.	188(87.7)	21(9.5)	13(5.8)	222
The meat may still contain some of the drug administered.	192(86.5)	32(14.4)	28(12.6)	222
The drug may have effect on my health	183(82.4)	29(13.1)	10(4.5)	222
Other reasons*				
The meat will be tasteless or unpalatable	9(4.1)	-	213(95.9)	222
Psychological/aesthetical reasons	3(1.4)	-	219(98.6)	222
The disease may still be there	2(0.9)	-	220(99.1)	222
The disease may be zoonotic	2(0.9)	-	220(99.1)	222
Total of other reasons	16(7.2)	-	206(92.8)	222

Table 28: Influence of respondent's relationship with meat/food animals on judgement over residue containing meat.

Relationship with meat/food animal	Meat could be sold			Meat could be consumed		
	YES	NO	Total	YES	NO	Total
Livestock producer	64	48	112	65	45	110
Meat seller/butcher	26	11	37	26	9	35
Consumer	99	87	186	86	95	181
Total	189	146	335	177	149	326
X^2	P>0.05			P<0.05		
No response	63			72		

4.3 Results Of Microbial Inhibition Test With *Micrococcus luteus* ATCC 9341 Of Slaughter Cattle And Chicken.

A total of 876 slaughtered animals were screened for the evidence of antimicrobial drug residues and 119(13.6%) were positive.

4.3.1 Cattle:

Out of the 500 cattle screened, 37(7.4%) had urine samples that were positive. One of the positive samples is illustrated in Plate I. Range of diameters of inhibition produced by positive urine samples was from 1.4-3.0cm. Urine pH from the slaughter animals ranged from 6.8-9.8, with a mean of 8.7(n=81). Seventy three(91%) of the pH determinations fell between 8.0-9.6.

The thirty four positive cases were found in 13 of the 466 Bunaji cattle, 2 of the 15 Rahaji, 1 in the 13 Gudali and none in the cross breed cattle(Table 29). The breed specific rates were 7.3%, 13.3%, 7.7% and 0 for Bunaji, Rahaji, Gudali and cross breed cattle respectively. There was, however, no significant association between breed and the occurrence of residues(Fisher's exact test, $P>0.05$).

Two hundred and forty two(48.4%) of the cattle screened were males; the remaining 258(51.6%) were females. Male cattle produced significantly higher number of positive cases than females(X^2, $P<0.01$)

Sex specific incidence rates were 11.1% for males and 3.9% for females. Four hundred and seventy seven(95.4%) were adults with ages of 3 years and above. Calves 2 years and below constituted only 4.6%(23). Age specific incidence rates were 0, 9.9, and 3.7% for age groups 1-2, 3-9 and over 9 years respectively. Positive cases were more likely in animals between 3-9 years of age(Fisher's exact test,

P<0.05). Body condition of 248(49.6%) of the animals were considered to be at least good, 180(36.0%) to be fair, and 72(14.4%) to be either poor or very poor. Most of the positive cases, 27 out of 37(73%), occurred in animals with body condition score of good to very good. One positive case was found in an animal with poor body condition score(Table 30).

Most of the animals(204) with at least good body condition score(248) were males. One hundred and fifty five of the 500 animals(31.0%) were graded as having a fair body score and were females out of a total of 180(86.1%) animals judged as having a fair body condition. Similarly 59 out of 72(81.9%) animals with less than fair(poor to very poor) were females. Males generally had better body condition scores than females(X^2, P<0.01).

Ante-mortem and post-mortem inspection were not carried out. However, 38 different pathologies and disease conditions were noted in the course of visual observation. The pathologies were lymphadenopathy 10(26.3%), dermatophilosis 6(15.8%), emaciation 5(13.1%), and 2(5.3%) cases each due to anaemia, trauma, suppurative lymph nodes and tuberculosis. One case each(2.6%) of abscesses, cysticercosis, fascioliasis, haemoglobinuria, lateral hernia, mycotic skin lesion, fat pigmentation, pimply gut and pyometra was noted. One(2.6%) positive urine sample was found in a case with lymphadenopathy.

Table 29: Distribution of antimicrobial substances in the urine of slaughter cattle by breed.

Breed	+ (%)	- (%)	Total(%)	Incidence rate(%)
Bunaji	34 (6.3)	432 (86.4)	466 (93.2)	7.3
Rahaji	2 (0.4)	13 (2.6)	15 (3.0)	13.3
Sokoto Gudali	1 (0.2)	12 (0.4)	13 (2.6)	7.7
Cross breed	0 (0.0)	6 (1.2)	6 (1.2)	0
Total	37 (7.4)	463 (92.6)	500(100.0)	

Fisher's exact test, $p>0.05$

Table 30: Distribution of antimicrobial substances in the urine of slaughter cattle by body condition score.

Body score	+(%)	-(%)	Total	Incidence rate(%)
Very good	4(0.8)	39(7.8)	43(8.8)	9.3
Good	23(4.6)	182(36.4)	205(41.0)	12.2
Fair	9(1.8)	171(34.2)	180(36.0)	5.0
Poor	1(0.2)	66(13.2)	67(13.4)	1.5
Very poor	0(0.0)	5(1.0)	5(1.0)	0
Total	37(7.4)	463(92.6)	500(100.0)	

Fisher's exact test, $P<0.05$.

4.3.2 Chicken:

Out of the 376 chicken, screened, 178(47.4%) were broilers, 72(19.1%) pullets, and 126(33.5%) were local chicken. A total of 82(21.8%) of the birds were positive for antimicrobial substances at slaughter(Table 31). Zones of inhibition ranged from 1.4-2.8cm, the mean for control discs was 2.7cm. Positive samples are illustrated in Plates II and III.

