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**CHANGES IN MICROBIAL ECOLOGY DURING POULTRY
PRODUCTION**

by

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“For the Lord giveth wisdom:
out of his mouth cometh knowledge”

AUTHOR OF ALL THINGS

This dissertation is dedicated to
my mother

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CHAPTER 1

LITERATURE REVIEW

1. INTRODUCTION

Poultry and its products are a major dietary item for a large proportion of the Southern African population (Bok *et al.*, 1986). The meat is a suitable substrate for microbial growth as the edible portion of the carcass contains ca. 70% water, has a water activity of 0.98 to 0.99, a protein content of ca. 20.5 % and a fat content of ca. 9.5% (Bryan, 1980).

The poultry industry comprises of two main constituent parts, namely the egg production and meat production (Bremner, 1977). Broilers represent by far the largest part of poultry meat production in the world. The modern poultry breeds, whether broiler or egg layer, achieve performance levels unheard of ten years ago. To achieve this potential, all the important aspects of husbandry, nutrition and health must be incorporated in a strictly coordinated fashion (Pattison, 1993).

According to Pattison (1993) the poultry industry can be represented as a pyramid. At the apex is a small group of primary breeders, with great-grandparent and grandparent stock immediately below them.

The breeding unit consists of the laying and the hatching part. Hens, as well as cocks, are placed together in one house from day of placement until day of slaughter. This term is called day-old to death. The cocks represent 10% of the total chickens placed in one house and are constantly reduced until 50 weeks of age resulting in 7%. The total lifespan of hens and cocks are about 65 weeks. Eggs are collected four times a day. These eggs are fumigated with formaldehyde

and store in a coldroom at 17°C for one day. The eggs are transferred to the hatchery where it will be set and hatch after 21 days. The temperature in the setter and in the hatcher is controlled at 37°C. At day of hatch, chickens are graded for defects and vaccinated with the prescribed vaccines. Transportation of these chickens to the broiler houses usually occurs on the same day.

The term broiler is applied to birds of the domestic fowl species which have been specially bred to grow rapidly and are slaughtered at seven to eight weeks of age depending on the weight of the bird required. They are housed intensively in large units or sites. Broilers are grown by companies organized on a vertically integrated basis. Such companies either own or are responsible for the breeding flocks from which the hatching eggs are produced, the hatcheries in which the eggs are incubated and the growing units, which are either managed by a farmer under contract to the company or the direct responsibility of the company. The birds are slaughtered in the company's own slaughter plant and finally sold by the sales division to wholesale or retail outlets (Pattison, 1993).

The vast majority of birds are reared and maintained under cover in poultry houses. These houses are environmentally controlled, including the temperature, ventilation and lighting. Heat is supplied to the chicks during the brooding period and the temperature of the house gradually declined over the first few weeks of life (Bremner, 1977). The temperature for day old chicks is about 35°C and is reduced by 3°C a week. The best performance is usually obtained if the house temperature is reduced from 30°C during the first week to 27°C in the second and 24°C in the third. Humidity should not be below 50 % (Bremner, 1977) whereas ventilation should be 0.7 m/h/kg body weight in the winter and 4 m/h/kg body weight in the summer. The lighting programme resulting in the best performance is as follows: 0 - 3 weeks - Continuous lighting (with 1 hour off in 24 hrs), 3 - 5 weeks - 3 hours on and 1 hour off, 5 - 7 weeks - 2 hours on two hours off (Bremner, 1977).

Poultry processing plants in South Africa are graded according to the maximum daily throughput of birds: Grade AP poultry abattoirs slaughter >10 000 birds per day; grade BP abattoirs, <10 000; grade CP abattoirs, < 800; grade DP abattoirs, < 300 and grade EP abattoirs, <150 birds per day (Government Gazette, 1989).

Birds are transported to the processing plant at the age of 35 to 42 days in plastic crates from the broiler farm. The birds are hung onto the shackles by their feet, and stunned electrically in a waterbath. After stunning the birds are slaughtered, bled for a minimum of 90 sec., scalded and defeathered by plucker machines. The next steps involve head and hock removing, evisceration, spraying with water and air- or spinchilled. Carcasses are finally packed as whole birds or portions, fresh, frozen or further processed.

2. MICROBIAL CONTRIBUTION

Infection/contamination is readily spread to domestic poultry by egg transmission, originated from various natural environmental sources (including other animals and humans), and through the consumption of contaminated feed (Williams, 1971).

2.1 Breeder farm and the hatchery

2.1.1 Eggs

Freshly laid eggs rarely contain microorganisms, but the egg could be infected during its formation, from either the ovaries or oviduct (Grau, 1986). The degree of egg contamination also appears to be a function of the cleanliness of the surface

onto which they are laid (Harry, 1963), and the manner in which the eggs are handled after laying, which induces the greatest effect when the egg is cracked (D'Aoust *et al.*, 1980). Environmental conditions such as temperature (Kinner *et al.*, 1981), period of storage, as well as washing (Lorenz and Starr, 1952) also have an effect.

The microflora of the egg shell is dominated by gram-positive bacteria which may originate from dust, soil or faeces (Board, 1964). Some of the most common contaminants are members of the genera *Alcaligenes*, *Pseudomonas*, *Proteus* and *Aeromonas* (Harry, 1957).

Bacteria isolated from newly laid eggs are mainly representatives of *Micrococcus* spp. which grow poorly at body temperature, suggesting that they may be environmental contaminants (Miller *et al.* 1953). Harry (1963), however, isolated *Lactobacillus* spp. and *Micrococcus* spp. from the ovarium of laying hens. It is, therefore, possible that the *Micrococcus* spp. isolated by Miller *et al.* (1953) originated from the ova and not from the environment.

As early as 1939, Haines (1939) observed that the egg is equipped with physical (cuticle, shell, shell membrane) and chemical defences (pH, Lysozyme, Coalbumen) against microbial infection. It has been suggested that these defences develop to protect the developing embryo. Contamination and rotting of eggs take place when such physical and chemical defences become overloaded. In general, in the absence of gross mishandling by humans, these defences are remarkably successful in preventing bacterial spoilage (Mayes and Takeballi, 1982).

2.1.2 Hatchery

The environment of a chick hatchery and its surroundings may become

contaminated with microorganisms from various sources. Microorganisms on/or in a few hatching eggs can easily be distributed throughout the hatchery by air movement during hatching and consequently contaminate or infect all other chicks in the hatchery (Magwood, 1964). Microorganisms typically responsible for contamination include *Escherichia coli*, *Staphylococcus* sp., *Streptococcus* sp. and *Aspergillus fumigatus* (Chute and Gersman, 1961).

Contamination of embryos occurs in the incubator but probably more frequently in the hatcher during pipping and hatching, and during transportation to the farm (Cox *et al.*, 1990). Bacteria can penetrate the egg shell (Maclaury and Moran, 1959). The existing conditions during incubation of a hatching egg, tend to favor the proliferation of these microorganisms. The invading bacteria do not usually cause extensive decomposition of the egg, and the chick usually hatches from the contaminated egg. This results in the establishment of extensive bacterial reservoirs in commercial hatcheries (Maclaury and Moran, 1959).

When the freshly egg cools from body temperature to nest, room, or cool-room temperature, a pressure differential occurs between the inside of the egg and the atmosphere. Motile bacteria penetrate the shell by this mechanism (Zander, 1978). The primary contamination of this nature is from enteric organisms, particularly *Salmonella* and coliforms as well as other types of bacteria and fungi.

Effective cleaning and sanitation programs are virtually needed in the poultry hatcher. Included in any hatchery sanitation program, is the application of effective disinfectants (Brake and Sheldon, 1990).

2.2 Broiler farm

Newly hatched chicks are microbiologically sterile, but microorganisms soon become established in and on different regions of the body (Mead, 1982).

Considering that the birds are placed in such close proximity, the potential for transmission is substantial (Stern *et al.*, 1995).

Saccharomyces boulardii, a typical yeast species showing probiotic activity, has demonstrated antagonistic activity against various bacterial pathogens both in vivo and in vitro (Brugier and Patte, 1975). *S. boulardii* has several attributes that could potentially be useful for reducing colonization of broiler chicks by *Salmonella typhimurium* and *Campylobacter jejuni* (Blehaut *et al.*, 1989).

Stress causes a disturbance of intestinal function and may lower the resistance of the live bird and increases spreading of intestinal bacteria. Pathogens that are orally consumed before and during crating and transportation, may colonize the caeca where they may be retained throughout processing (Moran and Bilgili, 1990).

Poultry thrush is a chronic wasting disease caused by yeast infection, mainly from *Candida albicans*, on the mucosa of the digestive tract (Chut, 1984). The disease has a high incidence in areas with a high humidity.

2.3 Abattoir

The procedure for converting a live bird into a safe and wholesome poultry product provides many opportunities for microorganisms to colonise the surface of the carcass (Bryan, 1980). Defeathering has been identified as a major contributor to cross contamination (Kaufman *et al.* 1972).

Broiler carcasses may be contaminated with the contents of the gastrointestinal tracts during processing (Baker *et al.*, 1987; May *et al.* 1990). One of the production management techniques frequently used to minimize carcass contamination, is the withdrawal of feed and water from broilers before catching,

loading, and transportation to the processing plant (Bilgili, 1988). During the withdrawal period, the crop and digestive tract are emptied, and there is less material available for contamination in the plant (Lyon *et al.* 1991).

During processing, most of the gram-positive bacteria originating from incoming birds is removed and replaced by a heterogenous population largely composed of gram-negative bacteria, including *Pseudomonads*, *Flavobacteria*, *Acinetobacter/Moraxella* and *Enterobacteriaceae* (Mead, 1989). Although not all of these microorganisms are involved in carcass spoilage or cause foodborne diseases, their presence in excessive amounts indicates on unsatisfactory processing, improper sanitary and hygienic practices in the plant, or both (Tompkin, 1983). It also signals that the finished product may contain high levels of spoilage-causing organisms, such as *Pseudomonas* spp. or pathogens (Cox *et al.* 1975).

Spoilage organisms are introduced into the slaughter plant in large numbers on the skin and feathers of the birds and in the dust that is scattered as the live birds are removed from the crates and hung on the line. Faecal contamination is also present on the feet, breasts and backs of many birds contributing to the presence of high numbers of mesophilic bacteria. The numbers of these bacteria are reduced during scalding but some remain on the carcass while others contaminate the environment. Other mesophilic bacteria in the intestinal content contaminate the carcass from the alimentary tract during evisceration (Mead, 1982). Another source of spoilage organisms, is the water. There is also a risk that the operatives may introduce bacteria on to the carcass during processing which may cause food poisoning outbreaks in humans. The personal hygiene of the workers is important in preventing this spread. *Salmonella* and *Staphylococcus* being the most commonest bacteria of consequence spread from humans to meat (Bremner, 1977).

To significantly reduce the level of contamination on processed broilers, pathogen-free or nearly pathogen-free birds, must be delivered to the processing plant (Bailey, 1993).

3. ENVIRONMENTAL CONTRIBUTION

The contribution of the environment with regards to the egg, chicken and the processed meat, substantially enhances spoilage.

3.1 Breeder farm and the hatchery

3.1.1 Eggs

Man

Because of his mobility, duties, curiosity, ignorance, indifference, and carelessness, humans constitutes one of the greatest potential causes of the introduction of diseases (Hofstad *et al.*, 1978). Most frequently, footwear is suspected as the means of transport of disease, but the hands can become contaminated with exudates when lesions and discharges are examined. Clothing can also become contaminated with dust, feathers and excrement (Hofstad *et al.*, 1978).

3.1.2 Hatchery

In order to minimize bacterial contamination of eggs and hatching chicks, the hatchery premises must be kept free of reservoirs of contamination which readily become air-borne (Magwood, 1964).

3.2 Broiler farm

The intestines of the chicks can become colonized from ingested faeces, from breathing contaminated aerosols or dust, eating and drinking infected food and water (Grau, 1986).

3.2.1 Environmental surfaces (Equipment)

Diseases and parasites can be carried on equipment. Poultry house equipment and vehicles usually have accumulations of litter and faeces which can be a threat (Zander, 1978). Limited studies, however, referred to the contribution of environmental surfaces to bacterial contamination on the farm.

3.2.2 Water

Water quality is an important consideration in the performance of broilers (Barton, 1996). Contamination of the watering equipment may persist, if it is not adequately cleaned between batches. The same *Salmonella* serotypes were found in both the chickens faeces and their drinking water (Morris *et al.*, 1969).

The presence of certain genera of bacteria in drinking water is undesirable, and generally indicates that faecal contamination of the water source took place. The type of bacteria, rather than the numbers, is therefore important in water analysis. Some bacteria may be detrimental to humans and chickens. This is particularly true for the coliform bacteria, such as *Escherichia coli* (Zander, 1978).

Bacterial contamination can be eliminated by removing the source of contamination, or by filtration and/or chlorination. Care must be taken when chlorination is implemented, since chlorine kills live vaccines. Enclosed

watering systems increase water quality by reducing bacterial contamination (Pattison, 1993).

3.2.3 Litter

The litter on the floor is usually wood shavings. The litter must be kept dry to assure that the bird can live under good conditions. Occasionally drinking troughs overflow causing damp patches in the litter. Damp litter can predispose the birds to disease conditions and tends to produce dirty birds with soiled feet and feathers (Bremner, 1977).

The majority of microorganisms present in litter can be assigned to in three groups, namely coryneform bacterium, micrococci or Gram negative types. Other bacteria such as aerobic spore formers, *Nocardias*, *Streptomyces* and *Streptococci* are found only occasionally (Shefferle, 1965), whereas the presence of Enterococci were found to be very low in litter. *Lactobacillus*, coliforms, moulds and yeasts occur in the range of 1000 to 200 000 cfu/g for unused litter and 600 000 to 3 000 000 for unchanged litter used for a period of one to eight weeks (Halbrook *et al.*, 1950).

When droppings of chickens are added to litter, rapid multiplication of bacteria appears to take place (Zander, 1978).

