A HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF CUTANEOUS NODULAR LESIONS AND OTHER SURFACE SWELLINGS OF KENYAN HORSES

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(Veterinary Pathology and Diagnostics)

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This thesis is dedicated to my parents, Nileshkumar Shah and Meena Shah; and to the Late Prof. T. A. Ngatia, a great mentor.

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LIST OF ABBREVIATIONS

AUD – Australian Dollar

BPV – Bovine Papillomavirus

BPV-1 – Bovine Papillomavirus-1

BPV-2 – Bovine Papillomavirus-2

DNA – Deoxyribonucleic acid

DPX - Di-N-Butylphthalate in Xylene

ECL – Epitheliotropic cutaneous lymphoma

EcPV-2 – *Equus caballus* Papillomavirus-2

EEG – Equine eosinophilic granuloma

EGT – Exuberant granulation tissue

EHV-2 – Equine Herpesvirus-2

EUR – Euro

GDP – Gross Domestic Product

H_2-receptor – Histamine H_2 receptor

HIER - Heat-induced epitope retrieval

IBH – Insect bite hypersensitivity

IgE – Immunoglobulin E

IHC - Immunohistochemistry

NECL – Non-epitheliotropic cutaneous lymphoma

NEMA – National Environment Management Authority - Kenya

p53 – Phosphoprotein p53/Tumour protein p53/Cellular tumour antigen p53

PCR – Polymerase Chain Reaction

SCC(s) – Squamous cell carcinoma(s)

A HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF CUTANEOUS NODULAR LESIONS AND OTHER SURFACE SWELLINGS OF KENYAN HORSES

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ABSTRACT

The objective of the study was to determine the trends of nodular cutaneous lesions and other surface swellings of the horse in Kenya and to relate the histological and immunohistochemical characteristics to the clinical parameters.

The study used the retrospective and prospective cases presented to the Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi for histological diagnosis and from which a diagnosis of cutaneous pathology was recorded. The procedure involved retrieval of diagnostic reports from retrospective cases and histopathological examination of both retrospective and prospective cases. Each case was evaluated for the type and frequency of histological lesions and clinical data. Parameters included analyses of age, sex, breed, geographical origin, diagnosis, location of neoplasms, the pathology of the lesion, and the clinical features presented. The histological features were compared between cases and cellular behaviour was correlated with clinical parameters. Immunohistochemistry was performed on morphologically related lesions.

A total of 141 cases were identified for the study at the Department, between 1967 and 2014. Neoplastic lesions accounted for 64.5% (91/141) while inflammatory lesions accounted for 31.9% (45/141). The most common neoplastic lesion was squamous cell carcinoma (35.2% - 32/91), followed by the equine sarcoid (20.9% - 19/91), melanocytic neoplasms (18.7% -

17/91) and fibroblastic neoplasms (14.3% - 13/91). The most common inflammatory lesion was granuloma (46.7% - 21/45), most of which were granulomas exhibiting the presence of numerous eosinophils, due to a likely parasitic aetiology. There were two cystic cases, an epidermoid cyst and a dermoid cyst. Two different biopsies submitted from a 3 year old male were associated with cutaneous lesions caused by *Besnoitia* spp. Other granulomas exhibited histological characteristics similar to those of a fibroblastic neoplasm. A few cases of "proud flesh" were also encountered.

Immunohistochemistry was performed according to the manufacturer's (Dako®) protocol and it resulted in adequate staining of the antigen on selected cases. The histological diagnosis was confirmed in most instances (90% - 18/20). Discrepancies were encountered between the histopathological diagnosis and the results of immunohistochemistry in 10% (2/20) of the cases.

This study is the first to document the cutaneous lesions and other surface swellings of horses in Kenya, describing the histological and immunohistochemical characteristics. The study has also established that commercially available standardized immunohistochemical kits for various antigens can be used effortlessly to help distinguish between various morphologically related lesions. The results form the basis for further studies on the application of immunohistochemistry in routine veterinary diagnostics in Kenya.