Fifty nine (33.1%) of the 178 broilers were positive. Class specific incidence rates for pullets and local chicken were 23.6% and 4.8% respectively. Broilers had the highest class specific incidence rate, while local chicken had the lowest. Class of birds is a risk factor; broilers significantly contain more residues(X^2, $P<0.01$) than the other classes.

Table 31: Distribution of cases of antimicrobial substances in slaughter chicken based on poultry class.

Class	+(%)	-(%)	Total(%)	Incidence rate(%)
Broilers	59(15.7)	119(31.7)	178(47.4)	33.1
Pullets	17(4.5)	55(14.6)	72(19.1)	23.6
Local chicken	6(1.6)	120(31.9)	126(33.5)	4.8
Total	82(21.8)	294(78.2)	376(100.0)	

X^2, P<0.01.

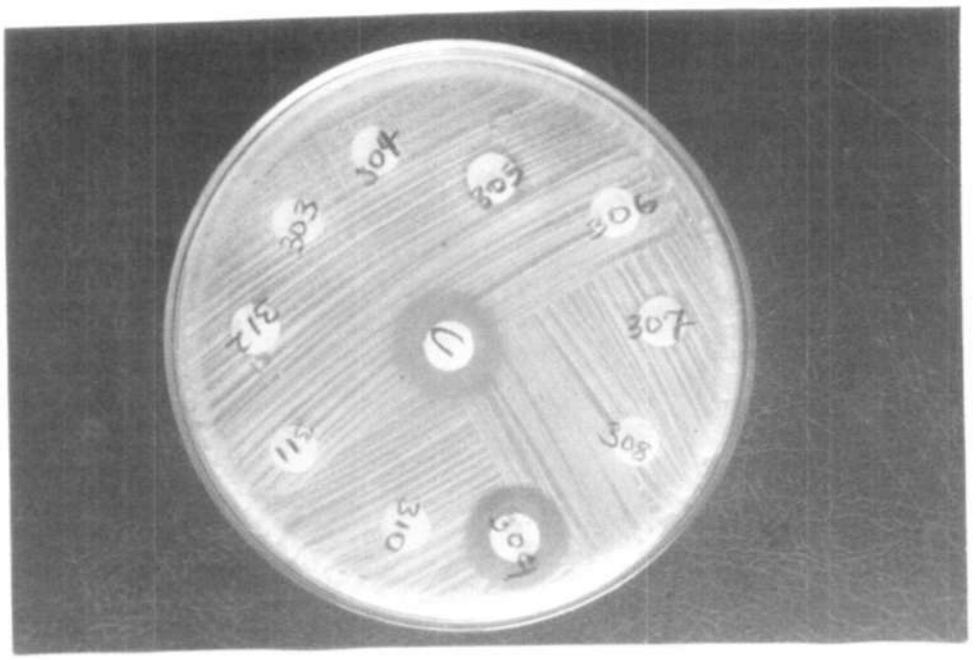


Plate 1 : positive microbial inhibition test(309) for the detection of antimicrobial substances in urine of slaughter cattle. Note control dise(C-30ug chloramphenicol) at centre.

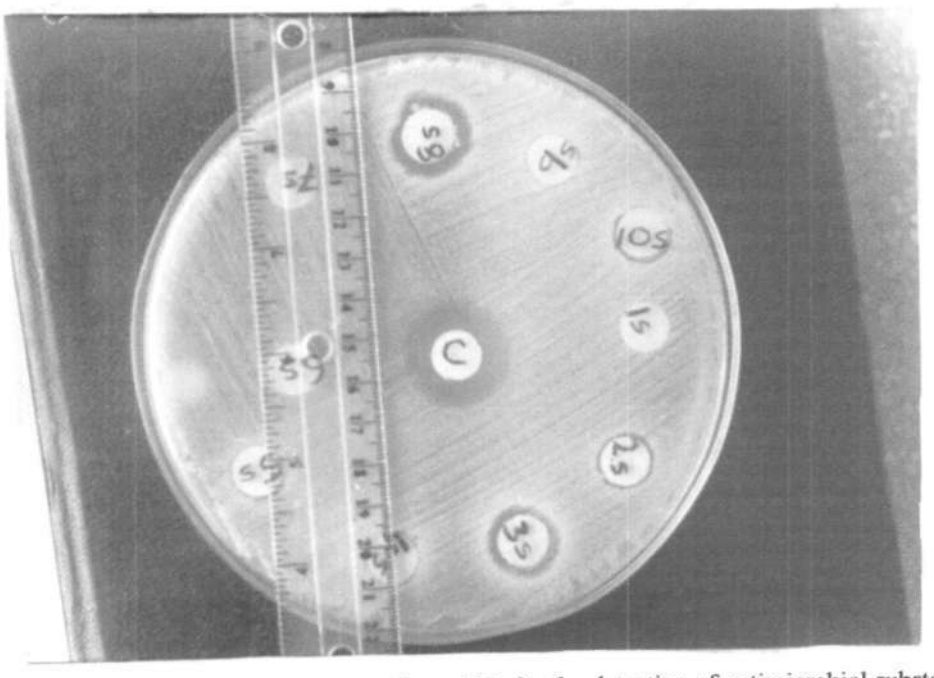


Plate II: positive microbial inhibition test(3s and 8s) for the detection of antimicrobial substances in feaces of broilers at slaughter. Note control disc(C-30ug chloramphenicol) at centre.