3.2.4 Feed

Feeds are favourable media for the growth of undesirable microorganisms, which can be further stimulated by environmental factors, such as elevated moisture and temperature. Deficient manufacturing practices and poor hygienic standards also contribute to undesirable contamination of animal feeds. Workers, birds, rodents and insects are the main sources of spoilage and pathogenic microorganisms

contaminating farm feeds (Durand *et al.*, 1990). Different processing methods such as heat treatment, irradiation, changes in the chemical composition and contamination during processing, can influence the pattern of microbial growth in feeds. Feed should be withdrawn from broilers between 8 - 12 hrs prior to slaughtering to prevent an increase in carcass contamination (Veerkamp, 1986).

A high initial microbial count, with resultant growth, results in reduced levels of nutrients and increased levels of toxic metabolites and moisture. Microbiologically safe feeds are essential to protect animals, humans and the environment against feed-borne pathogens (Durand *et al.*, 1990).

3.2.5 Air

Bacteria, like *Escherichia coli*, in the environment of a chicken house constantly colonize the respiratory tract of poultry and invade the bloodstream (Jensen *et al.*, 1987).

3.3 Abattoir

3.3.1 Equipment surfaces

Rubber fingers are the main source of microbial contamination in the "dirty" area due to the warm, humid condition induced by the scalding process, thus allowing microbial growth in channels and cracks (Mead and Dodd, 1990). *Micrococcus* spp. are predominantly associated with the rubber fingers (Geornaras *et al.*, 1998).

3.3.2 Water

The high microbial counts in the scalding tank represents a major site of contamination attributed to the microbial loads on the external surfaces of the birds entering the scalding tank (Mulder and Veerkamp, 1974). Predominant isolates associated with the scalding tank water comprises *Micrococcus* spp. Enterobacteriaceae and lactic acid bacteria (Geornaras *et al.*, 1998). According to Mulder and Veekamp (1974), gram positive bacteria are the main bacterial group present in the water of the scalding tank, and their survival depends on factors such as bacterial identity and temperature. The water within the spin chiller should not be a source of contamination if the chlorine levels and temperature of the water are controlled.

3.3.3 Air

Microbiological contaminants occur in the air as aerosols, defined as solid or liquid particles suspended in the air. Coliforms and *Salmonella* are frequently observed from air samples taken in the vicinity where live birds are hung, killed, scalded and picked (Zottola *et al.*, 1970). The air in the dirty area is heavily contaminated attributed to the scattering of feathers at the defeathering units (Patterson, 1973), whereas the air in the clean area is generally acceptable. *Micrococcus* spp., Enterobacteriaceae and *Corynebacterium* spp. are common air contaminants associated with spoilage (Geornaras *et al.*, 1998).

4. PATHOGENS ASSOCIATED WITH POULTRY

If pathogenic bacteria are present at all, they are represented at low numbers with heterogenous distributions, requiring extensive sampling to have even modest confidence in negative results (Cason *et al.*, 1997).

4.1 *Salmonella*

Salmonella is a zoonosis, typically associated with poultry and poultry products (Aho, 1994). Clinically healthy animals carrying *Salmonella* and other pathogens may increase their shedding of the organisms if an external factor upsets the equilibrium of their intestinal flora (Mulder, 1995). Most *Salmonella* infection in poultry arise from the ingestion of these organisms. Ingested organisms proceed through the alimentary tract, where interaction with the mucosal surfaces at the Payer's patches may occur, and penetration or adhering into the intestinal epithelial cells take place. After proceeding through the intestinal wall and into deeper tissues, some *Salmonella* species can invade, survive and multiply in the reticuloendothelial system and disseminate to other tissues, causing serious systemic diseases (Barrow *et al.*, 1987). Contamination of carcasses depends on various factors including: (1) duration of intestinal carriage in the live animal; (2) stress during transportation; (3) time spend in lairage and (4) control of hygiene during slaughter (Mead, 1994).

The epidemiology of *Salmonella* infection in poultry is complex and although live birds become infected from a variety of sources, the animal protein fraction, is recognised as being the most important (Williams, 1971).

Salmonella can be introduced into a slaughter plant by either in the viscera or intestinal content, or on the outside of the bird by means of faecal contamination. These bacteria survive for some time in the scalding tank, depending on the temperature of the water within the scalding tank. At 10°C the number of *Salmonella* species will double in about 11 hrs (Bremner, 1977).

The most direct indicator of potential carcass contamination is the *Salmonella* status of the caecal contents since the caeca provide the best evidence of colonisation of the alimentary tract (Fanelli *et al.*, 1971; Linton *et al.*, 1985).

Doughert (1976) found that feeds are frequently contaminated with *Salmonella*, but that breeder/multiplier flocks could also pass contamination to their progeny. The presence of *Salmonella* serotypes in production represents a consumer risk and is an indicator for the occurrence of more pathogenic serotypes (Palmu and Camelin, 1997).

4.2 *Listeria*

Listeria is a ubiquitous environmental microorganism, often found in the faeces of animals (Gray and Killinger, 1966). Younger birds are more susceptible to colonization with *L. monocytogenes* than older chickens (Bailey *et al.*, 1989).

Listeria monocytogenes causes serious and sometimes fatal diseases in humans. The species is widespread in the environment and is sometimes carried in the intestines of healthy animals, capable of growth under chill conditions (Gray and Killinger, 1966; Mead, 1994). In extreme cases of listerioses, young chicks can develop disorders of the central nervous system as well as gross and histological lesions in the liver, spleen, heart and kidneys (Basher and Fowler, 1984). Healthy carriers of *Listeria* among chickens, have been demonstrated (Dijkstra, 1987). This contamination could be carried into the processing plant. Cross-contamination at the mechanized slaughter line plays an important role in the spread of *Listeria* (Bailey *et al.*, 1989).

The ecological characteristics of the organism, its ability to multiply at low temperatures and over a broad pH range as well as at low water activity value, enable it to multiply readily in the environment and in feeds and foods (Skovgaard, 1988).

4.3 *Escherichia coli*

Large numbers of *E. coli* organisms are produced in the intestines of poultry. Diseases caused by *E. coli* infection, are probably the most common and economically significant problem in broilers world-wide. As a normal inhabitant of the intestinal tract, the numbers of *E. coli* in the environment of the poultry house can build up very rapidly. The organism survives better in dry conditions and are harboured in large numbers in litter and dust (Pattison, 1993).

There are two routes of infection. Firstly, the presence of *E. coli* in faeces can result in the contamination of the shell of hatching eggs, resulting in yolk sac infection. Secondly, *E. coli* also infects poultry by the respiratory route. Respiratory infection results in haemorrhagic tracheitis and pneumonia, with air sacculitis involving the abdominal and thoracic air sacs (Pattison, 1993).

According to Barnes and Gross (1997), the most frequent causes of *E. coli* infection are infectious bronchitis. Exposure to excess ammonia resulting from poor ventilation or overcrowding disrupt mucosal barriers, impairs antibacterial defence systems and interferes with normal immune responses causing secondary *E. coli* infection (Awan and Matsumoto, 1998). Edens *et al.* (1997) indicated that *E. coli* type 1 and 2 are capable of causing severe diarrhea, dehydration, and mortality in poultry kept on warm wet litter.

4.4 *Staphylococcus aureus*

Staphylococcus species are normal inhabitants of the skin and mucous membrane of animals. In poultry, *Staphylococcus aureus* is known to cause various diseases from acute septicemia to chronic osteomyelitis (Skeeles, 1997).

Certain strains of staphylococci produce a toxin responsible for symptoms of food poisoning in humans after consuming food. A time period for growth in the food, however, is needed before sufficient toxin is produced to cause disease. Although staphylococci are present on the skin of poultry carcasses when they leave the slaughter plant, the commonest source of infection is the human food handler during further processing or in the catering establishment. Staphylococci can be found in the nose and on the hands of many humans and it is difficult to remove all of them by ordinary washing. Boils are also a major source of staphylococci infection since the toxin is not destroyed by cooking due to its resistance to heat. Staphylococci will not multiply at a temperature below 7°C and at 10°C multiplication is very slow; the optimum temperature for growth being 35 - 39°C, which is also the optimum for production of toxin (Bremner, 1977).

Osteomyelitis and synovitis in young chicks were found to be predominantly associated with *S. aureus* (Nairin, 1973; Skeeles, 1997).

5. MICROORGANISMS ASSOCIATED WITH SPOILAGE

Microbiological spoilage of poultry results in economic losses to retailers and processors. It is therefore important to increase the product shelf life by proper handling and processing methods. Spoilage bacteria are able to grow on the surfaces of cut muscle tissue and therefore the temperature of the product must be kept as low as possible (Mead, 1982).

5.1 Bacteria

Spoilage bacteria most frequently associated with poultry processing are *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Alteromonas putrefaciens*, *Corynebacterium*, *Flavobacterium*, *Micrococcus* and *Enterococcus* (Bryan, 1980).

These microorganisms are spread over the skin (during scalding and defeathering) and on the inner and outer carcass surfaces (during evisceration and further processing) and may lead to the spoilage of the product (McKeekin *et al.*, 1982). It is necessary to keep the initial bacterial numbers low to improve the shelf life of the product, since psychrotrophic bacteria continue to multiply during chilled storage (Mead, 1989). Off-odours become noticeable when total counts reach 10^{10} bacteria per cm^2 of skin and slime formation occurs when total counts exceed 10^8 bacteria per cm^2 (Mead, 1982).

5.2 Yeasts

Yeasts are generally not considered to be of major importance in the spoilage of meat products since their numbers in these products are highly variable relative to bacterial numbers (Jay and Margitic, 1981). Barnes *et al.* (1978), however, reported a large increase in the numbers and proportions of yeasts present on spoiled, polyethylene-wrapped, air chilled turkey carcasses stored at -2°C . Most yeast spoilage probably occur on meat that is either completely or partially thawed, the casual species can grow at temperatures down to -5°C (Lowry and Gill, 1984). According to Viljoen *et al.* (1995) and Kobatake *et al.* (1992), species of *Candida*, *Cryptococcus*, *Debraryomyces*, *Yarrowia* and *Trichosporon* are associated with the spoilage of poultry.

CHAPTER 2

MICROBIAL CONTAMINATION OF THE HEN'S EGG ASSOCIATED WITH BREEDING AND HATCHING

INTRODUCTION

The quantification of microbiological contamination on hatching eggs is of the utmost importance to the poultry breeder (Grau, 1986). Contaminated hatching eggs can lead to a reduction in hatchability and the contamination may also spread to other areas in the hatchery resulting in infection of newly hatched chicks (Humphrey, 1994). The level of contamination on the eggs varies with the standard of hygiene (Board, 1966).

Fertile eggs could carry a variety of bacterial populations when leaving the breeder house, both those on the shell surface and others that have penetrated beneath the shell via pores (Williams and Dillard, 1968). In addition to surface contamination, freshly laid eggs that are wet and warm are susceptible to rapid penetration by microorganisms, and these contaminated eggs possess the potential for spreading *Salmonella* in the hatchery by means of fluff (Williams and Dillard, 1968).

Egg shells become contaminated with *Salmonella* and other bacteria at oviposition as a result of infection of the oviduct or by faecal contamination (Board, 1977). Dirty nesting material also contributes to egg contamination (Williams and Dillard, 1968). Most eggs are contaminated at oviposition and it is

believed that the major (but not all) contamination of the egg is of external origin. The generalization that roughly 90% of newly-laid eggs are free from contamination is generally accepted, and the true figure may even be higher (Board, 1977). Contamination of the oviduct occurs from the cloacal region and the contaminants are dominated by *Micrococcus* spp, *Streptococcus* spp, and coli-aerogenes (Harry, 1963). *Pseudomonas* species readily penetrate the shell of poor quality eggs (Sauter and Petersen, 1969). *Escherichia coli*, *Staphylococcus* spp., *Streptococcus* spp. and *Aspergillus* are also typical contaminants often found on the surface of egg shells. All these microorganisms are suspected of being associated with early chick mortalities (Magwood, 1964). According to Moore and Madden (1993) and Board (1964), the microflora of an egg shell consist mainly of gram-positive bacteria derived from dust and faeces. Egg shells are also contaminated with *Salmonella enteritidis* as a result of intestinal carriage (Humphrey, 1994) and *Listeria* due to faeces and the breeder house environment (Leasor and Foegeding, 1989).

The environment of a chick hatchery and its surroundings may become contaminated with microorganisms from various sources. Microorganisms on, or in a few hatching eggs are easily distributed throughout the hatchery by air movement during hatching, leading to contamination or infection of all other chicks in the hatcher (Pienaar *et al.*, 1995). The wet navel of the newly hatched chicks acts as a port to entry for environmental contaminants which lodge in the nutrient-rich yolk sac. This causes omphalitis and yolk-sac infection, causing mortalities in newly hatched chicks (Pienaar *et al.*, 1995).

Once the egg has been laid, it is usually wet and become soiled at the same time. The presence of dirt in the surrounding environment of the breeder house adds to the number of contaminating organisms (Board *et al.*, 1964). The only pathway through which bacteria can get into the interior part of the egg is via pores (Board, 1963). The shell is pervious to microorganisms such as *Escherichia coli*,

Salmonella typhi, *Serratia marcescens* and *Pseudomonas aeruginosa* (Garibaldi, 1958). Contamination of embryos can occur in the incubator by means of contaminated surfaces and air, but occurs more frequently in the hatcher during pipping and hatching as well as during transportation to the farm (Cox *et al.*, 1990).

The objectives of this study were to determine the incidence, extent, and serotypes of different pathogens on and in freshly laid eggs as well as incubated eggs in the broiler breeder hatchery. In addition, the number of viable yeast cells and bacteria were also determined.

MATERIALS AND METHODS

Description of the breeder farm and the hatchery

Breeder farm

The breeder farm consisted of three rearing sites with four houses per site and four layer sites with six houses. The production capacity were 9 200 chickens per house. The first 20 weeks of the chicken's lifespan are called the rearing stage and the last 45 weeks the laying stage. The production cycle of a hen starts at 25 weeks of age.

The houses consisted of automatic roll-away nest boxes with rubber inserts. Collection of eggs took place at least four times a day. The eggs were fumigated on the farm within 1/2 hour after collection, using formaldehyde. Freshly laid eggs were separated on the basis of gross contamination, and classified as clean or dirty (soiled). Soiled eggs were not used for hatching. Eggs were stored in a

coldroom for one day at 17°C and transported to the hatchery once a day in a closed, chilled and fumigated vehicle.