Key words: Equine, Horse, Cutaneous, Neoplastic, Non-neoplastic, Equine sarcoid, Squamous cell carcinoma, Granuloma, Besnoitia spp., Histopathology, Immunohistochemistry

CHAPTER ONE: INTRODUCTION

1.1 Background information and problem statement

Equine practice in Kenya has largely remained at the periphery of the mainstream veterinary practice with most of the professionals focused in non-equine practice. Consequently, there is limited published information on diseases of the horse in Kenya.

Diseases of the skin of the horse affect the aesthetic value of the horse. A healthy skin of the horse manifests a smooth flat coat with a conspicuous sheen that changes notably as a result of disease. Changes in looseness and direction of the hair fibres may indicate malnutrition, heavy worm burden or onset of disease (Edwards, 1991). There are many diseases that can affect a horse's skin; it is a common site of pathological masses and lesions (Crabbe and Carter, 2007; Gore *et al*., 2008). The lesions range from inflammatory to neoplastic nodules.

Inflammatory cutaneous lesions may include hypersensitivity reactions (such as urticaria, hives and eosinophilic granuloma), staphylococcal cellulitis, pemphigus foliaceus, and pyoderma (folliculitis). Cutaneous neoplasms of the horse include primary lesions such as equine sarcoid, fibroma and fibrosarcoma, melanoma and melanosarcoma, squamous cell carcinoma; and secondary ones like lymphosarcoma. Other cutaneous lesions and surface swellings of the horse include seroma, haematomas, abscesses, papillomatosis, and chronic progressive lymphedema (Crabbe and Carter, 2007; Gore *et al*., 2008). Cutaneous lesions contribute significantly to the total disease burden in horses and they often impact negatively on their productivity and performance.

Diagnosis of cutaneous swellings in the horse mainly involves the evaluation of clinical manifestation, histopathology on tissue biopsies and necropsy specimen, and immunohistochemistry. The major indication of histopathology ante-mortem, in equines, is the identification of abnormal masses or growths on or within the body (Crabbe and Carter, 2007; Gore *et al*., 2008).

Globally, neoplasms are the most commonly encountered cutaneous lesions of the horse compared to non-neoplastic conditions with regard to abnormal cutaneous masses and growths. Among the neoplasms, sarcoids and squamous cell carcinoma are invariably the most common diagnosis (Foy *et al*., 2002; Meuten, 2002; Valentine, 2005; Valentine, 2006; Meierhenry, 2008). Non-neoplastic skin conditions of the horse are less frequently encountered, but in this category, eosinophilic granulomas are more commonly encountered (Valentine, 2005; Schaffer *et al*., 2013).

Treatment of cutaneous conditions in the horse depends on the type of lesion. Most inflammatory lesions can be treated medically while neoplastic lesions may require a combination of surgical and medical management.

Results from the present study will reveal the common cutaneous conditions of the horse and therefore provide reference data to support the pathological diagnosis required in good management of these conditions.

1.2 Hypothesis

Cutaneous lesions of horses are not frequently diagnosed at histopathology and immunohistochemistry in Kenya.

1.3 Objective

1.3.1 Broad Objective

To characterize the pathology of the cutaneous nodular lesions and surface swellings of the horse in Kenya using a retrospective and prospective study of cases submitted to the Department of Veterinary Pathology, Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Nairobi.

1.3.2 Specific Objectives

1. To determine the types of cutaneous nodular lesions and surface swellings of the horse in Kenya using histopathological characterisation.
2. To characterize the pathology of cutaneous nodular lesions and surface swellings of the horse in Kenya that have close histological features using immunohistochemistry.

1.4 Justification

Horse owners are sensitive and passionate about their animal's health and aesthetics. At the moment there is no published work on neoplastic and non-neoplastic cutaneous lesions and other surface swellings of the horse in Kenya. Most of the diagnosis has been based on histopathology which may not differentiate between neoplasms that show close morphological similarities. In this case, immunohistochemistry with specific markers can be used to confirm diagnosis of morphologically similar neoplasms. Some non-neoplastic masses may occasionally be misdiagnosed as neoplastic clinically. This justifies a histological and immunohistochemical confirmatory study.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview on Horses

Horses (*Equus caballus*) belong to the Genus Equus and the Family Equidae. They were domesticated about 5500 to 6000 years ago, mainly for draught power and transport. Lately, horses are gaining popularity for sports and leisure riding. Most of the horses in the world are kept in the Northern hemisphere, especially in the United States of America, where both wild and domesticated horses are found. In the Tropics, horses are usually kept in cooler areas and at higher altitudes. There is only one species of domestic horse, but around 400 different breeds, all of which are grazers (Cliarlottetown, 2003; Pearson *et al.*, 2005).