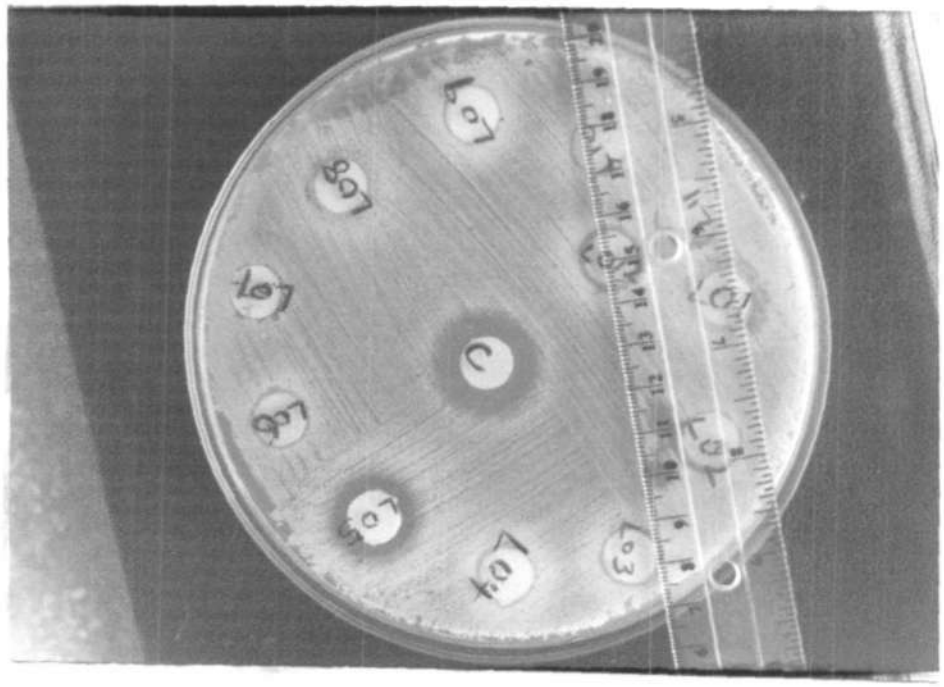


Plate III: Positive microbial inhibition test(L012 and L05) for the detection of antimicrobial substances in feaces of local chicken at slaughter. Note control disc(C- 3Oug Of chloramphenicol) at centre.

CHAPTER FIVE

5.0 DISCUSSION

A good response rate was obtained to the two distinct questionnaires used in this study. Up to 72% and 79% of the questionnaires in Appendix I and II respectively were returned. Failure to respond to 28% and 21% respectively may not be unconnected with inefficient postal services, loss in transit, lack of interest in the study, disregard for questionnaires or a general reluctance to release information some respondents may consider personal.

The results of this study confirmed that infectious diseases are the most important cause of drug use in animal production. Fifty percent(50%) of cases of treatment of animals going for slaughter were against infections. The same need to prevent infectious diseases was responsible for the introduction of drugs during production in 56.3% of cases. Similarly, 93% of veterinary personnel believed that antibiotics are misused or abused in Nigeria.

The misuse or abuse is a factor of drug use. In Nigeria, cattle is the most important slaughter animal. Estimated population of cattle is put at 13.9 million and chicken at 13.9m(Bourn *et al.*, 1994). The cattle remain largely in the hands of Fulanis concentrated in the northern part of Nigeria, and has remained the primary source of meat. While poultry is found evenly, and are substantially reared under commercial and intensive conditions(Bourn *et al.*, 1994). In this study two classes of animals namely cattle and poultry, raised under two different systems free range (nomadic) ruminant production and intensive poultry production respectively, have received the

highest accusation for common abuse/misuse of veterinary drugs by 79.9% and 40.3% of respondents.

The nomadic nature of the Fulanis makes them inaccessible to social services, good veterinary care and health programs. They are therefore more prone to unqualified drug usage and treatment of their own animals. Eventhough use of drugs under free range practice is uncommon, when used the drugs may not be used according to recommendations of dosage, frequency of dosing, or observance of withdrawal period. Up to 70.8% of veterinary personnel believed that over dosage would be the likely cause of residues in edible animal tissues, followed by use of long acting preparations(52.8%) and failure to observe withdrawal time(91.0%). Oxytetracycline long acting preparation is enjoying tremendous patronage by both qualified and unqualified personnel and owners it is given as a single injection in 4 days, therefore convenient under field conditions. Mercer *et al.*(1970), among others implicated long acting drug preparations as the cause of residues. This problem is associated, in this study, with lack of knowledge about the drug, refusal to obey instructions, and lack of veterinary laws on drug usage. When such animals are slaughtered, there is a high chance of encountering residues, and 69.4% of veterinary personnel believed that this practice is common and is capable of leading to residue occurrence. In this survey 17.9% of the animals that had undergone treatment in the recent past found in the abattoir were treated by owners; 21.4% of these animals were treated less than 24 hours before slaughter, and 32.1% in less than 7 days before slaughter. It is highly probable that those animals treated less than 24 hours before slaughter harbour high concentration