Hatchery

Prior to setting, the eggs were transferred into a preheating room and fumigated with clinafarm (Enilconazole, Janssen Pharmaceutical). The eggs (15552 per day) were then set in an incubator at 37°C for 18 days. During this period, the incubators are fumigated with clinafarm on a daily basis. After 18 days, the eggs were transferred to a hatcher and kept for another 3 days at 37°C. The developing time for an embryo was 21 days.

After the chickens have hatched, they were sorted for defects, vaccinated with an oil base (Broiler Plus, Anchopharm) as well as with a water base spray against the New Castle virus (at the time of sampling there was a severe New Castle challenge on the broiler farm). There-after the chicks were transported to the broiler farm with a ventilated chicken truck.

Sampling procedure

The same batch of eggs were sampled at the hatchery one day after laying and after 18 days in the incubator. Individual eggs were wiped with a soft paper towel to remove any particulate material before placing in the incubator. Eggs were handled with sterile metal tongs and placed separately into a sterile Whirl Pak bag (Nasco, USA) to prevent contamination. Twenty clean and 20 dirty eggs were sampled one day after been laid for surface contamination as well as for content contamination.

Six surface and equipment samples of a hatcher were sampled after the chicks were removed and the hatcher was cleaned thoroughly, before transferring the eggs. Rodac contact plates (60 x 15 mm) (Nunc, Amersham) containing 3 different media (Table 2.1) were used for sampling and 25 cm² sampled on each surface. Five samples were taken of each surface, and the means calculated (Geornaras *et al.*, 1994).

Microbial numbers of the air within a hatcher after cleaning were quantified by duplicate settle plates containing three different media (Table 2.1), using an exposure time of 30 min. All samples were refrigerated at 4°C and transported to the laboratory in cooler boxes for microbial analysis on the same day.

Egg samples, surface samples as well as environmental samples were taken on five separate occasions over a period of 7 months from March to September.

Sample processing and analysis

Sterile Bacto Peptone (Difco, Laboratories, Detroit, MI) (10 ml) were poured into a sterile Whirl Pak bag (Nasco, USA). To prevent contamination, 20 sampled eggs, randomly selected from the nestbox, were handled with sterile metal tongs. Individual eggs were positioned in a sterile bag at an angle making sure the entire egg was covered with Bacto Peptone (Difco). Each egg was individually rubbed (through the bag) for 1 min to suspend surface residue in the Bacto Peptone (Difco). Each egg was removed by forcing it out through the top of the bag. Samples (0.1 ml) were transferred to three different media (Table 2.1) in duplicate using the spread plate technique. Similar procedures were followed for clean and dirty eggs as well as for the eggs examined after 18 days in the incubator.

Internal egg contamination was determined by soaking the egg in 70% ethanol solution for 30 sec (Jones *et al.*, 1994), blotting the egg dry on a clean paper

towel, cracking the egg with the blunt end of flamed forceps, and collecting the contents of the egg in a sterile glass jar. Five eggs were pooled. The contents were blended in a Warrick blender for 2 min. Portions (25 g) were transferred to a sterile glass bottle and 250 ml of Bacto Peptone (Difco) were added (Jones *et al.*, 1994). This procedure was only performed on the freshly laid eggs. Since the development of the embryo at 18 days was nearly completed for the subsequent sampling, these were omitted.

Tenfold serial dilutions in Bacto Peptone (Difco) were prepared as required and the samples plated in duplicate by the spread plate technique onto three different media (Table 2.1).

Plates were incubated aerobically. Plates containing between 30 and 300 colony forming units (CFU) (or the highest number if below 30) were counted and the means determined from duplicate plates.

Microbial counts for eggs, surfaces and environmental samples were converted to logarithms as indicated in Table 2.2 and 2.3. Microbial counts obtained from the eggs were analysed statistically by analysis of variance (ANOVA) using the Genstat 5 Computer Programme (1987) (after counts were converted to logarithms).

The prevalence of *Salmonella*, *Listeria*, *Escherichia coli* and *Staphylococcus aureus* was determined for all egg samples (egg shell as well as egg contents).

a) *Salmonella*

Similar procedures were followed for the detection of *Salmonella* as performed for bacterial detection (Gentry and Quarles, 1972), with the exception of the pre-

enrichment broth. Buffered Peptone Water (Oxoid, Basingstoke, UK) was used instead of Bacto Peptone (Difco) and incubated at 37°C for 24 h. After 24 h incubation, 0.1 ml of the pre-enriched broth were transferred into 10 ml Rappaport-Vassiliadis Soya Peptone Broth (Oxoid) (Ogonowski *et al.*, 1984) for enrichment and incubated at 42°C for 24 h. After incubation, the enrichment broth was streaked onto Modified Brilliant Green Agar with *Salmonella* Sulphamandelate supplement (Oxoid) (42°C for 24 h) (Geornaras *et al.*, 1994) and xylose lysine desoxycholate agar (XLD) (37°C for 24 h) (Bok *et al.*, 1986). Presumptive *Salmonella* colonies were serotyped by the South African Institute for Medical Research (SAIMR). To determine the presence of *Salmonella* within the eggs, 25 g portions of the blended eggs used for microbial counts were added to 225 ml Buffered Peptone Broth (Oxoid) and analysed for *Salmonella* as described for the eggshell above.

b) *Listeria*

Similar isolation procedures for *Listeria* as described for bacterial isolation were performed. University of Vermont *Listeria* enrichment broth (Oxoid) supplemented with *Listeria* primary selective supplement enrichment (UVM I) (Oxoid) (Bailey *et al.*, 1988) substituted the Bacto Peptone (Difco). The pre-enrichment broth was incubated at 35°C for 24 h. After incubation, 0.1 ml of the pre-enrichment broth were transferred into 10 ml Fraser Broth (Oxoid) supplemented with Fraser supplement (Oxoid) and incubated at 35°C for 24 h. The enrichment broth was streaked onto *Listeria* selective agar base (Oxford formulation) supplemented with *Listeria* selective supplement (Oxford formulation)(Oxoid) and incubated at 30°C for another 48 h (Dykes *et al.*, 1994). Isolates showing a dark brown colour change on the agar were selected from plates showing presumptive *Listeria* growth and purified on Tryptone soya agar

(Oxoid) with 0.3% yeast extract (Merck) (Dykes *et al.*, 1994). Presumptive isolates were evaluated for tumbling motility at 20°C (ICMSF, 1990). Colonies showing tumbling motility were identified to species level as described by Skovgaard (1988). To determine the presence of *Listeria* within the eggs, 25 g samples of the blened eggs used for microbial counts were added to 225 ml *Listeria* enrichment broth (Oxoid) and analysed for *Listeria* as described for eggshells above.

c) *Staphylococcus aureus*

The prevalence of *Staphylococcus aureus* on the egg shells and the egg content was determined by spread plating, in duplicate, 0.1 ml sample of the original eggshell and egg content homogenate used to determine microbial numbers, onto Baird-Parker agar (Oxoid) + Egg Yolk-Tellurite Emulsion (Oxoid) (37°C for 24 h). The identity of some presumptive *S. aureus* colonies, taken randomly from the plates, was confirmed by the coagulase test (Dodd *et al.*, 1988).

d) *Escherichia coli* type 1

Coliform colonies with deep red halos selected from Violet Red Bile Agar (Oxoid) were confirmed as *E. coli* type 1 based on the IMViC test (Harrigan and McCance, 1966). Typical *E. coli* type 1 species were characterized by the Eijkman (+), indole (+), methyl red (+), Voges-Proskauer (-) and citrate (-) tests (Harrigan and McCance, 1966).

RESULTS AND DISCUSSION

Eggs

The highest microbial numbers on the egg shells one day after been laid were consistently obtained by aerobic total plate counts (APC), followed by coliform (CF) and yeast counts (Table 2.2). A significant difference ($P>0.05$) was observed between bacterial counts obtained from clean shell eggs compared with dirty shell eggs (Fig. 2.1). The bacterial populations, obtained on aerobic plate count agar (PCA), being present on the egg shells decreased from day one after been laid to day 18 in the incubator at 37°C by 1.55 and 2.88 log units respectively for clean and dirty eggs (Fig. 2.2). Gentry and Quarles (1972) reported that clean eggs were exhibit by average bacterial counts of 3.2×10^3 which were much lower than reported by Rosser (1942), obtaining mean bacterial counts of 7.1×10^4 . Based on the results obtained in this study, bacterial counts of 2.15×10^3 were observed. According to Gentry and Quarles, 1972, dirty eggs represent average counts of 4.1×10^5 whereas counts of 9.52×10^5 were obtained in this study (Table 2.1).

Incubation of the eggs at 37°C for 18 days in the incubator resulted in a huge reduction in the numbers of bacteria on the egg shells (APC counts on clean eggs decreased from log 5.19 to log 3.64 and from log 7.18 to log 4.90 on dirty eggs) (Table 2.2 and 2.3). The reduction in bacterial numbers corresponds with the results obtained by Gentry and Quarles (1972). Accordingly, only a portion of the contaminating bacteria survived on the egg shell (2% of the original counts were viable) in an environment exhibiting temperatures of 37°C and 80 % relative humidity.

Evaluation of the egg contents revealed no bacterial or yeast growth. Consequently, no pathogens were present. The absence of bacterial growth corresponds with results obtained by Mayes and Takeballi (1983) who reported that the interior contents of eggs are free of *Salmonella* at the time of lay. Similar findings were reported by Jones *et al.* (1994) and Baker *et al.* (1980).

The high numbers of microbial populations on both the clean and dirty egg shells may be attributed to dirty nesting material (should be cleaned frequently), contaminated hands (should be washed more frequently), wet and dirty litter (in case of floor eggs) and frequent gathering of eggs. Gathered eggs should be stored in a clean, dry and dust-free area (Hofstad, 1978). Data revealing the absence of bacteria and yeasts in the eggs, do not necessarily mean no infection was present. If the sampling of contents has been performed after 72 h in the setter when the pores opened again and air movement was active, positive results might have been observed.

The yeast counts obtained on the eggshells were much lower (APC for clean eggs was log 5.19 and 2.18 for YC, APC for dirty eggs log 7.18 and 2.18 for YC) compared to the total aerobic plate counts (Table 2.2). The inhibition of yeasts was attributed to the fumigation with formaldehyde which proved extremely effective against yeasts and molds (Deak and Beuchnat, 1996).

Equipment surfaces

Relatively low bacterial counts were observed on the surfaces after cleaning. The aerobic plate counts (PCA) consistently exhibited the highest levels, whereas no yeasts and coliforms were obtained. The highest bacterial populations were obtained from the floor, fan and trolleys (Table 2.4).

Air samples

Settle plates indicated the presence of only aerobic bacterial populations (5 cfu/30 min). No coliforms or yeast populations were detected (Table 2.4).

Incidence of foodborne pathogens

Escherichia coli type 1 and *Listeria* species were isolated from 40% and 20% respectively of eggs one day after been laid and after 18 days in the incubator (Table 2.5). *L. innocua* was present on dirty eggs and *L. murayi* on the clean eggs (Table 2.5). Moore and Madden (1993) found *Listeria* in liquid eggs. *Listeria* species represented more than 30% of the total pathogenic species, dominated by species of *L. innocua* and *L. murayi*. Moore and Madden (1993) claimed that the contamination of eggs could be attributed to the environment. This was confirmed by Leasor and Foegeding (1989) and Foegeding and Stanley (1990). No other pathogens were detected on the eggs. The absence of *Salmonella* species on the egg surfaces corresponds with the results obtained by Meller and Banwart (1965) and Ross et al. (1964). However, Gibbons and Moore (1946), Bains and MacKenzie (1974), Perales and Audicana (1989), and Jones *et al.* (1994) disagreed. Gibbons and Moore (1946) reported that hens produce eggs contaminated with salmonellae, whereas Meller and Bandwart (1965) and Ross et al. (1964) reported that contamination of eggs with salmonellae originated from external sources. They also stated that an effective control measure could be the sanitizing of egg shell surfaces shortly after been laid. A USDA (1993) survey confirmed the presence of *Listeria innocua* (nonpathogenic to humans) on egg shells and other surfaces. No *Staphylococcus aureus* strains were detected. The absence of the species is primarily due to the high content of lysozyme of the inner shell membrane (Baker, 1974).

The substantial reduction in bacterial counts on the surface after 18 days in the setter, could be attributed to the frequent fogging of the setter with clinafarm (Enilconazole, Janssen Pharmaceurical). This could lead to the decrease in microorganisms in the air and on the eggshells. The high bacterial populations present on the egg shells could be ascribed to the automatic roll-away nestboxes being very small with a rubber insert and uncomfortable for the hens. In the houses implementing manual nestboxes, the eggs are much cleaner. The high incidence of *E. coli* species on the dirty eggs is due to faeces contamination and environmental surfaces (hands, belts etc.). *E. coli* is commonly associated with faecal contamination. The presence of *Listeria* on eggs is attributed to the species resistance against egg fumigation and sanitation (Laird *et al.*, 1991). *Listeria* loads on egg shells, however, decreased more rapidly when stored at 20°C for a few days, than at 5°C (Bräckett and Beuchat, 1992).

The absence of *Salmonella* and *S. aureus* species in this study may be related to the investigation of an insufficient number of eggs, or the lack of multiple repetitions. The deduction in the bacterial populations present on the egg shells during the 18 day incubation is ascribed to the constant fogging of the environment with clinafarm. Therefore, the major problem concerning egg shell contamination, originated from the breeder farm due to the frequent handling, collecting, treatment and improper environmental house standards.

Table 2.1: Culture media, temperature and times of incubation used for the microbiological analysis of eggshell surfaces and egg contents at a broiler breeder hatchery

	Incubation		Growth media
	Time (h)	Temperature (°C)	
Aerobic Plate count (APC)	72	20	Tryptone Soya agar (Oxoid) + 0.3 % Yeast Extract (Merck) (Geornaras et al. 1994)
Coliform count (CF)	24	30	Violet Red Bile Agar with overlay (Oxoid) (Harrigan and McCance, 1966)
Yeast count (YC)	120	25	YGC Agar (Oxoid) (Welthagen and Viljoen, 1998)

Table 2.2 Microbial counts present on egg shells one day after been laid.