2.2 Management of Horses

Horse rearing and management is based on the intended use that the animal will be subjected to. Most draught horses are kept in free-range and receive no supplementation. They are usually kept in barns either singly or in groups. Sport and leisure horses are usually maintained on a balanced feed which supplements their grazing in paddocks and are usually housed in individual stables (Cliarlottetown, 2003; Pearson *et al.*, 2005; Varma, 2012).

In health management, owners of sport and leisure horses are more pro-active in ensuring that optimal health programmes are applied to their horses. Regular health-checks are scheduled and the veterinarian is heavily involved in the care of sport and leisure horses.

Additionally, such owners are knowledgeable and involved in first aid for the horses. This diversity in management of horses creates challenges for those practicing equine medicine and management whereby owners of draught horses may refrain from calling the clinician until the case becomes terminal while, on the other hand, owners of sport and leisure horses may call the clinician on a regular basis (Cliarlottetown, 2003; Pearson *et al.*, 2005; Varma, 2012).

2.3 Horses and Health

The major constraints that face the horse industry in general are mainly associated with inadequate nutrition, injuries and diseases. Nutritional problems arise from inadequate supplementation and imbalanced diets. Injuries are common to all working horses due to the strains placed on them, for example, orthopaedic injuries in racing horses. Several organisms cause disease in the horse, of which, the most important are vector-borne diseases such as Piroplasmosis, African Horse Sickness, Eastern Equine Encephalitis and West Nile Virus. Reviews of the common diseases of the horse have been documented by several authors (Traub-Dargatz *et al*., 1991; Wilson, 1993; Madigan and Pusterla, 2000; Radostits *et al*., 2007; Onmaz *et al*., 2013; Kane, 2014). These diseases can affect the performance and productivity of the horse manifesting as reduced growth rates, reduced exercise tolerance, abortions and other forms of infertility. The diseases impart financial burden to the owners through cost of treatment, loss of production and in severe cases, loss of the animal (Cliarlottetown, 2003; Pearson *et al.*, 2005; Gore *et al*., 2008; Varma, 2012).

Globally, research and studies on horse health and disease are widespread, ranging from orthopaedic injuries to diseases (Traub-Dargatz *et al*., 1991; Wilson, 1993; Madigan and Pusterla, 2000; Kidd *et al*., 2001; Williams *et al*., 2001; Ross and Dyson, 2003; Naeini and Niak, 2005; Radostits *et al*., 2007; Dyson, 2007; Cogger *et al*., 2008; Ramzan and Palmer, 2011; Onmaz *et al*., 2013; Kane, 2014). Studies on cutaneous lesions are extensive and mainly related to neoplasms (Pascoe and Summers, 1981; Schuh, 1986; Fleury *et al*., 2000; Valentine, 2006). Studies on non-neoplastic conditions are comparatively fewer (Wilkie *et al*., 1985; Ramos-Vara *et al*., 1996; Pusterla *et al*., 2003; Valentine, 2005).

2.4 Horses in Kenya

The total population of horses in Kenya is about 4,000 and the most common breed kept is the Thoroughbred (Varma, 2012). Most of the horses are found within the Karen suburb of Nairobi City, Laikipia County and Nakuru County and are mainly used for leisure riding and sports. A few horses are attached to the police service and isolated private companies for the purpose of security (Varma, 2012). This implies that the Kenyan horse industry is mainly focussed around sporting, leisure riding and, to limited extent, security. Most sporting horses are used for racing and polo. Most horse owners in Kenya value their animals and are passionate for their health and aesthetics (Pearson *et al.*, 2005; Varma, 2012). As a result, management of sporting horses in Kenya is to a great extent appropriate and few managemental problems are encountered. The major problems reported from sporting horses are orthopaedic injuries and cutaneous lesions. However, there is limited documentation on the occurrence of these lesions.