of residues, since commonly used drugs like penicillin and oxytetracycline have pre-slaughter withdrawal periods of 5 and 20 days respectively(Booth, 1977b). Failure to observe withdrawal times of drugs is the leading cause of residues worldwide(Booth, 1977b; Bishop *et al.*, 1984; Bevill, 1984; Ausburg, 1989; Van Dresser and Wilcke, 1989). Often, the withdrawal period is ignored completely where the animal is slaughtered almost immediately after treatment, probably because the animal otherwise would die or the prognosis is poor, 86.8% of veterinarians and other veterinary staff surveyed believed that this practice is common in Nigeria and would lead to residues. Commercially raised poultry are often sent to slaughter while undergoing treatment with a drug in feed or water. A number of poultry diseases may warrant immediate depopulation to control losses due to morbidity or mortality, these birds usually end up in slaughter places and are processed for human consumption. From this study 77.8% of veterinary staff believed that this is common in Nigeria and would lead to residues at slaughter. Use of higher than normal dosage or extra-label drug administration has accounted for residue occurrence in many studies (Kaneane *et al.*, 1987; Van Dresser and Wilcke, 1989; Krainock, 1991; Mc Ewen *et al.*, 1991). Over 70% of veterinary personnel interviewed by the present study consider over dosage as explanation for the presence of residues in Nigerian slaughtered animals.

Broilers had the highest class specific incidence rate, probably because of the requirement for rapid growth and use of drugs to facilitate weight gain as well as to prevent stress conditions and diseases. Residues have been reported in broiler tissues as s result

of feed mill contamination of feed and recirculation of drug via litter(Righter *et al.*, 1971a; Kan, 1991). Feeding chloramphenicol in drinking water to broilers left residues in muscle, fat, skin, liver and kidney(Sissodia and Dunlop, 1972a) and chloramphenicol metabolites were found in broiler tissues at slaughter(Anadon, 1994). Amuda-Giwa(1994) has demonstrated that oxytetracycline used in poultry feed in Nigeria could cause residues in both tissues and eggs even at recommended doses. This may explain the higher incidence of residues found in this study in intensively raised chicken(33.3% of broilers and 23.6% in layers), compared to local birds(4.8%). Overall, the proportionately higher incidence noticed in Nigerian slaughter chicken in comparison to cattle is in support of earlier reports of higher residues in poultry from other parts of the world. Huber *et al.*(1969) found 25% of chicken at slaughter to have residues, compared to 6% in mature beef cattle. The lower incidence (7.4%) reported for slaughter cattle in this study, is a reflection of the low drug usage in extensively raised cattle compared to intensively raised cattle in other parts of the world. Calves are frequently reported to harbour residues(Huber *et al.*, 1969; Van Dresser and Wilcke, 1989; Guest and Paige, 1991) and culled dairy cows(Mc Ewen *et al.*, 1991; Fajo *et al.*, 1995; Norman *et al.*, 1995). At the same time the results shows the poor control of veterinary drugs even at the low drug usage level.

Results of this study showed that breed is not a risk factor. Age and sex are risk factors. Age was significant in other surveys, where contrary to this study, residues are more likely in calves than in adult animals(Van Dresser and Wilcke, 1989). However, animals with good body condition are more likely to contain residues($P<0.01$)

compared to those with poorer body scores. This may mean that exposure to drugs is probably higher in animals with good body conditions. It is common practice in northern Nigeria to fatten bulls within short periods of time, the use of antibiotics for this purpose cannot be ruled out. It is known that drugs are used to enhance weight gain(Beeson, 1969) and have been implicated in residue causation(Huber, 1971a; 1971b; Van Dresser and Wilcke, 1989). Cattle from Fulani herds are more likely to have poorer body condition particularly during the dry season the period of this study. Thus a number of the animals with good and very good body scores may have originated from small scale producers that undertake fattening programs. Traction bulls may be slaughtered after the farming season and have constituted part of the males slaughtered with good body condition. Male animals have a greater tendency to have better body conditions than females($P<0.01$), due to androgenic influence of the male hormone testosterone(Schimedaly *et al.*, 1992; WHO, 1988). The female animals taken to slaughter are usually old, spent cows or cows milking under poor dietary conditions, so have a poorer body condition score. Systematic ante-mortem inspection and assessing appearance and disposition of slaughter culled dairy cows could predict animals likely to contain residues(Norman *et al.*, 1995).

Meat inspection procedure is hardly carried out in most Nigerian abattoirs. Determination of the extent and type of pathologies beyond visual inspection was not done. The conditions reported in this study are the same as reported usually in this region. Some of the conditions such as dermatophilosis, pyometra, lymphadenopathy(evidence of infection), are conditions that often require chemotherapy with

antibacterial drugs. Lymphadenopathy may be due to septicaemia or parasitaemia; it had the highest incidence of 26.3% and produced one positive result. Residues have been reported in animals that could not be passed because of disease conditions(Oka *et al.*, 1991). Diseases may alter body physiology and interfere with drug metabolism and elimination from the body(Ahrens, 1978; Groothius *et al.*, 1978; Jenkins and Friedlander, 1989). The occurrence of diseases like tuberculosis and the slaughter of emaciated animals suggests a very bad hygiene status of Nigerian meat.