SAMPLES	Microbial numbers (log cfu/egg)		
	AEROBIC PLATE COUNT (APC)	COLIFORM COUNT (CF)	YEAST COUNT (YC)
CLEAN	5.19	<2.00	2.18
DIRTY	7.18	3.11	2.18

Results are the mean microbial numbers of aerobic plate count (APC), coliform count (CF) and yeast count (YC) on egg shells one day after been laid

Table 2.3: Microbial counts present on egg shells 18 days after been set.

SAMPLES	Microbial numbers (log cfu/egg)		
	AEROBIC PLATE COUNT (APC)	COLIFORM COUNT (CF)	YEASTS COUNT (YC)
CLEAN	3.64	<2.00	<2.00
DIRTY	4.90	<2.00	<2.00

Results are the mean microbial numbers of aerobic plate count (APC), coliform count (CF) and yeast count (YC) on egg shells 18 days after been laid

Table 2.4: Microbial counts per 25 cm² of surface samples after the cleaning of the hatcher immediately before transferring of the eggs

AREA	Microbial numbers (cfu/25 cm ²)		
	AEROBIC PLATE COUNT (APC)	COLIFORM COUNT (CF)	YEAST COUNT (YC)
FLOOR	116	0	0
WALL	6	0	0
ROOF	6	0	0
FAN	37	0	0
TROLLEY	38	0	0
	cfu/30 min		
AIR	5	0	2

Table 2.5: Prevalence of pathogens on the surface of egg shells at day of set and after 18 days in an incubator at 37°C

SAMPLE	NO. composite samples	Positive samples							
		Salmonella		Listeria		S. aureus		E. coli	
		No.	%	No.	%	No.	%	No.	%
CLEAN EGGS - DAY OF SET	5	0	0	1	20	0	0	0	0
DIRTY EGGS- DAY OF SET	5	0	0	1	20	0	0	2	40
CLEAN EGGS- AFTER 18 DAYS	5	0	0	1	20	0	0	0	0
DIRTY EGGS- AFTER 18 DAYS	5	0	0	1	20	0	0	0	0

CHAPTER 3

MICROBIAL POPULATIONS ASSOCIATED WITH THE CAECUM AND LIVER OF BROILERS, AND ENVIRONMENTAL SAMPLES

INTRODUCTION

The caecum of chickens has been shown to contain the largest number of bacteria, most of which are anaerobes (Barnes and Impey, 1970). Crops and caeca are also major sites for *Salmonella* colonization in poultry (Impey and Mead, 1989). The livers of chickens are not a major source of bacteria, whereas the caecum has long been considered the primary source of *Salmonella* within the chicken (Fanelli *et al.*, 1971).

The bacterial population of live birds (feathers, feet and gut) consists mainly of gram-positive bacteria (Mead, 1989). Barnes and Impey (1970) found *Bacteriodes fragilis* and *Bacteriodes hypermegas* to be among the dominant caecal flora of young chickens. Mead and Adams (1975) and Barnes and Impey (1970) studied the development of the caecal flora of chickens from hatching until maturity and indicated that up to 100 % of the caecal bacteria are uric acid utilizers. The majority of caecal flora in the newly hatched chicken are represented initially by *Streptococcus faecalis* and coliforms. After two weeks of age, these bacteria are replaced by obligate anaerobes, whereas the number of uric acid decomposers decreases to 1-10% of the flora.

Modern poultry husbandry includes regional concentration of the industry, high stock densities, uniform age-distribution of birds on a single farm and continuous

feeding. This leads to an increase in horizontal transmission of animal diseases (Aho, 1994).

Salmonella infection in poultry arises from ingestion of the organism (Brown *et al.*, 1987). Invasive serotypes, mainly *Salmonella enteritidis* PT4 and *Salmonella typhimurium*, can cause recognizable diseases in birds and serious disorders in humans. Non-invasive serotypes, spreading mainly via horizontal routes, cause disorders which may be particularly dangerous for people with a poor immunity system (Aho, 1994). It has been suggested that poultry flocks with high aerobic plate counts (APC) before processing, are more likely to be *Salmonella*-positive during processing (Clouse, 1995).

Listeria monocytogenes is a ubiquitous environmental microorganism which is often found in animal faeces, and is an occasional animal pathogen (Gray and Killinger, 1966). Young chicks develop disorders of the central nervous system, as well as gross and histological lesions in the liver, spleen, heart and kidneys in extreme cases of listeriosis (Blaser and Fowler, 1984). The species is very resistant against a wide range of disinfectants. Dijkstra *et al.* (1988) reported that even after broiler houses were disinfected, some of the houses remained positive for the presence of *Listeria*.

Escherichia coli is widespread in nature and is a normal inhabitant of the intestinal tract of poultry (Gross, 1994). Pathogenic serotypes of *E. coli* are frequently isolated from the intestinal tract of healthy birds supporting the claim that *E. coli* develops as a secondary or opportunistic pathogen. Faeces and dust in chicken houses are important sources of pathogenic *E. coli*. Potential routes of infection could be either ingestion or inhalation (Gross, 1994). Birds between 7 and 28 days of age appear to be most vulnerable for Poultry Enteritis and Mortality Syndrome (PEMS) (Barnes *et al.*, 1996).

Staphylococci species are normal inhabitants of the skin and mucous membranes of animals (Skeeles, 1997). *Staphylococcus aureus* is considered as an opportunistic or secondary pathogen due to the required predisposing conditions before allowing them to enter into and multiply within a host (Johnson and Wadstrom, 1993). The increased mortality of birds during the final two weeks of the growing period is of great concern (Awan and Matsumoto, 1997).

Numerous potential pathogenic sources exist in integrated broiler operations. These include breeder flocks, incubators and hatchers of hatcheries, contaminated feed and water, as well as environmental sources such as litter, air, rodents (Bailey, 1993).

To our knowledge, limited information available is concerning the microbial populations of the chicken caecum. Consequently, the objectives of this study were to determine the incidence, extent and serotypes of different pathogens in the caeca, liver, feed, and litter in a broiler house. In addition, the microbial contamination of the above, as well as water, air and surface samples were also determined.

MATERIAL AND METHODS

Description of broiler farm

The broiler farm consisted of nine sites with eight houses per site. The average number of birds in a chicken house was approximately 27 000. Closed houses were used, equipped with nipple drinkers, gas burners, and wood shavings on the floor as litter. Broilers were allowed ad libitum access to water and food. A granulated balanced feed, manufactured by the company feedmill, was fed to the broilers. A standard lighting programme was implemented as well as a medication program (see appendix). The birds are normally slaughtered at 39

days of age, but due to a severe Newcastle challenge they were slaughtered at 35 days of age during this survey.

After every cycle (39 days) the litter was removed and a thorough cleaning and disinfection programme on the house and the site were performed. Remaining food was not carried over to a new site. Each cycle started with clean litter and fresh feed. The water was treated with milk powder before medication to neutralize the free chlorine present in the water.

Sampling procedures

Samples of feed, water, litter, air and surfaces were collected prior to arrival of chicks at broiler houses to provide a baseline of contamination. To determine whether caecal or liver colonization of poultry with microorganisms occurred prior to arrival at the broiler house, samples of the caeca and livers of newly hatched chickens were sampled at the hatchery. Feed, water, litter, caeca and liver samples were collected at days 5, 12, 19, 26 and 33. The entire experiment was repeated 5 times in 5 different broiler houses over a period of seven months from March until September.

Caeca and liver sampling of broilers

At day 0, broiler chicks were obtained from the hatchery after hatching and placed at a commercial density of approximately 21 birds/m² in a broiler house. Birds were killed by cervical dislocation. The birds were dipped under 70% ethanol and aseptically opened with sterile scissors and forceps, and the livers and caeca removed (Jones *et al.*, 1990). The blind end of the caeca was snipped, and the caecal contents of 15 birds emptied directly into a sterile Whirl Pak bag (Nasco, USA). Before removal of the caeca, 10 g of the liver was aseptically removed with sterile scissors and forceps, and placed into a sterile glass blender

jar. The livers of the 15 birds were pooled and blend in a Warrick blender for 2 min.

Feed

Feed samples, ca. 250 g, were collected in the flow from the three augers to the hoppers directly into sterile Whirl Pak bags. Samples, 25 g, were aseptically transferred to sterile glass bottles for thorough mixing (Jones *et al.*, 1991).

Water

Water was sampled from the main lines (100 ml) in each broiler house after the contact surfaces of the lines were sanitized with 70% ethanol. Sterile glass bottles were used for sampling. Prior to sampling, water was flowing for 30 sec to avoid accidental contamination.

Litter

Five litter samples, ca. 150 g, were collected from the upper 10 cm of the litter in each house and combined to produce composite samples in sterile Whirl Pak bags (Jones *et al.*, 1991). The collecting spots were chosen to represent the total floor space of each broiler house. Composite litter (25g) samples from five locations in each house were transferred to a sterile glass bottle (for thorough mixing) using sterile forceps.

Air

Bacterial numbers present in the air of the five chicken houses, prior to placement, were quantified to provide a baseline. Duplicate settle plates

containing 3 different media (Table 3.1) and an exposure time of 30 min were used.

Surface samples

Eight surface and equipment samples located at different areas in each broiler house were sampled (Table 3.2) using Rodac plates (60 x 15 mm) (Nunc, Roskilde, Denmark) (Favero *et al.*, 1968) after cleaning and disinfection. The means of 5 samples representing similar areas within each broiler house were calculated and are indicated in Table 3.2.

Samples were refrigerated at 4°C and transported to the laboratory for analysis on the same day.

Sample processing and analysis

Caeca samples, comprising of 7.5 g of the pooled caeca contents, were transferred to a sterile glass bottle. Bacto Peptone (67.5 ml) (Difco Laboratories, Franklin Lakes, NJ) were added to the contents, shaken vigorously and analyzed. Feed, water and liver samples (25 g) were added individually to 225 ml Bacto Peptone (Oxoid, Basingstoke, UK) and thoroughly mixed for microbiological analysis. Sodium thiosulphate (0.1ml of a 10% solution) was added to 100 ml water samples for the inactivation of residual chlorine (Patterson, 1968).

Tenfold serial dilutions in Bacto Peptone (Difco) for all the samples were prepared and plated in duplicate by the spread plate technique using five different media (Table 3.1). Plates were incubated aerobically, except for the lactic acid bacteria and anaerobic counts (Table 3.1).

Plates containing between 30 and 300 colony forming units (CFU) (or the highest

number if below 30) were counted and the means determined from duplicate plates. Counts were converted to logarithms.

The prevalence of *Salmonella*, *Listeria*, *E. coli* and *S. aureus* was determined in all caeca, liver, feed and litter samples.

a) *Salmonella*

The caeca sample, comprising of 7.5 g of the pooled caeca contents, was aseptically transferred to a sterile glass bottle. Buffered Peptone Water (Oxoid) (67.5 ml), implemented as pre-enrichment broth, were added to the caeca contents, shaken vigorously and incubated at 37°C for 24 h (Geornaras *et al.*, 1994). After 24h incubation, 0.1 ml of the pre-enrichment broth were transferred into 10 ml Rappaport-Vassiliadis Soya Peptone Broth (Oxoid) (Ogonowski *et al.*, 1984) for enrichment and incubated at 42°C for 24 h. After incubation, the enrichment broth was streaked onto Modified Brilliant Green Agar with *Salmonella* Sulphamandelate supplement (Oxoid) (42°C for 24 h) (Geornaras *et al.*, 1994) and xylose lysine desoxycholate agar (XLD) (37°C for 24 h) (Bok *et al.*, 1986). Presumptive *Salmonella* colonies were serotyped by the by South African Institute for Medical Research (SAIMR). To determine the presence of *Salmonella* in the livers (from the pooled sample), feed and litter, 25 g of each were separately added to 225 ml Buffered Peptone Water and analyzed for *Salmonella* as described for the caeca above.

b) *Listeria*

Similar isolation procedures for *Listeria* from the caeca as described for the isolation of *Salmonella* were performed. University of Vermont *Listeria* enrichment broth (Oxoid) supplemented with *Listeria* primary selective supplement (UVM I) (Oxoid) (Bailey *et al.*, 1988) substituted the Buffered Peptone Water. Caeca samples (7.5 g) were submerged in the pre-enrichment

broth and incubated at 35°C for 24 h. After incubation 0.1 ml of the pre-enrichment broth were transferred into Fraser Broth (Oxoid) (Bailey *et al.*, 1988) and incubated at 35°C for 24 h. The enrichment broth was streaked onto *Listeria* selective agar base (Oxford formulation) supplemented with *Listeria* selective supplement (Oxford formulation) (Oxoid) and incubated at 30°C for another 48 h (Dykes *et al.*, 1994). Isolates showing a dark brown colour change on the agar were selected from plates showing presumptive *Listeria* growth and purified on Tryptone soya agar (Oxoid) with 0.3% yeast extract (Dykes *et al.*, 1994). Presumptive *Listeria* isolates were evaluated for tumbling motility at 20°C. Colonies showing tumbling motility were identified to species level as described by Skovgaard (1988). To determine the presence of *Listeria* in the livers (from the pooled sample), feed and litter, 25 g of each were separately added to 225 ml of University of Vermont *Listeria* enrichment broth and analyzed for *Listeria* as described for the caeca above.

c) *Staphylococcus aureus*

The prevalence of *Staphylococcus aureus* in the caeca, liver, feed and litter was determined by spread plating in duplicate 0.1 ml samples of the original caeca, liver, feed and litter homogenate used to determine microbial numbers onto Baird-Parker agar (Oxoid) + Egg Yolk Tellurite Emulsion (Oxoid) (37°C for 24 h). The identity of some presumptive *S. aureus* colonies, taken randomly from the plates, was confirmed by the coagulase test (Dodd *et al.*, 1988).

d) *Escherichia coli*

Coliform colonies with deep red halos selected from Violet Red Bile Agar (Oxoid) were confirmed as *E. coli* type 1 based on the IMViC test (Harrigan and McCance, 1966). Typical *E. coli* type 1 species were characterized by the Eijkman (+), indole (+), methyl red (+), Voges-Proskauer (-) and citrate (-) tests (Harrigan and McCance, 1966).