Most of the veterinary practice in Kenya concentrates on dairy, meat and small companion animals, with very little participation in equine practice especially horses. This may be associated with limited attention given to the horse in legislative documents. The Animal Diseases Act (1965), one of the most important legislation affecting veterinary practice in Kenya, pays limited attention to the horse as compared to other livestock, mainly ruminants. Another reason could be as a result of the small population of horses in Kenya relative to those of other livestock and other companion animals. Equine practice is an important field in Kenya and it should be supported since it offers many opportunities for the development of the animal industry in the country.

The horse industry in countries where it is well developed has contributed to the economy in a positive manner. In Australia, the horse industry contributed over AUD 6.3 billion to the GDP in 2000 as a result of expenditures on the animal, business and events; and government revenue from taxes on bets and owner income (Gordon, 2001). In Europe, direct economic contribution from the horse industry in countries like Sweden and Austria has been estimated at EUR 2.0 billion annually (Liljenstolpe, 2009). In these countries, management and legislation are targeted at ensuring that the industry grows and develops by implementing legislation that protects and supports the industry (Häggblom *et al*., 2009; Stull *et al*., 2010; Elgåker, 2011; Zeverte-Rivza and Paula, 2014). Such studies on the economic impact of the horse industry in Kenya have not been done.

2.5 The skin of the horse

The skin is the largest organ in the horse accounting for 12-24% of the animal's total weight. It serves a number of functions including protection of underlying tissues, thermoregulation, excretion of water and salts through sweat glands, environmental sensation (pressure, temperature, etc.), and synthesis of vitamin D. Table 1 shows the difference between certain characteristics of the skin of the horse, ox and dog (Wakuri *et al*., 1995; Aughey and Frye, 2001; Scott, 2004; Wong, 2005).

The skin of the horse consists of various cellular and tissue components as illustrated in Plate 1. The skin can generally be divided into the epidermis and the dermis, with the subcutis serving as the innermost layer overlying the muscles. The epidermis is the outer layer and the dermis is the inner layer. The epidermis of the horse has an average thickness of 0.053mm made up of several cell layers. The thickness of the dermis varies between 1-6mm being thicker on the dorsum and thinner on the ventrum and on the medial aspects of the limbs. The two layers are attached by collagenous and elastic connective tissue. The subcutis, also referred to as the hypodermis, is composed of loose connective tissue which is either fatty or fibrous. It functions to insulate the body, absorb external shock to protect internal structures and as an energy reserve for the body as it is composed of adipose tissue. The thickness of the subcutis can vary throughout the body and between individual horses (Wakuri *et al*., 1995; Aughey and Frye, 2001; Scott, 2004; Wong, 2005; Samuelson, 2007).

Table 1: Differences in some skin characteristics of the horse, ox and dog. Adapted from Aughey and Frye (2001).

	HORSE	**OX**	**DOG**
THICKNESS	Varies from 1-5mm Thickest on dorsal surface of tail, mane attachment	Greater than that of any domesticated animal Average 3 - 4mm, 6 - 7mm at brisket, 5mm at root of tail and hock	Variable, breed-dependent
CUTANEOUS GLAND	Larger than that of other domesticated animal Numerous in number	Less developed Fewer in number	More developed Numerous
SEBACEOUS GLAND	More developed Specially developed on lips, prepuce, mammary gland, perineum, labia of vulva	Well developed Best developed on udder, none on teats	More developed, best developed in short and rough-haired breeds Largest and most numerous at lips, anus, sterna region
SWEAT GLANDS	More developed Coiled Occur diffusely Largest in lateral wing of nostril, flank, mammary glands, free part of penis	Well developed Not coiled Location is breed dependent; most of the functional sweat glands of *Bos taurus* are located in the nose, while those of *Bos indicus* are located in the nose and loose skin areas like the dewlap	More developed Coiled Best developed in long and fine-haired breeds Largest in digital pads
NASO-LABIAL GLANDS	Less	More	Absent or very scanty
HAIRS	Fine Constantly shed and replaced	Extremely variable Curly in frontal region, form brush in tail	Usually arranged in groups of three