Results of this study compliments the work of Ogboebulem and Fidelis(1996) that reported antimicrobial residues in the tissues of chicken fed medicated feed and in 2 slaughtered cattle in Nsukka, Nigeria. Screening cattle and poultry provides a spectrum of the residue status of slaughter animals. Beef is the primary meat source in Nigeria. According to this study 115 out of 180(63.8%) respondents to the questionnaire in Appendix II were in contact with or in possession of beef. On the other hand poultry is produced intensively across Nigeria in small to large commercial farms. Majority of respondents in this study opined that the animal product with the highest risk of containing residues is meat. In many countries slaughter animals are routinely screened at ante-mortem and post-mortem for drug and chemical residues(Cordle, 1989; Salisbury *et al.*, 1989; Norcross and Brown, 1991; Corrigan, 1992). Under these surveillance programs the sample used in screening slaughter cattle predominantly is urine(Huber *et al.*, 1969; Bevill *et al.*, 1984) using microbial inhibition test. Some of these tests commonly used for screening include swine test on premises(STOP)(Fajo *et al.*,

1995), sulfa on site(SOS) test used for the control of SMZ in swine(Bane *et al.*, 1984; Ausburg, 1989; Guest and Paige, 1991), calf antibiotic sulfa test(CAST) for screening of veal calves for residues(Korsurd and McNeil, 1988) and other microbial inhibitions tests. However, EEC regulations requires screening of tissue samples for residue monitoring, particularly in exported meat (Council, 1986; Wilson *et al.*, 1991a).

Different workers have employed different test organisms for the screening procedure. The organism used in this study *Micrococcus luteus*(*Sarcina lutea*)ATCC 9341 was employed by Loftsgaard *et al.*(1967) for a plate cylinder monolayer technique. Huber *et al.*(1969) and Van Schorthorst and Peelen-knol(1970) used *M. luteus* to screen urine and kidney of slaughter animals for antimicrobial residues and for residues in milk(Katz and Fassbender, 1978; Brady and Katz, 1989). The organism detected 0.01 ug/ml of drug in urine containing penicillin, oxytetracycline and dihydrostreptomycin, 0.04 iu/ml of penicillin and 0.8 ug/ml of oxytetracycline in kidney tissue(Van Schorthorst and Peelen-knol, 1970). In milk *M. luteus* consistently detected 0.01 units of penicillin/ml and levels as low as 0.0075 units/ml(Katz *et al.*, 1974). The positive urine samples in this study may contain antibacterial drugs at similar or even higher concentrations. Comparatively, *M. luteus* is more sensitive to penicillin and oxytetracycline than *Bacillus subtilis*(Huber *et al.*, 1969), and would thus be suitable for testing animals where these drugs are extensively used as in Nigeria. However *M. luteus* failed to detect 14% of known antibiotic positive urine(Huber *et al.*, 1969) a

number of positive samples may test negative thereby lowering the sensitivity and specificity.

This study could not identify the particular antibacterial substances involved; chromatographic methods would have established the identity of the agents involved. According to market availability of drugs, oxytetracycline is the commonest antibiotic used and it is probably more used. It is used clinically because of its effect on Gram positive and Gram negative bacteria and rickettsia(Huber, 1977b). In Nigeria it is abundantly used as a long acting injectable preparation and as a soluble powder for poultry(Agrovet,1989).

False positive results do occur. In one study using different tests, 5-22% of cowside tests inappropriately identified cows as having residues in milk(Sischo and Burns, 1993). No one screening technique is accurate enough to truly identify all positive cases of residues this result to inaccurates test results and losses due to condemnation and discarding of good meat and milk respectively (Gardner *et al.*, 1996). High urine osmolarity was blamed for 20% of false positive results obtained in live animal swab test for oxytetracycline residues in calves(Ter Hune and Upson, 1989). In this study urine osmolarity was not specifically determined so its effect could not be quantified. Urine pH of up to 10 did not inhibit the growth of the bacterium used for testing urine(Huber *et al.*, 1969). The results of the present study showed a urine pH range of 6.8-9.8(mean 8.7), thus zones of inhibition are more likely to have been produced by antimicrobial substances contained in the urine rather than antimicrobial effect of urine pH.

In MIT procedures where *M. luteus* demonstrated higher sensitivity(Korsrud and Mc Neil, 1988), Antibiotic Medium and Saskatoon Antibiotic Medium were used for growing the test organism, while Mueller Hinton agar is usually employed for CAST. In this study *M. luteus* was grown on Mueller Hinton agar. Mueller Hinton agar is a suitable medium for the assay of antibiotics. It has a pH of 7.4 and is devoid of metallic salts that could influence test results. Most reports of residue screening employ different types of Antibiotic Medium. In a comparative study *M. luteus* grown at 37°C on Mueller Hinton agar and *Bacillus megatarium* under the same conditions were more sensitive to antimicrobial substances than were other three test organisms(Read *et al.*, 1971). Mueller Hinton is the best medium for the assay of sulfa drugs(Read *et al.*, 1971). Since many antibiotics are incorporated into poultry feed as coccidiostats and antidiarrheal drugs, this medium was most ideal.

Faeces was used as test sample for screening poultry since birds excrete urinary products as solid waste in faeces. Terminal faeces was considered suitable. Huber et al(1969) used faeces to screen poultry for antimicrobial substances. The use of phosphate buffer(pH 7.0) eliminates the effect of uric acid and pH dependent antimicrobial substances in the test. Since there is no specific legal provision for residue monitoring in Nigeria, it is more difficult and much more expensive to collect tissue samples for residue determination. Urine and faeces are easier to obtain without apprehension on the part of the butchers. Residues in urine and faeces at the time of slaughter are indicators of tissue residues(Van Schothorst and Peelen-Knol, 1970; TerHune and Upson, 1989). Other residue screening procedures

like immunoassay methods are expensive and unavailable. Physico-chemical methods require expertise and complex instrumentation.