RESULTS AND DISCUSSION

Caeca of broilers

A substantial decrease in the microbial population present in the caeca, namely the anaerobic plate counts, lactic acid bacterial counts and coliform counts (1.6, 0.7 and 1.4 log cfu/g respectively) were observed from day 0 to day 19 (Fig. 3.1). These microbial numbers, however, subsequently increased significantly after 19 days by 0.7, 0.4 and 0.7 log cfu/g respectively (Fig. 3.1). The variance in the lactic acid counts was less compared to the total bacterial counts after 19 days (log 9.1 to 8.4). Kovalenco *et al.* (1989) and Jin *et al.* (1997) reported that lactic acid bacteria are the most dominant bacteria in the gut of young chickens (17 days), comprising the species *Lactobocillus acidophilus*, *Lactobacillus fermentum* and *Lactobacillus brevis*. The lactic acid bacteria, however, were replaced by the dominance of anaerobes in older chicken. According to Jim *et al.* (1997), nearly the entire microbial population present in the caeca of older chickens are representatives of anaerobes. In contrast to these authors, the results obtained in this study indicates that the proportional microbial populations present in the caeca remained unchanged for the entire period of 33 days prior to slaughtering.

The decrease in microbial numbers in the caeca observed for the first 19 days, could be induced by the vaccination of the birds against Newcastle 3 days prior to the survey, since vaccination caused a stress situation among the chickens. According to Mead and Adams (1975), the replacement of the uric acid utilizers by obligate anaerobic bacteria, also contribute to lower microbial numbers. The subsequent increase in the anaerobic plate count is attributed to a change in the type of feed, implemented after 19 days (Fig. 3.2). The number of yeast populations, however, continued to increase reaching a maximum after 33 days exceeding 7 log units. The progression of the yeasts may be due to better

resistance against antibiotic substances or the absence of competition for available nutrients in the caeca (Deak and Beuchnat, 1996). Yeasts therefore, will develop as secondary microflora and may add to the inhibition of bacterial loads.

Feed

The highest microbial numbers in the chicken feed were consistently obtained by aerobic plate counts, followed by yeast and coliform counts. Except for the high aerobic plate count of 4.7 log cfu/g obtained after 5 days, the overall microbial population up to 19 days showed no substantial variation. Feed samples, however, exhibited substantial increases in aerobic plate counts (by 1.5 log cfu/g) after 19 days up to 33 days (Table 3.2). The increase in total bacterial loads, observed after 19 days, was reflected in the increase in bacterial numbers in the caeca (Fig. 3.1). Yeast counts obtained within the feed over a period of 33 days fluctuated between log 3 units and 3.8 (Fig. 3.2), whereas a significant decrease in the number of coliforms was obtained from day 0 up to 33 days (3.2 to 2.6 log cfu/g) (Fig. 3.2). Since the microbial surveys were performed on fresh feed, administered daily, no relation between the microbial loads in the caeca and feed was observed, except for the increase in bacterial numbers after 19 days. It is noteworthy, that despite the constant number of yeasts obtained from the feed, the numbers increased within the caeca indicating that the feed was not the major source of yeast contamination. The tendency of decreasing numbers of coliforms within the feed, was also not reflected in the caeca.

Litter

Aerobic plate counts consistently exhibited the highest counts, followed by yeast counts (except at 12 days when coliform counts were higher) and coliform counts (Fig. 3.3). Extremely high bacterial loads on the litter were observed before

placing of the chickens (log 6.5 cfu/g) and as expected continued to increase reaching counts exceeding log 9.5 units. The high bacterial loads are attributed to constant excretion of fluids from the chickens and the perfect environmental conditions that prevailed (high humidity, temperature etc.) stimulating bacterial growth. The results obtained (Fig 3.3) correspond with the findings of Halbrook *et al.* (1950), who indicated on a definite increasing trend of bacterial and yeast counts in poultry litter up to 8 weeks.

Despite the lower initial numbers of coliforms and yeasts (log 2.5 and 4.9 log cfu/g) present in the litter, these numbers also continued to increase upto 4.9 and 7.4 log cfu/g. Again, the increase in loads is attributed to the excretion of faeces and fluid by the birds.

Surfaces and the air

The low microbiological counts on the surfaces within the broiler houses prior to placing of the chickens revealed that proper cleaning and disinfection had been implemented (Table 3.2). Low microbial counts were obtained on all surfaces. The highest counts (APC, CF, and YC of 34, 5 and 16 cfu/25 cm², respectively) were obtained in the water fond, whereas both the pole and feeder hopper represented the lowest bacterial counts of all surfaces monitored (APC, CF and YC of 1, 0 and 0 cfu/25 cm²). After the chickens had been placed in the broiler house it was practically impossible to continue with surface sampling.

Aerobic plate counts analyzed by means of settle plates as an indication of bacterial air contamination prior to chicken placing revealed that low bacterial (20 cfu/30 min exposure) and yeast numbers (2 cfu/30 min exposure) were present. These population numbers, however, will increase after placing of the chickens, but again due to practical reasons further sampling was impossible.

Water

The microbial populations associated with the drinking water of the chickens within the broiler houses are indicated in Fig. 3.4. Low initial populations were evident prior to placing of the chickens (2 log units for coliforms and total aerobic counts). These populations, however, increased rapidly after placing of the birds reaching maximum numbers after 12 days (APC and CF of 6.8 and 3.5 log cfu/ml). The subsequent decrease in the total bacterial count is due to the cleaning of the watering system after 18 days. Similarly, the coliform numbers showed a slight decrease after 18 days.

Incidence of pathogens

The prevalence of pathogens in the caeca and liver of broiler chickens and environmental samples (feed and litter) is shown in Tables 3.3; 3.4; 3.5 and 3.6. From the results obtained it is evident that *E. coli* species pre-dominated, whereas lower incidences of *Salmonella*, *S. aureus* and *Listeria* in that order were observed.

The dominance of *E. coli* in the caeca was expected, since the pathogen is a natural inhabitant of the caeca of chickens and therefore, also being present in the litter due to faecal excretion. *E. coli* was isolated from all the caeca and liver samples, the litter (except prior to placement) and none from the feed. *Salmonella* species colonized in the caeca introduced by means of the shavings, which probably originated from the workers, water, rodents, birds, etc. Representative *Salmonella* species isolated from the caeca comprised of *S. infantis* (5) and *S. livingstone* (1). In contrast to the absence of *Salmonella* species in the feed obtained in this survey, Morris *et al.* (1969) reported that feed was the most frequently contaminated source of *Salmonella* species. Furthermore, Dougherty (1976) reported that day-old chicks are more frequently contaminated

with *Salmonella* compared to broilers prior to processing. No *Salmonella* positive results in day-old chickens were observed, but positive samples were detected prior to processing. *S. infantis* (3) isolates were isolated after 19, 26 and 33 days obtained from the liver. The litter also contained strains of *S. infantis* (6) isolated at regular intervals, whereas *S. livingstone* (1) was isolated after 33 days. Bhatia et al. (1979) also found *S. infantis* in litter samples and reported that the presence of *Salmonella* isolates in litter was a reliable indicator of flock contamination.

A low incidence of *S. aureus* was detected in the caeca of chickens only after 26 and 33 days whereas no *Listeria* isolates were observed. *Listeria* was isolated only on one occasion from feed samples, represented by *L. innocua*.

No traceability of the pathogen sources could be detected. Based on the circumstances prevailing in a broiler house, representative samples are collected with difficulty due to the quantity and uniformity of the chicken samples. Therefore, low incidences of pathogens may be misleading. The reliability can be increased by frequent examinations including more samples. The inclusion of the workers, dust, water and the surfaces of the broiler house would also add to a better representative collection.

Table 3.1: Culture media, temperature and times of incubation used for microbiological analysis of caeca, feed, water and surfaces in a broiler house

COUNT TYPE	Incubation		Growth Media
	Time (h)	Temperature (°C)	
Aerobic Plate Count	72	20	Tryptone Soya agar (Oxoid) + 0.3% Yeast Extract (Merck) (Geornaras et al. 1994)
Anaerobic Plate Count	48	35	* Reinforced Clostridia Agar (Oxoid) (William-Smith, 1961)
Lactic Acid Bacteria Count	48	35	* MRS agar (De Man, Rogosa, Sharpe) (Oxoid) (Welthagen and Viljoen, 1998)
Coliform Count	24	30	Violet Red Bile Agar with overlay (Oxoid) (Harrigan and McCance, 1966)
Yeast Count	120	25	YGC Agar (Oxoid) (Welthagen and Viljoen, 1998)

* MRS and Reinforced Clostridia agar - incubated under anaerobic conditions with 5 % CO₂

Table 3.2: Surface samples taken after the cleaning of the broiler house, immediately before placing the chickens

SAMPLES AREA	Microbial count (cfu/25 cm ²)		
	AEROBIC PLATE COUNT (APC)	COLIFORM COUNT (CF)	YEAST COUNT (YC)
WATER FOND	34	5	16
WALL	3	2	0
ROOF	8	0	0
FEEDER	11	0	0
CURTAIN	15	0	0
FEED FOND	9	0	0
POLE	1	0	0
FEED HOPPER	1	0	0

Table 3.3: Pathogens in the caeca of chickens

Sample	No. composite samples	Positive samples							
		Salmonella		Listeria		S. aureus		E. coli	
		No.	%	No.	%	No.	%	No.	%
Post hatch caeca from clean egg	5	0	0	0	0	0	0	5	100
Post hatch caeca from dirty eggs	5	0	0	0	0	0	0	5	100
Caeca 5 days	5	0	0	0	0	0	0	5	100
Caeca 12 days	5	0	0	0	0	0	0	5	100
Caeca 19 days	5	3	60	0	0	0	0	5	100
Caeca 26 days	5	2	40	0	0	2	40	5	100
Caeca 33 days	5	1	20	0	0	2	40	5	100

50

Table 3.4: Pathogens in the liver of chickens

Sample	No. composite samples	Positive samples							
		Salmonella		Listeria		S. aureus		E. coli	
		No.	%	No.	%	No.	%	No.	%
Post hatch liver from clean eggs	5	0	0	0	0	0	0	2	40
Post hatch liver from dirty eggs	5	0	0	0	0	0	0	2	40
Liver 5 days	5	0	0	0	0	0	0	2	40
Liver 12 days	5	0	0	0	0	0	0	1	20
Liver 19 days	5	1	20	0	0	0	0	1	20
Liver 26 days	5	1	20	0	0	0	0	4	80
Liver 33 days	5	1	20	0	0	0	0	2	40

Table 3.5: Pathogens in the feed of chickens

Sample	No. composite samples	Positive samples							
		Salmonella		Listeria		S. aureus		E. coli	
		No.	%	No.	%	No.	%	No.	%
Day 0	5	0	0	0	0	0	0	0	0
Day 5	5	0	0	1	20	0	0	0	0
Day 12	5	0	0	0	0	0	0	0	0
Day 19	5	0	0	0	0	0	0	0	0
Day 26	5	0	0	0	0	0	0	0	0
Day 33	5	0	0	0	0	0	0	0	0

51

Table 3.6: Pathogens in the litter of chicken houses

Sample	No. composite samples	Positive samples							
		Salmonella		Listeria		S. aureus		E. coli	
		No.	%	No.	%	No.	%	No.	%
Day 0	5	0	0	0	0	0	0	0	0
Day 5	5	1	20	0	0	0	0	5	100
Day 12	5	0	0	0	0	0	0	5	100
Day 19	5	2	40	0	0	0	0	5	100
Day 26	5	0	0	0	0	0	0	5	100
Day 33	5	3	60	0	0	0	0	5	100

1
151
201
32

Fig 3.1: Microbial populations in chicken caeca from day of hatch until 33 days of age

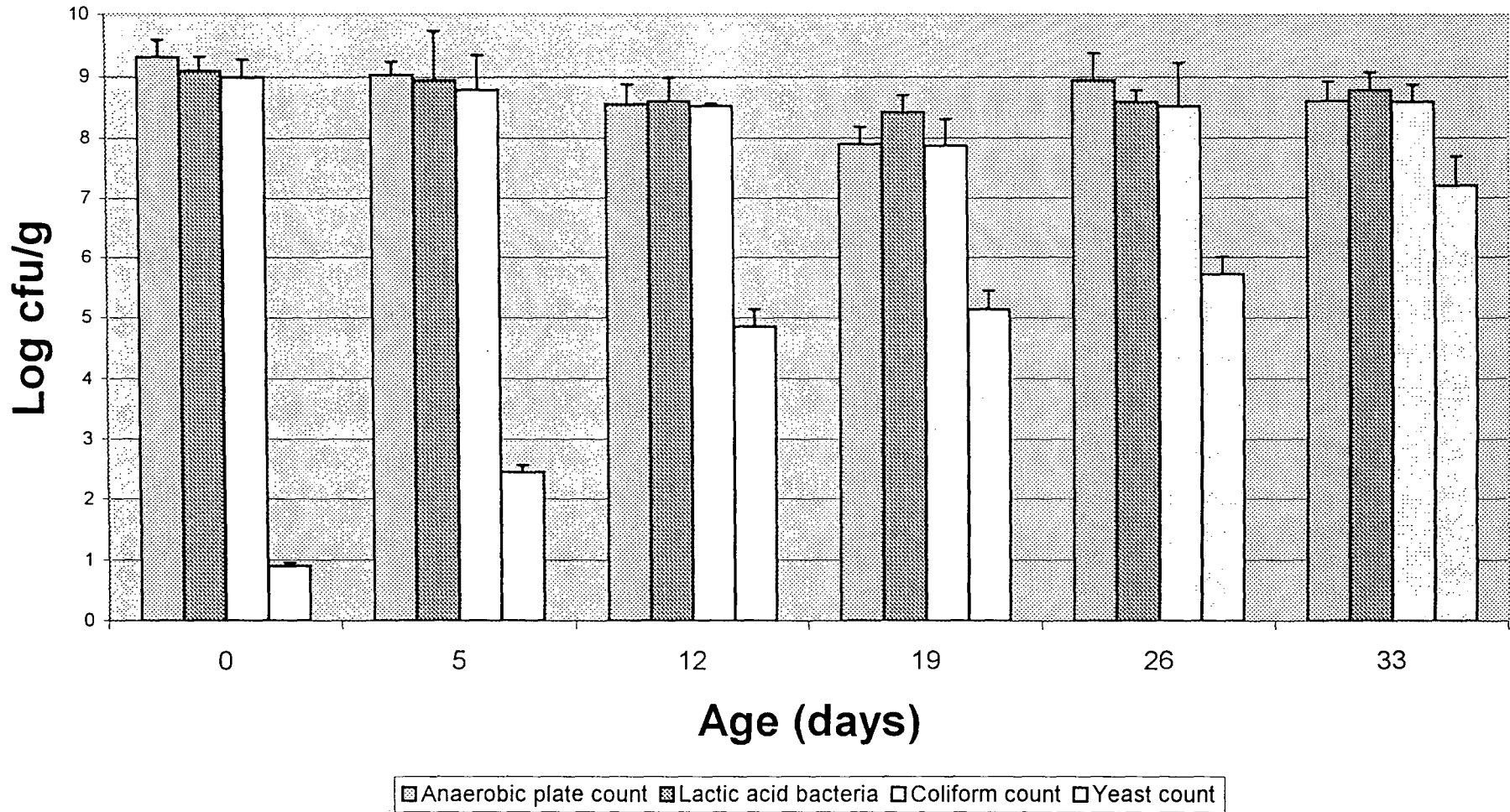


Fig 3.2: Microbial populations in chicken feed from 1 day before chick arrival until 33 days of age

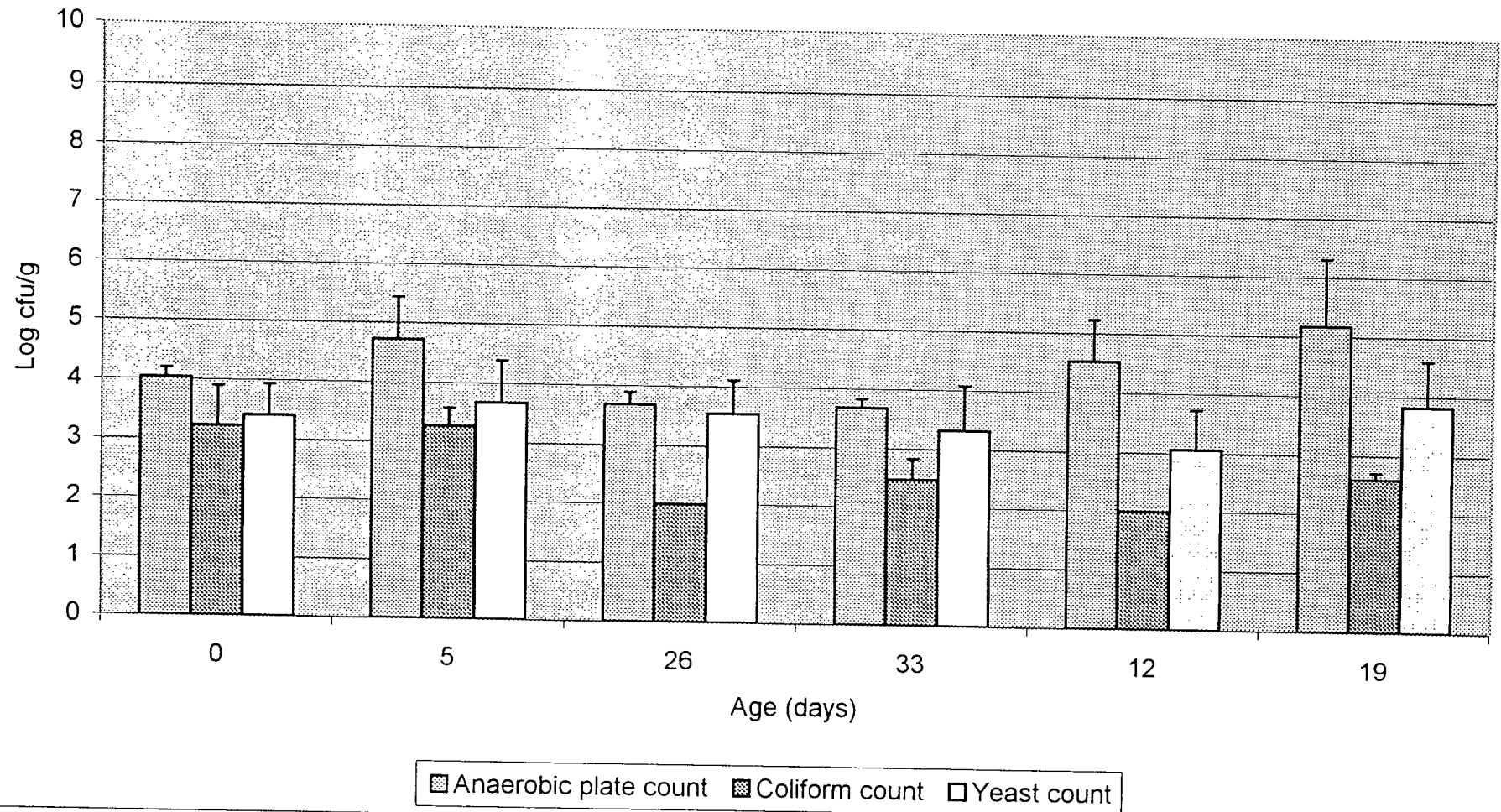


Fig 3.3: Microbial populations present in the litter of broiler houses 1 day before chick arrival until 33 days of age

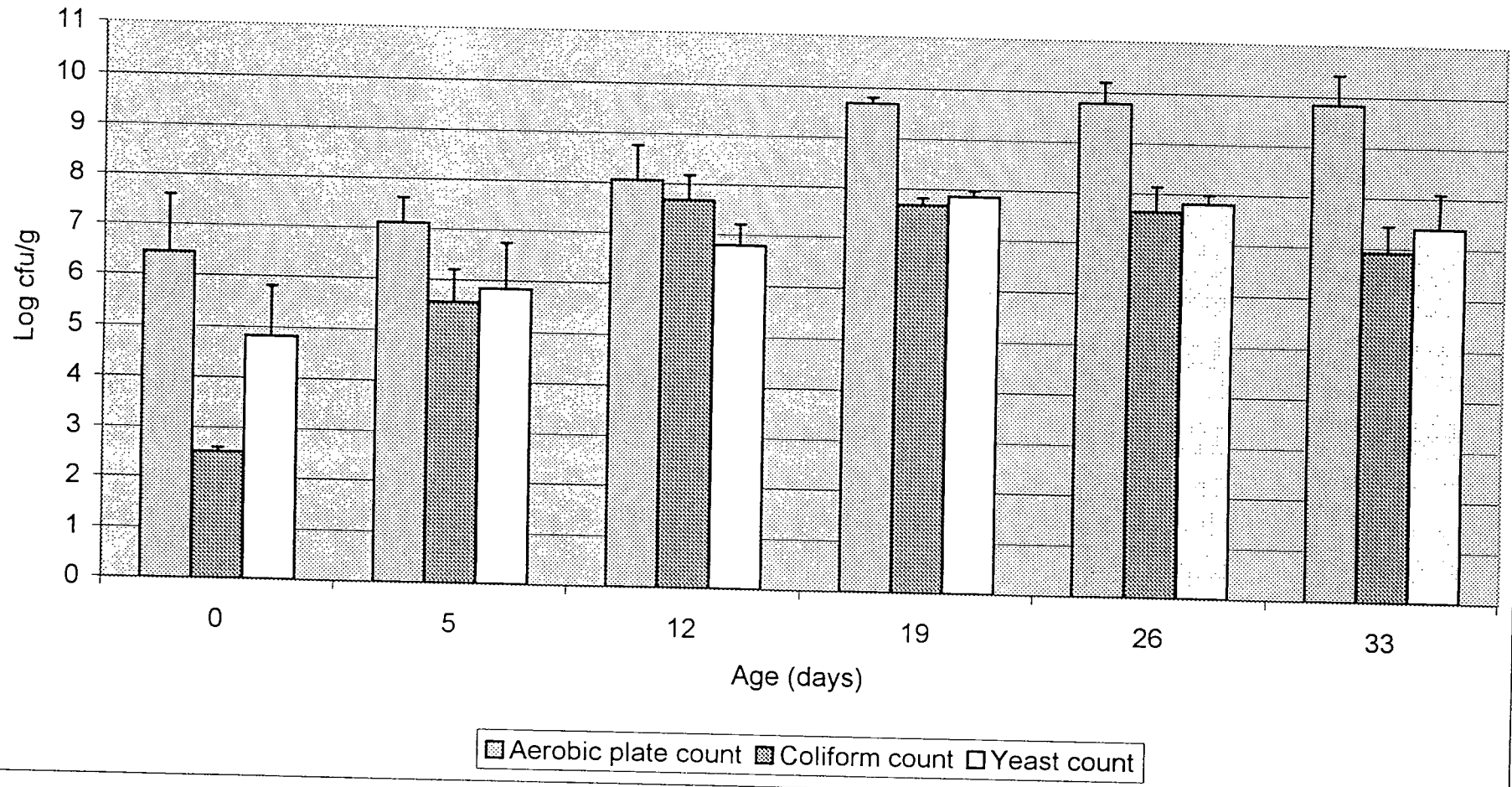
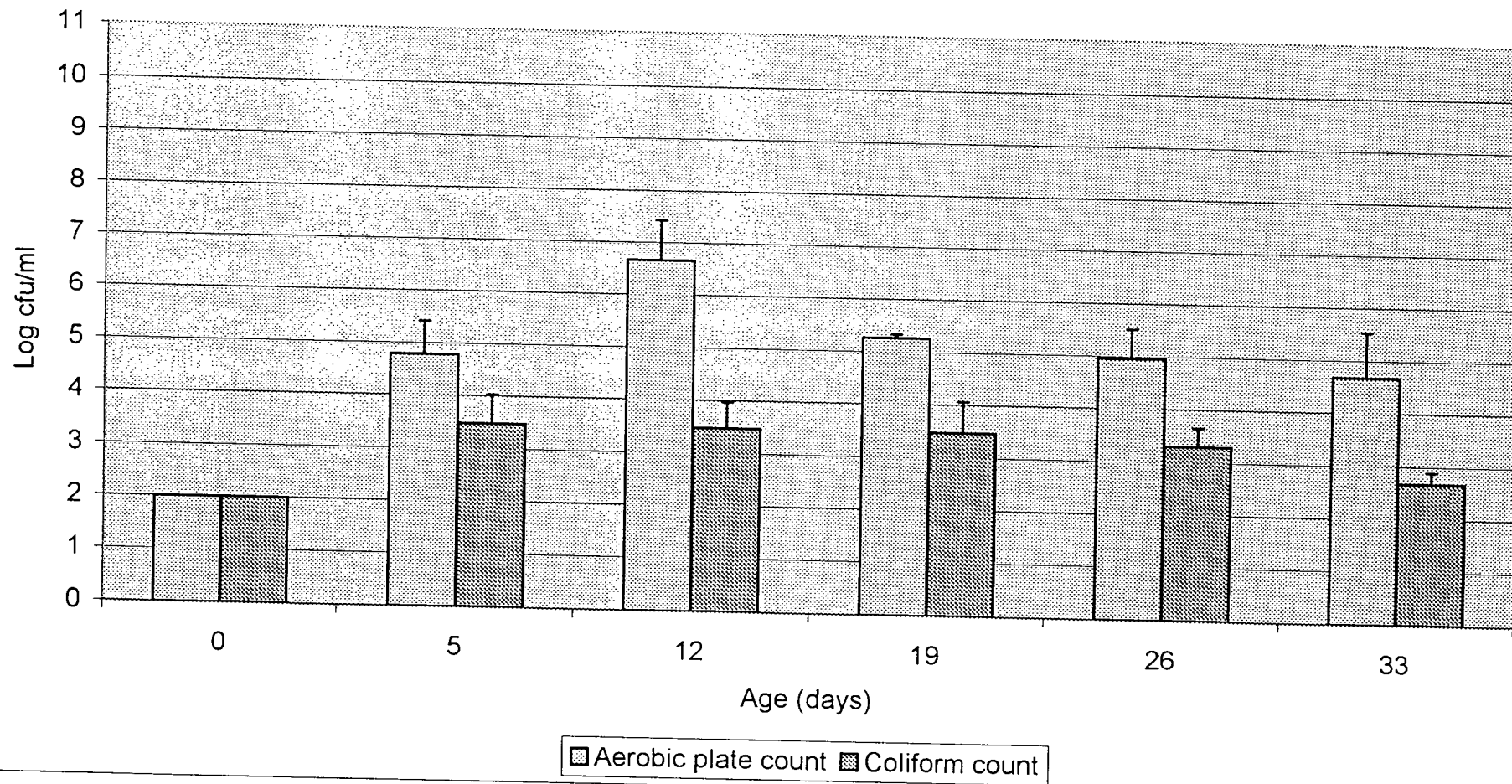


Fig. 3.4: Microbial populations present in the water of broiler houses 1 day before chick arrival until 33 days of age



CHAPTER 4

MICROBIAL POPULATIONS ASSOCIATED WITH BROILER MEAT AND ENVIRONMENTAL SOURCES IN THE ABATTOIR

INTRODUCTION

Increased public concern over foodborne diseases associated with poultry has highlighted the need to monitor and improve control of the presence of microorganisms in the poultry marketing chain (Willis and Murray, 1997). However, it should be remembered that food can never be completely safe. There will always be a small group in any population (human) who are at risk for some part of the food supply (Waites *et al.*, 1991).

At the processing plant, the microbiological quality of freshly processed poultry carcasses depends on the level of contamination involving the live birds. These numbers and types of microorganisms introduced lead to the spreading of contamination enhanced by the technical design and type of processing equipment, efficiency of processing methods, temperature control, and sanitary and hygienic practices in the plant (Bailey *et al.*, 1987).