Where animals are found to be containing antimicrobial residues, at ante-mortem inspection the slaughter of such animals is delayed until tested and found negative. At post-mortem the carcass is often detained and determinative test carried out to ascertain the type and quantity of residues. The EU does not allow residues in slaughter animals while different countries establish tolerance limits for each drug used in animal production according to the WHO recommendations. All cases exceeding these tolerance levels are condemned. There are no such provisions in the Nigerian law, the draft Meat Hygiene(Public Health) decree consider residue containing meat as adulterated and should thus be condemned, but no specifications on type or tolerance levels(Federal Ministry of Agriculture, 1992).

The judgement over residue containing meat is determined by socioeconomic status of average consumer, literacy level and the presence or absence of viable legislations. Concern by consumers over safety of drugs and chemicals in the food largely determines the existence and enforcement of legislations on producers and marketers of animal food products. Concern over health hazards like residues may also be influenced by availability of the product in the society. Where there is scarcity there is little demand for quality. Results of this study show that 55.8% of respondents are concerned about consuming the meat of an animal exposed to or treated with a veterinary drug soon before slaughter. Similarly, 86.5% of these 222 respondents are concerned because they felt that the meat may contain

residues of these drugs. In 82.4% of cases they believed that these residues may have effect on their health. Only 47.5% and 44.5% of 398 respondents agreed that the meat be sold to the public and be consumed in their households respectively. This suggests that people would favour legislations on control of residues and residue containing meat. Females showed greater concern, thus at the level of the household residues may be unacceptable. The place where people buy or sell meat did not affect their being concerned($P>0.05$), thus the concern cuts across socioeconomic classes. But, proportionately more urban dwellers were concerned than rural people($P<0.05$), thus residue control may be more feasible in urban areas. Similarly, literacy level was found to be a very important determinant, people that were not literate were more likely to be unconcerned than literate people($P<0.01$); Therefore improving literacy level would facilitate residue concern. Public health education would be an important tool in drug residue control, since 97.2% of vet personnel support public health education as a means of controlling residues. Those respondents that believed in allowing the sale and consumption of meat containing residues believed that unnecessary losses may be incurred by condemning such meat(35.7% of cases), and that such meat would not have any effect on their health(36.7% of cases). Five percent(5%) opined that such meat should be allowed because there is scarcity of meat in Nigeria, and 2% agreed that there has not been any report of ill-health as a result of consumption of residue containing meat. There has not been reports of reactions to residues in Nigeria. This problem has neither been specifically determined, nor is information of allergy to antibacterial agents in human chemotherapy documented.

One obvious problem however is the increasing cases of drug resistant bacterial infections including *Salmonella* and *Escherichia coli* infections in both man and animals. These phenomena grow subtly. Legislations controlling the sale of antibiotics for human use are attempted, but the use of veterinary completely lacks enforcement. There is no specific agency in charge of veterinary drugs. Only recently did the NAFDAC respond to international call for ban on use of nitrofuran drugs in livestock production(NAFDAC, 1996), while the ban on chloramphenicol(WHO, 1988) has remained unenforced. Veterinary personnel are often ignorant of these recommendations. This study showed that 71.5% of vet personnel are aware of legislations regarding veterinary drugs and drug residues at all levels of governance, and only 23.6% of the vet personnel knew that chloramphenicol is not suitable for use in food animals. This ignorance cuts across all employment classes of veterinarians and other veterinary staff($P>0.05$). This means that some veterinarians working in government administration and regulatory agencies are unaware. Exposure to laboratories and literature (in universities and research centres) and to regulatory information (in the case of veterinarians working in the regulatory sector) is expected to at least in part confer greater awareness about issues on drug residues. Dessimination of regulatory information is an important component of residue control(Van Dresser and Wilcke, 1989; Sundlof *et al.*, 1991).

5.1 CONCLUSION

This study has demonstrated the evidence of antimicrobial drug residues in Nigerian slaughter animals, in the form of antimicrobial substances excreted at slaughter. It confirmed the beliefs of veterinary personnel, that there is gross abuse and misuse of veterinary drugs in Nigeria. The abuse or misuse could lead to the occurrence of residues of these drugs in animal products particularly meat produced from intensively raised chicken and routinely slaughtered cattle. It also showed that there is absence and lack of enforcement of basic meat hygiene regulations, therefore residues are not determined in slaughter animals. The public is concerned about the possibility of drug residues in meat particularly in urban areas, and would favour legislations for the control of residues. Public health education would be an essential tool for the control of residues. Control of residues would reduce veterinary drug abuse and misuse in animal production, and would strengthen qualified veterinary practice and consumer safety.

5.2 RECOMMENDATIONS

The following measures are recommended as a result of this study to control drug residues in Nigerian slaughter animals:

1. Prohibiting the indiscriminate sale of veterinary drugs. Only registered veterinarians should have the right to purchase veterinary drugs, drug companies should sell directly and only to registered veterinarians.

2. The draft meat (Vet, Public Health) decree should promptly be signed into law, and reviewed from to time.

3. A unit under the NAFDAC should be developed to cater for the quality and safety of veterinary drugs and animal food products, and to evolve regulations that would balance actual needs and quality concern.

4. Public health education should be promoted to increase awareness of producers, butchers, meat sellers and consumers about drug residues and their effect on marketability of animal products, and on health of consumers.

5. Further studies should be carried out to determine the incidence of residues in milk and eggs. The rate of allergic reactions to antibacterial drugs in human chemotherapy should be documented. Occurrence of drug resistant bacteria in man and animal populations should also be documented.

6. The socioeconomic status of consumers must be improved for any meaningful development.

7. Routine screening of slaughter animals (cattle and poultry) and meat for antimicrobial drug residues-as part of professional veterinary meat hygiene programme.