Defeathering, using hot water and a common bath scald system, has been identified as a major contributor to cross-contamination due to the accumulation of faecal matter and bacterial loads (Kaufman *et al.*, 1972). Carcasses entering the scald tank carry high numbers of bacteria on the feathers, feet, skin and in the faeces (Bryan, 1980; Mead, 1989). The survival of these bacteria in the water depends on factors like the temperature of the water as well as the bacterial types (Mulder *et al.*, 1989). Higher scalding temperatures, above 60°C, will result in a decrease in total bacterial counts and *Salmonella* contamination (Mulder and

Veerkamp, 1974; Mulder *et al.*, 1978). Scalding at higher pH values was found to cause a substantial reduction in the level of microbial contamination of chicken carcasses before and after plucking (Humphrey *et al.*, 1984). Scalding of chicken carcasses has also been implicated as a major site for cross-contamination with *Campylobacter* (Wempe *et al.*, 1983).

The defeathering machine is also considered as a major site of bacterial cross-contamination between carcasses (ICMSF, 1980; Mulder *et al.*, 1978). The rubber fingers of defeathering machines are well-recognized sites of contamination, mainly because of warm, humid conditions provided by the scalding process. Consequently, microbial growth in the channels and cracks of the rubber fingers is induced (Mead and Dodd, 1990). The microorganisms present are spread over the skin during scalding and defeathering leading to contamination of the inner and outer carcass surfaces during evisceration and further processing (Harrigan and McCance, 1976).

Evisceration can result in further contamination of the carcass by means of rupturing of the intestinal tract allowing faecal material to contaminate the carcass surface (Mead, 1982). Before the chilling process, the carcasses are washed with water at ambient temperature to reduce microbial contamination. As a result, aerobic plate counts, Enterobacteriaceae and coliforms decrease by 50 - 90% (May, 1961). After washing, the carcasses are chilled, either by air - or immersion chilling. This process reduces the temperature of the carcass and delays or prevents the growth of psychrotrophic bacteria and pathogens (Bryan, 1980).

During the various processing operations at a typical processing plant, opportunities also exist for contamination of carcasses from the environment, equipment, the hands of workers and by cross-contamination from other carcasses (Bryan, 1980; Connor *et al.*, 1987).

Most of the gram-positive bacteria, originating from incoming birds, are removed and replaced by a heterogeneous population largely composed of gram-negative bacteria, including *Pseudomonas*, *Flavobacteria*, *Acinetobacter/Moraxella* and Enterobacteriaceae (Thomas and McMeekin, 1980). Although not all these microorganisms are involved in carcass spoilage or the cause of foodborne diseases, their presence in excessive numbers, indicates unsatisfactory processing, improper sanitary and hygienic practices in the plant (Tompkin, 1983). It also signals that the finished product may contain high levels of spoilage organisms, such as *Pseudomonas* spp. or pathogens, primarily *Salmonella*, *Campylobacter*, *Clostridium perfringens* and *Staphylococcus aureus* (Cox *et al.*, 1975).

The objectives of this study were to determine the incidence, extent and serotypes of different pathogens on whole chicken carcasses and equipment. In addition, the viable yeast cells and bacteria populations were also determined.

MATERIALS AND METHODS

Description of processing plant

The plant was a Grade A poultry abattoir with a capacity of ca. 7200 birds per hour (ca. 64 800 birds per day). Birds were scalded at 54 - 56°C and feathers removed by three defeathering machines in series. The evisceration process was performed manually. Eviscerated carcasses were spray washed with unchlorinated water followed by a pre-washing in a spin washer. Carcasses were subsequently immersion chilled in a counterflow system for 30 min. The temperature of the water varied between 2 - 4°C with a chlorine level of 30 p.p.m. Finally, carcasses were packed as whole birds, portions or individually quick frozen (IQF) portions.

Sampling procedure

Samples (neck skins, water, equipment surfaces and environmental) were taken after a midmorning rinse at five separate occasions, over a period of seven months from March to September. Neck skin samples were aseptically collected using sterile scissors and forceps and placed into sterile Whirl pak bags (Nasco, USA). Neckskin samples were taken from 10 randomly selected packaged whole carcasses (ca. 2 g per carcass).

Approximately 100 ml of water were sampled from the scalding tank and the spin chiller in sterile glass bottles. Sodium thiosulphate (0.1 ml of a 10% solution) was added to all water samples to inactivate residual chlorine (Patterson, 1968).

Equipment surfaces (rubber fingers, chute to evisceration, shackle, evisceration equipment, packing funnel and packing chute) were sampled for microbial analysis by swabbing adjacent surface areas of ca. 25 cm² for 30 sec, each using five replicate swabs moistened in Bacto Peptone Water (Difco, Laboratories, Detroit, MI)

Microbial numbers in the air in the vicinity of the defeathering area and in the packing area of the processing plant were quantified by duplicate settle plates (90 mm Petri dishes) containing three different media (Table 4.1) using an exposure time of 30 min.

Samples were refrigerated at 4°C and transported to the laboratory for analysis on the same day.

Sample processing and analysis

Composite 20 g samples of neck skin were prepared (ca. 2 g per carcass) from

the packed whole birds and homogenized for 2 min in 180 ml Bacto Peptone Water (Difco) using a Colworth 400 Stomacher (London, UK). Equipment surface samples were prepared for microbiological analysis by suspending each set of 5 swabs per surface in a sterile McCartney bottle containing 10 ml Bacto Peptone Water and shaking vigorously for 30 sec.

Tenfold serial dilutions in Bacto Peptone Water were prepared as required and plated in duplicate by the spread plate technique onto three different media (Table 4.1). Plates were incubated aerobically at the required temperatures as indicated in Table 4.1.

Plates containing between 30 and 300 colony forming units (CFU) (or the highest number if below 30) were counted and the means determined from duplicate plates. Counts were converted to logarithms.

The prevalence of *Escherichia coli*, *Listeria*, *Salmonella* and *Staphylococcus aureus* was determined on neck skins samples and equipment surfaces.

a) *Salmonella*

Composite 20 g neck skin samples obtained from 10 randomly selected packed whole carcasses were added to 180 ml Buffered Peptone Water (Oxoid, Basingstoke, UK), homogenized for 2 min in a Colworth 400 Stomacher (London, UK) and pre-enriched at 37°C for 24 h. After 24 h incubation, 0.1 ml of the pre-enrichment broth were transferred into 10 ml Rappaport-Vassiliadis Soya Peptone Broth (Oxoid) (Ogonowski *et al.*, 1984) for enrichment and incubated at 42°C for 24 h. After incubation, the enrichment broth was streaked onto Modified Brilliant Green Agar with *Salmonella* Sulphamandelate Supplement (Oxoid) (42°C for 24 h) (Geornaras *et al.*, 1994) and xylose lysine desoxycholate agar (XLD) (37°C for 24 h) (Bok *et al.*, 1986). Presumptive *Salmonella* colonies were serotyped by the South African Institute for Medical Research (SAIMR). To

determine the presence of *Salmonella* on equipment surfaces, 5 replicate swabs per surface, moistened in Buffered Peptone Water, represented by 25 cm² adjacent surfaces areas per swab were (added to 100 ml of Buffered Peptone Water) shaken vigorously for 30 sec and analysed for *Salmonella* as described for the neck skin samples above.

b) *Listeria*

Sampling for *Listeria* comprised of 20 g neck skin samples from 10 randomly selected packed whole birds (ca. 2 g per carcass). Neckskin samples were submerged in 180 ml University of Vermont *Listeria* enrichment broth supplemented with *Listeria* primary selective supplement (UVM I) (Oxoid) (Bailey *et al.*, 1988), and homogenized for 2 min. in a Colworth 400 Stomacher (London, UK). Samples were incubated at 35°C for 24 h. After incubation 0.1 ml of the pre-enrichment broth were transferred into 10ml Fraser Broth (Oxoid) (Bailey *et al.*, 1988) supplemented with Fraser supplement (Oxoid) and incubated at 35 °C for 24 h. The enrichment broth was streaked onto *Listeria* selective agar base (Oxford formulation) supplemented with *Listeria* selective supplement (Oxford formulation) (Oxoid) and incubated at 30°C for another 48 h (Dykes *et al.*, 1994). Isolates showing a dark brown colour change on the agar were selected from plates showing presumptive *Listeria* growth and purified on Tryptone soya agar (Oxoid) with 0.3% yeast extract (Merck). Presumptive *Listeria* isolates were evaluated for tumbling motility at 20°C (ICMSF, 1990). Colonies showing tumbling motility were identified to species level as described by Skovgaard (1988). To determine the presence of *Listeria* on the equipment surfaces, 5 replicate swabs per surface (moistened in *Listeria* enrichment broth base), of 25 cm² adjacent surfaces areas per swab, were added to 100 ml of *Listeria* enrichment broth, shaken vigorously for 30 sec and analysed for *Listeria* as for neck skins samples above.

c) *Staphylococcus aureus*

The prevalence of *Staphylococcus aureus* on neck skins samples and equipment was determined by spread plating in duplicate, 0.1 ml samples of the original neck skin and equipment homogenate (used to determine microbial numbers) onto Baird-Parker Agar (Oxoid) + Egg Yolk-Tellurite Emulsion (Oxoid) (37°C for 24 h). The identity of some presumptive *S. aureus* colonies, taken randomly from the plates, was confirmed by the coagulase test (Dodd *et al.*, 1988).

d) *Escherichia coli* type 1

Coliform colonies with deep red halos selected from Violet Red Bile Agar (Oxoid) were confirmed as *E. coli* type 1 based on the IMViC test (Harrigan and McCance, 1966). Typical *E. coli* type 1 species were characterized by the Eijkman (+), indole (+), methyl red (+), Voges-Proskauer (-) and citrate (-) tests (Harrigan and McCance, 1966).

RESULTS AND DISCUSSION

Neck skin samples

The highest microbial number on neck skin samples were consistently obtained by aerobic plate counts, followed by coliforms and yeasts represented by 5.0, 3.92 and 3.91 log cfu/g respectively (Table 4.2). Despite the high frequency of aerobic plate counts obtained from neck skin samples in this survey, these numbers are significantly lower than the counts reported by Geornaras *et al.* (1994) and Abu-Rawaida *et al.* (1994). Abu-Rawaida *et al.* (1994) obtained aerobic plate counts and coliform counts of 6.4 and 5.0 log cfu/g respectively whereas Geornaras *et al.* (1994) reported aerobic plate counts of 6.2 log cfu/g. Bacterial counts obtained in this study, however, correspond to the results reported by Olivier *et al.* (1996)

and Cason *et al.* (1997). Olivier *et al.* (1996) obtained aerobic plate counts of log 5.4 cfu/g and Cason *et al.* (1997) counts of 5.3 log cfu/g. Bacterial numbers of the product, however, may vary between abattoirs (Abu-Rawaida *et al.*, 1994). The microbial contamination present on the necks skins of the packed bird, originated from the spin chiller, manual handling and contaminated equipment. The lower microbial loads observed in this study may be attributed to the effective cleaning of the abattoir after production, as well as the good hygiene and housekeeping conditions during production.

Equipment surfaces

Aerobic plate counts were the highest, followed by the coliform and the yeast counts (Table 4.2). The highest aerobic plate count and coliform count were obtained from the rubber fingers {7.33 (APC), 4.81 (CF) and 3.8 (YC) log cfu/cm²} and the shackle {6.81 (APC), 3.8 (CF) and 5.84 (YC) log cfu/cm²} (Table 4.2) whereas the lowest microbial counts were obtained on the packing chute to the Moba packing machine (3.33 (APC), 3.11 (CF) and 3.08 (YC) log cfu/cm²) (Table 4.2). The high microbial numbers observed on the rubber fingers corresponds with results obtained by Geornaras *et al.* (1994), who reported microbial numbers of 7.7 (APC) log cfu/cm². Rubber fingers are difficult to clean due to the survival of microorganisms in the cracks and channels on the surfaces (Mead, 1982; Lindsay *et al.*, 1996). High counts on the rubber fingers are expected due to the warm, humid conditions, as well as residual organic material (blood, feathers) serving as nutrients for microbial growth (Mead and Dodd, 1990). The high microbial counts obtained on the shackle were attributed to the absence of a shackle brush in the abattoir. An effective shackle brush and water mix with an effective detergent may result in decreasing microbial counts. High bacterial loads in the packing funnels (log 5.05 cfu/cm²) may also contributed towards the contamination of the birds when they were packed.

Water samples

Bacterial counts within the spin chiller were low (1.1 log cfu/ml APC) whereas the presence of coliforms could not be proved. The aerobic plate counts as well as the coliform counts of the water within the scalding tank were represented by 6.97 and 4.92 log cfu/ml respectively (Table 4.3). Data obtained from water in the scalding tank by Abu-Rawaida *et al.* (1994) were significantly higher (log 8 cfu/ml on APC and log 5.9 cfu/ml on CF). Since the skin and feathers of live birds contain high microbial loads (Mulder and Veerkamp, 1974; Mead, 1989; Katula and Pandya, 1995), increased microbial contamination within the scalding tank water was expected. Geornaras *et al.* (1994) also found high microbial counts in the scalding tank, whereas Schmitt *et al.* (1988) found lower microbial counts. Lower temperatures of the water (53 - 55°C) in the scalding tank applied in this study may be responsible for the enhanced bacterial numbers. In the survey performed by Schmitt *et al.* (1988), higher water temperatures (60°C) were applied contributing to the inhibition of bacterial growth. The lower aerobic plate counts obtained from the water within the spin chiller, could be the result of the chlorine level set at 30 - 50 p.p.m.

Air samples

Settle plate counts indicated the presence of bacteria in the "dirty" {>300 (APC), 112 (CF) and 121 (YC) cfu/30 min.} and "clean" {79 (APC), 9 (CF) and 10 (YC) cfu/30 min.} areas of the abattoir (Table 4.3). The higher counts in the "dirty" area were expected attributed to the defeathering process causing spreading of microorganisms (Slavik *et al.*, 1995). The effect of microbial contamination derived from the air on the final product is insignificant according to Patterson (1973) due to the quick flow of the product, but the adverse could be possible if product build-up appears (Patterson, 1973).