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APPENDIX I

Department of Veterinary Public Health
and Preventive Medicine
Ahmadu Bello University, Zaria.

Dear Respondent,

DRUG RESIDUES IN NIGERIAN ANIMAL PRODUCTS

This questionnaire is designed as part of a scientific study of drug residues in Nigerian animal products.

The name and identity of respondent would not be required and all information provided would be used solely for this purpose.

Thank you for your kind assistance.

Dr. Junaidu Kabir.

GUIDE

Kindly respond to all questions as you find appropriate.

1. All questions require only one response
2. In some instances explanations pertaining to a particular question are provided
3. Please circle or tick an appropriate option boldly.
4. Where boxes are provided a bold tick will suffice.

Please tick the appropriate option

1. Age: i) Under 20 years
ii) 21 - 40 years
iii) 41 - 60 years
iv) Over 60 years

2. Sex: i) Male
ii) Female

3. Relationship with food animals or meat (choose one only)

i) Veterinarian
ii) Non - Veterinarian

4. Duration of relationship

i) Less than 10 years
ii) 10 - 20 years
iii) 21 - 30 years
iv) Over 30 years

5. Location of practice

i) Urban
ii) Rural

6. Choose ONE of the following to describe yourself, please.

i) Veterinarian
ii) Livestock Superintendent
iii) Veterinary assistant

7. What is the nature of employment ? (Choose only ONE)

i) Working in a veterinary clinic
ii) Government administration / legislation
iii) Pharmaceutical industry
iv) Academic
v) Other reasons (Please specify)----------
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8. If (i) above in (7), what is the description of the clinic ?

i) Public (government) clinic
ii) University/research centre clinic
iii) Private clinic

9. Range of animals handled in your professional practice/clinic?

i) Food animals mainly
ii) Non food animals mainly

If (ii) in (9) above go to 11

10. If (i) in (9) above which of the food animals do you handle mostly in your clinic ?

i) Ruminants
ii) Poultry
iii) Pigs
iv) Others (please specify).....
.....

Answer YES or NO, by ticking the appropriate box/opinion

11. There is gross abuse/misuse of veterinary drugs in Nigeria YES NO

12. If YES above in (11), who would you consider responsible for the above misuse/abuse ?

i) Owners of the animal/clients
ii) Veterinary assistants/Superintendents
iii) Veterinarians
iv) All of the above

Please answer the following by ticking the appropriate option. Based on the drug category which of these would you consider appropriate in describing the pattern of the abuse/misuse ?

Drugs	Commonly	Uncommonly	Not abused/misused
13. Antibiotics/ antibacterials			
14. Anthelminthics			
15. Antiprotozoals			
16. Hormonal preparations			
17. Insecticides and acaricides			
18. Others (please specify)			

Based on management system, what would you consider to be the pattern of the abuse/ misuse of veterinary drugs ?

Management system	Commonly	Uncommonly	Not abused/ misused
20. Intensive ruminant production			
21. Semi intensive ruminant production			
22. Free range(nomadic) rum. production			
23. Intensive poultry production			
24. Semi intensive poultry production			
25. Free range poultry production			
26. Intensive pig production			
27. Semi intensive pig production			
28. Others (please specify)			

29. Do you think that the nature and extent of the abuse/ misuse could result into the occurrence of residues of these drugs in edible animal tissues ?

i) Yes
ii) No
iii) Not sure
If (ii) or (iii Go to 44

30. If YES in(29) above, which of these animal products would you consider more likely to contain these residues ?

i) Eggs
ii) Meat
iii) Milk

Please answer by ticking the appropriate box. Which of the following would you consider as a likely explanation of how these residues come about ?

31.	Use of a particular drug in a species, while the drug is not recommended for use in the particular specie ?	YES	NO
32.	The right drug in the right animal but over dosing the animal ?	YES	NO
33.	Mode of administration such as the use of long acting injectable preparations ?	YES	NO
34.	Mode of administration such as the use of implants or slow release preparations ?	YES	NO
35.	Failure to observe withdrawal time of the drug ?	YES	NO

On the part of the user which of the following would you consider may lead to the occurrence of such residues ?

36. Insufficient knowledge about the drug ? YES NO

37. Refusal to obey instructions and recommendations regarding the use of the drug ? YES NO

38. Absence or inadequacy of laws on the use of drugs ? YES NO

39. Absence or inadequacy of laws on the use of veterinary drugs ? YES NO

With respect to slaughter practices, which of the following would you consider common and likely to result in residue occurrence ?

Slaughter practice	Common would lead to residues	Common would not lead to residues	Uncommon
40. Slaughter of animals with poor prognosis undergoing treatment with a drug?			
41. Slaughter of animals treated by owners ?			
42. Slaughter of poultry consuming medicated feed or water ?			
43. Others (please specify)			

Please tick the appropriate box to answer the following: What would be your judgement on the meat of an animal slaughtered while undergoing treatment with a veterinary drug soon before slaughter ?

44. Such meat could be consumed ? YES NO

45. Such meat could be sold to the public? YES NO

46. Such meat should be condemned ? YES NO

47. Are you familiar with legislations locally, nationally or internationally on the use of veterinary drugs and drug residues in animal products ?

i) Yes
ii) No

If NO go to 50.

48. If YES above in (47), which of the following antibacterial drugs would you consider not appropriate to use in food producing animals (choose one only)

i) Penicillin
ii) Chloramphenicol
iii) Tetracycline
iv) Sulfanamides
v) None of the above

What measure(s) would you consider appropriate in order to limit the occurrence of drug residues in Nigerian edible animal products ?