Incidence of pathogens

Salmonella, *Escherichia coli* and *Staphylococcus aureus* strains were isolated from neck skins (Fig 4.4, whereas no *Listeria* species was isolated. *E. coli* and *S. aureus* were isolated from all the equipment surfaces, while *Salmonella* was present only on the shackle. No *Listeria* was isolated on equipment surfaces (Table 4.4). The pathogens most frequently isolated from neck skins and the equipment surfaces comprised of *Escherichia coli* and *Staphylococcus aureus*. The *Salmonella* serotypes found on the shackle and rubber fingers were representatives of *S. muenchen* and *S. schwarzengrund*, and on the neck skin samples *S. muenchen* (40%) and *S. typhimurium* (20%) (Table 4.4).

Despite the absence of *Listeria* species in the abattoir and in the caecum of live chickens during this study (Chapter 3), it is not an indication of the total absence of the species. *Listeria* species might have been encountered if more representative samples were selected. Bailey *et al.* (1988), found *Listeria* on processed chicken. According to literature the presence of *Salmonella* on the neck skins may be related to the *Salmonella* strains isolated on the farms. Poultry obtained from the farm often introduce *Salmonella* infection into the processing plant (Dougherty, 1974). Such contamination could result in widespread dissemination of *Salmonella* during processing and may lead to contamination of the final product. The *Salmonella* serotypes found on the farm, however, were not related to those found in the abattoir in the current study. Other sources responsible for the contamination, might have induced the contamination on the rubber fingers or the shackle. In this study, no faeces of workers were taken, but it could also be a source of infection. Furthermore, since *Salmonella* was present in the caeca, the species are exposed into the air leading to enhanced contamination (Mead, 1989).

The presence of *S. aureus* on all the equipment surfaces indicated on the substantial contribution in pathogenic organisms derived from equipment in such

environments. Since positive *S. aureus* isolates in the caeca were collected at the farm at age 26 and 33 days, indicating the birds were positive for *S. aureus* prior to arrival at the abattoir. Again, positive *S. aureus* isolates obtained from the caeca might contribute to air contamination and as a result contaminate the feathers of the birds (Mead, 1989). Consequently, infected birds could have been responsible for the contamination of the equipment. *S. aureus* has the ability to attach to rubber fingers and once established, persists for months or even years (Gibbs *et al.*, 1978). Contamination of neckskins and equipment surfaces by *E. coli* was expected, since *E. coli* is a normal inhabitant of the gut of chickens and could easily be distributed to neckskins and equipment by faecal contamination during evisceration.

The low microbial numbers present on the neckskins samples, and the low incidence of pathogens obtained in this study may be attributed to good hygienic practices. The high microbial counts observed on the equipment, however, should be reduced by frequent rinsing of the abattoir as well as the installation of a shackle brush.

Table 4.1: Culture media, temperatures and times of microbiological analysis of neckskin samples, equipment surfaces, water and air in a Grade A poultry abattoir

COUNT TYPE	Incubation		Growth Media
	Time (h)	Temperature (°C)	
Aerobic Plate count	72	20	Tryptone Soya agar (Oxoid) + 0.3% Yeast Extract (Merck) (Geornaras et al. 1994)
Coliform count	24	35	Violet Red Bile Agar with overlay (Oxoid) (Harrigan and McCane, 1966)
Yeast count	120	25	YGC Agar (Oxoid) (Welthagen and Viljoen, 1998)

Table 4.2: Aerobic plate count, coliform count and yeast counts on equipment surfaces as well as on neckskin samples taken after a midmorning rinse in a Grade A poultry abattoir

SAMPLE TYPE	MICROBIAL COUNT (LOG CFU/25 CM)		
	TOTAL AEROBIC PLATE COUNT (APC)	COLIFORM COUNT (CF)	YEAST COUNT (YC)
RUBBER FINGERS	7.33	4.81	3.80
CHUTE TO EVISCERATION	5.50	3.99	3.52
SHAKLE	6.81	3.80	5.84
EVISCERATION EQUIPMENT	4.92	2.85	3.22
PACKING FUNNEL	5.05	2.90	3.35
PACKING CHUTE	3.33	3.11	3.08
NECK SKIN SAMPLES	5.00	3.92	3.91

Table 4.3: Microbial numbers present in the environmental samples of a Grade A poultry abattoir

		TOTAL AEROBIC PLATE COUNT	COLIFORM COUNT	YEAST COUNT	E. coli type 1
*WATER	SPIN CHILLER	<2.0	<2.0	NOT DETECTED	NEGATIVE
	SCALDING TANK	6.97	4.92	NOT DETECTED	POSITIVE
** AIR	DIRTY AREA	>300	112	121	NOT DETECTED
	CLEAN AREA	79	9	10	NOT DETECTED

Table 4.4: Prevalence of pathogens on equipment surfaces and on neckskin samples in a Grade A poultry abattoir

SAMPLE	No. composite samples	Positive samples							
		<i>Salmonella</i>		<i>Listeria</i>		<i>S. aureus</i>		<i>E. coli type 1</i>	
		No.	%	No.	%	No.	%	No.	%
RUBBER FINGER	5	1	20	0	0	3	60	4	80
CHUTE TO EVISCERATION	5	0	0	0	0	3	60	4	80
SHACKLE	5	1	20	0	0	5	100	3	60
EVISCERATION EQUIPMENT	5	0	0	0	0	2	40	1	20
PACKING FUNNEL	5	0	0	0	0	2	40	1	20
PACKING CHUTE	5	0	0	0	0	1	20	2	40
NECK SKIN SAMPLE	5	3	60	0	0	4	80	5	100

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Poultry and its products are a major dietary item for a large proportion of the Southern African populations (Bok *et al.*, 1986). The meat of poultry, however, is a suitable substrate for microbial growth as the edible portion of the carcass contains ca. 70% water, has a water activity of 0.98 tot 0.99, a protein content of ca. 20.5% and a fat content of ca. 9.5% (Bryan, 1980). Consequently, the composition of the meat renders an ideal medium for microbial spoilage, making the product prone to microbial contamination.

No local studies has been reported on the sources, incidence and levels of pathogens and spoilage organisms associated with poultry production from the egg up to the final processed poultry. The effect of breeding of broiler eggs, hatching of these eggs, rearing of the broilers up to slaughtering as well as slaughtering on the incidence and levels of pathogens and bacterial populations were subsequently investigated by replicate microbiological surveys. The incidence of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* and *Listeria* on the eggs, in the caeca and liver of broilers, as well as the neckskins and equipment surfaces in the processing plant were also investigated.

THE MICROBIAL CONTAMINATION OF THE HEN'S EGG ASSOCIATED WITH BREEDING AND HATCHING

The quantification of microbial contamination on the hatching egg is of the utmost importance to the poultry breeder, since the contamination can lead to a reduction in hatchability, spread to other areas in the hatchery resulting in

infection of newly hatched chicks (Board, 1966). Freshly laid eggs rarely contains microorganisms, but the eggs could be infected during its formation, from either the ovaries or oviduct (Grau, 1986) or from the immediate environment into which they are laid (Harry, 1963). The primary contamination of the eggs originates from enteric organisms, particularly *Salmonella* and coliforms (Zander, 1978). The mode of infection is through penetration.

The results obtained in this survey proved that the highest microbial contamination on the egg shells, one day after been laid as well as after 18 days in the incubator, is consistently caused by aerobic bacterial populations. A significant difference ($P>0.05$) was observed between bacterial counts obtained from clean shell eggs compared with dirty shell eggs. The only pathogens observed on the surfaces of the eggs were *Listeria* and *Escherichia coli*. No *Salmonella* and *Staphylococcus aureus* species were detected. The high numbers of microbial populations on both the clean and dirty egg shells may be attributed to dirty nesting material, dirty hands of egg collectors and gathering of eggs. Frequent changing of nesting material (wood shavings), better hygiene control of the hands of egg collectors and frequent gathering of eggs can overcome or inhibited the contamination. The incidence of *E. coli* species on the eggs is due to faecal contamination and environmental surfaces (hands, etc.), whereas the presence of *Listeria* on eggs, is attributed to the species resistance against egg fumigation and sanitation (Laird *et al.*, 1991).

MICROBIAL POPULATIONS ASSOCIATED WITH THE CAECUM AND LIVER OF BROILER AND ENVIRONMENTAL SAMPLES

The caecum of the chicken has been shown to contain the largest number of bacteria, most of which are anaerobes (Barnes and Impey, 1970), while the livers of chickens are not a major source of bacteria (Fanelli *et al.*, 1971).

Newly hatched chicks are microbiologically sterile, but microorganisms soon become established in and on different regions of the body (Mead, 1982). Modern poultry husbandry includes high stock density, uniform age-distribution of birds on a single farm and continuous feeding. This leads to an increase in horizontal transmission of animal diseases and contamination (Aho, 1994). Pathogens are usually orally consumed before and during crating and transportation and subsequently colonize the caeca where they may be retained throughout processing (Moran and Bigili, 1990).

The results obtained in this study showed that a substantial decrease in the microbial populations present in the caeca, namely the aerobic plate counts, lactic acid bacterial counts and coliform counts, were observed from day 0 to day 19. These microbial loads, however, subsequently increased significantly after 19 days. The decrease in microbial numbers in the caeca observed within the first 19 days, could be induced by the vaccination of the birds against Newcastle 3 days prior to the survey, since vaccination caused a stress situation among the chickens. After 19 days, the feeding was changed, which resulted in an increase in microbial populations.

Escherichia coli pre-dominated in the caeca, as expected, since the pathogen is a natural inhabitant of the caeca of chickens and therefore, also being present in the litter due to faecal excretion. Low incidences of *Salmonella*, *S. aureus* and

Listeria were observed. *E. coli* was isolated from all the caeca and liver samples, litter and none from the feed. *Salmonella* and *S. aureus* were occasionally isolated from the caeca and litter but none from the feed. *Listeria* was only isolated once on a single occasion from the feed but none was recovered from the caeca, liver or litter.

MICROBIAL POPULATIONS ASSOCIATED WITH BROILER MEAT, EQUIPMENT SURFACES AND WATER IN THE POULTRY ABATTOIR

Increased public concern over foodborne diseases associated with poultry has highlighted the need to monitor and improve control over the presence of microorganisms in the poultry marketing chain (Willis and Murray, 1997). At the processing plant, the microbiological quality of freshly processed poultry carcasses depends on the level of contamination evolving from the live birds (Bailey *et al.*, 1987). Defeathering and evisceration has been identified as major contributors to cross-contamination due to accumulation of faecal matter on the feathers (Kaufman *et al.*, 1972) and by means of rupturing the intestinal tract (Mead, 1982).

In this study the highest microbial numbers on the neckskin samples, equipment, air and water were consistently obtained by aerobic plate counts, followed by coliforms and yeast counts. The microbial contamination present on the neckskin of whole carcasses, originated from the spin chiller, manual handling and contaminated equipment. The isolated pathogens from both the neckskin samples and equipment were *Salmonella*, *S. aureus* and *E. coli*, whereas *no Listeria* was isolated.

The low incidence/absence of some pathogens in this study may be misleading. The reliability can be increased by frequent examinations including more samples of eggs, broiler caecums and livers as well as neckskin samples of whole carcasses. No traceability of the pathogen sources could be detected, although the litter in the broiler houses played a definite role in the contamination of carcasses with *Salmonella*, *S. aureus* and *E. coli*.

CHAPTER 6

SUMMARY

Microorganisms, especially pathogens, play an important role in the deterioration of poultry meat and its products causing spoilage or food poisoning. The meat renders an ideal medium for the growth and progression of microorganisms originating from the environment on the farm (eggs and broiler), transport to the abattoir as well as the abattoir. As far as we know, no attempts were undertaken to determine the total microbial spectrum, including bacterial and yeast populations from the egg unto the chicken carcass after slaughtering. A historical review of the incidence and extent of microorganisms associated with poultry and its environment is given in Chapter 1. The background of poultry production and contribution of microorganisms (for example pathogens and yeasts) are highlighted.

In Chapter 2 a survey was undertaken to determine the incidence, extent and serotypes of different pathogens on and in freshly laid eggs as well as incubated eggs in the breeder broiler hatchery. In addition, the number of viable cells of bacteria and yeasts were also determined. Microbial counts on the dirty eggs predominated. A major decline in microbial numbers on the egg shells, however, were observed after 18 days in the incubator. The deduction in bacterial populations present on the egg shells after 18 days in the incubator, is ascribed to the constant fogging of the environment inside the incubator with clinafarm. The irregular gathering of eggs as well as the dirty hands of the egg collectors could have been the major contributors of the high counts on these eggs. The only pathogens obtained were *Listeria* and *E. coli* type 1.

In Chapter 3 the microbial populations associated with the caecum and liver of broilers as well as with the environment were determined. Anaerobic plate counts reflecting the dominance of bacterial populations were constantly the highest ascribed to the contents of the gut. *Salmonella*, *Staphylococcus aureus* and *E. coli* type 1 were isolated from the caecum, however no *Listeria* were isolated. *Salmonella* and *E. coli* type 1 were isolated from the liver. The high incidence of pathogens associated with the broilers is an indication of the pathogens that enter the abattoir. These levels of pathogens, further increased during transport to the abattoir.

In Chapter 4 the incidence and extent of microbial populations associated with broiler meat and the environment in the abattoir were evaluated. All the pathogens present on the farm were also observed in the abattoir. The main reasons for the similarity in the incidence of pathogens on the farm and the abattoir were ascribed to the transport from the farm to the abattoir, the process of slaughtering, equipment surfaces in the abattoir and people handling the meat. Aerobic plate counts predominated on the neckskin samples, equipment surfaces as well as in the water and air of the abattoir. *E. coli* type 1 clearly predominated on the neckskin samples as well as on the equipment surfaces, followed by *Staphylococcus aureus* and that by *Salmonella*. No *Listeria* isolates was, however, isolated from either the broiler farm or the abattoir, but was indeed observed on the egg shells from the breeder farm.

Although a pattern reflecting the incidence of pathogens could be established between the broiler farm and the abattoir, no comparison could be made with the eggs from the breeder farm and hatchery. This may be blamed on the sampling of insufficient number of eggs, or the lack of multiple repetitions. Despite the inability to detect the primary sources of pathogens the distinct possibility remained that the broiler farm was the main contributor towards microbial contamination and infection.

Therefore, more effort is needed to control the diseases and infections on broiler farms, because in an abattoir you only get out what you put in.

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