49.	Restricting the sale of veterinary drugs?	YES	NO
50	Enforcing laws restricting the treatment of animals exclusively to veterinarians ?	YES	NO
51	Restricting the manufacture and marketing of medicated poultry feed ?	YES	NO
52.	Educating veterinary staff on the absorption, distribution, and elimination from the body of those veterinary drugs at the risk of forming residues ?	YES	NO
53.	Promulgating and enforcing laws regarding the sale and consumption of animal products containing residues ?	YES	NO
54.	Public education on the hazards of residues in animal products	YES	NO
55.	Educating animal owners on the proper use of veterinary drugs ?	YES	NO

56. Others (please specify).....
.....
.....

Please tick the appropriate option

1. Age:
   i) Under 20 years
   ii) 21 - 40 years
   iii) 41 - 60 years
   iv) Over 60 years

2. Sex
   i) Male
   ii) Female

3. Relationship with food animal or meat (choose one only)

   i) Livestock/ Poultry producer or owner
   ii) Meat seller or butcher
   iii) Consumer only

4. Duration of relationship

   i) Less than 10 years
   ii) 10 - 20 years
   iii) 21 - 30 years
   iv) Over 30 years

5. Location

   i) Urban
   ii) Rural

6. Are you at the moment in contact with or possession of meat?

   i) Yes
   ii) No

   If NO go to 13

7. Type of meat ?

   i) Beef
   ii) Mutton
   iii) Goat meat
   iv) Poultry meat
   v) Pork
   vi) Camel meat
   vii) Others (Specify please) .....
   .....

8. Do you have any knowledge of the health condition of the animal from which the meat was obtained?

   i) Yes
   ii) No

   If No go to 13

9. If Yes above (8), was the animal recieving treatment with a drug preparation before slaughter?

i) Yes
ii) No

If No go to 13

10. If Yes in (9), what was the time of the last treatment before the animal was slaughtered ?

i) Less than 1 day
ii) Between 1 - 3 days
iii) Between 4 - 7 days
iv) Between 8 - 14 days
v) Morethan 14 days

11. Who administered the treatment ?

i) A veterinarian
ii) A veterinary assistant
iii) Owner
iv) Others

12. For what purpose was the drug given?

i) For injury or trauma
ii) Against an infection
ii) Against a nutritional or dietary condition
iv) To make the animal gain more weight
v) Others
vi) Don't know

13. Do you produce or own food animals ?

i) Yes
ii) No

If No go to 25

14. What is the species or type of the animal ?

i) Cattle
ii) Goat
iii) Sheep
iv) Poultry
v) Pig
vi) Camel
vii) Others

15. Method of production employed ?

i) Intensive
ii) Semi - intensive
iii) Free range

16. What would you describe as the way veterinary drugs get introduce to your animals ?

i) Applied directly on the animal
ii) Added to feed
iii) Added to drinking water
iv) In medicated commercial feed
v) Morethan one way

17. For what purpose do you introduce drugs into the production system ?

i) Treatment and prevention of diseases
ii) Enhancing growth and productivity
iii) Both treatment, prevention and to enhance growth and productivity
iv) Others(Specify please).....
.....

18. Are(were) the drugs used always recommended by a vet. ?

i) Yes
ii) No

If No go to 20

19. How was(were) the drug(s) obtained ?

i) Provided by the vet.
ii) Obtained in the market
iii) Supplied by a drug company agent
iv) Others (please specify).....

20. What would you describe as the frequency of drug usage in your animals ?

i) Commonly
ii) Uncommonly

If **uncommonly** go to 25

21. If COMMONLY in(20) who among the following administers the drugs?

i) Veterinarian or vet assistant
ii) Self or farm help
iii) Both (I) and (ii)

22. If (ii) or (iii) in 21 above, how was the drug(s) administered?

i) As recommended by a vet
ii) As recommended by manufacturer
iii) As deemed fit

23. If you choose(iii) in 22 above what is your reason?

i) No instructions were available.
ii) Instuctions were available but were not clear.
iii) Others (Please specify).....

24. Which of the following describes the fate of the animal after treatment?

i) Sold or slaughtered in less than 7 days.
ii) Sold or slaughtered in greater than 7 days.
iii) Retained on farm.

25. With respect to your meat source/ outlet where do you buy or sell meat?

i) Abattoir
ii) Local market
iii) Meat shop
iv) Others(Please specify).....

26. What is your feeling towards the consumption of the meat of an animal that was exposed to or treated with a veterinary drug soon before slaughter ?

i) Concerned
ii) Not concerned
iii) Not decided

If concerned above, what may be your reason for being concerned. Answer by ticking YES or NO.

27.	The animal must have been sick.	YES	NO
28.	The meat may still contain some of the drug administered.	YES	NO
29.	The drug may have effect on my health.	YES	NO

30. Other reasons (Please specify)----------

With respect to the meat what would describe as your judgement?

31. The meat could be sold to the public. YES NO

32. The meat could be consumed in my household. YES NO

If you think the meat should be sold or consumed, which of the following agrees with your reason(s) ?

33. The meat should be sold to avoid unnecessary loss to the butcher, meat seller or owner. YES NO

34. Such meat would not have any consequence on the health of the consumer. YES NO

35. Other reasons (Please specify)----------

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36. Literacy level ?

I) Not literate
ii) Adult education
iii) Primary school education
iv) Secondary school education
v) Higher education
vi) Other reasons (Please specify)----------