The epidermis is ectodermal in origin and is classified as a stratified squamous epithelium. It is made up of several layers namely; stratum basale, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum. The stratum basale is mainly composed of keratinocytes that undergo mitosis to supply cells to the overlying layers. The stratum spinosum has keratinocytes that produce numerous cytoplasmic molecules and these cells are connected to each other by desmosomes that act as intercellular bridges. In the stratum granulosum, the keratinocytes lose their organelles and secrete their cytoplasmic molecules in to the extracellular space. The stratum lucidum, a three to five cell thick layer, is present in areas where the skin is thick. It is translucent in nature but may be pigmented depending on the density of melanocytes within it. The stratum corneum is the outermost layer and is composed of 10-30 layers of polyhedral anucelated keratinocytes which are referred to as corneocytes (Wakuri *et al*., 1995; Aughey and Frye, 2001; Scott, 2004; Wong, 2005; Samuelson, 2007).

The main activity of the epidermis is to produce two types of protein, keratin and melanin. Keratin is produced by keratinocytes and is the principal component of the epidermis. It is a simple insoluble protein with a fibrous structure. It serves a supportive and protective function, including the shedding of water. Melanin is the dark, shapeless pigment of the skin and hair. Melanin is produced by melanocytes, a cell of neural origin. The melanocytes have dendritic extensions that attach to keratinocytes. Another cell type in the epidermis is the Langerhans cell that is active in the immune response and possibly in the regulation of keratin formation. The most important part of the epidermis is the superficial layer, known as the stratum corneum, since much of the functional activity of the skin resides here. The proper functioning of this superficial layer depends on the structural arrangement of the keratin it contains and on its lipids. Lipids and keratin

combine to waterproof the skin and prevent various agents from entering the body (Wakuri *et al*., 1995; Aughey and Frye, 2001; Scott, 2004; Wong, 2005; Samuelson, 2007).

The dermis is mesodermal in origin and is made up of connective tissue (collagen, elastic and reticular fibres). In horses, it is divided into the superficial (papillary) layer, deep (reticular) layer and the cordovan layer which is a unique layer found in areas such as the dorsum and lateral neck. It is made up of fine collagen, elastin and reticular fibres. Also, hair follicles, sebaceous glands, sweat glands, blood vessels, lymph vessels and nerves occur in the dermis as illustrated in Plate 1 (Wakuri *et al*., 1995; Aughey and Frye, 2001; Scott, 2004; Wong, 2005; Samuelson, 2007).

The hair follicles in a horse's skin are simple, with a single hair emerging from each pore. The hair that emerges from these pores serves as frontline protection for the horse by acting as a filtering system. For example, ultraviolet light is filtered by the hair coat and is absorbed by melanin granules in the epidermis and hair. Hair is composed of keratinized epithelial cells. Each hair consists of a shaft and a root that is contained in the hair follicle. The colour of a horse's hair is determined by a genetic process involving the amount of pigment in the hair. The great variation in colour has to do with combinations or absences of the pigments. Sebaceous glands and bundles of smooth muscle connecting the side of the hair to the dermis are structures associated with the hair follicles. The sebaceous glands secrete sebum into the follicles and eventually to the epidermal surface. This lubricates the skin to prevent excessive evaporation that can result in dry skin (Wakuri *et al*., 1995; Aughey and Frye, 2001; Scott, 2004; Wong, 2005; Samuelson, 2007).

There are two types of sweat glands in the horse, the apocrine and the eccrine glands. The apocrine glands are spread throughout the skin, while the eccrine glands are found only in the frog of the hoof and, thus, play a very limited role in the cooling process. The horse sweats the most of any of the domestic species. The sweat glands are simple, coiled, tubular glands that open independently of the hair follicle. Since sweating is the major method of thermoregulation, horses do much better in cool, temperate climates as compared to the hot, humid tropics (Wakuri *et al*., 1995; Aughey and Frye, 2001; Scott, 2004; Wong, 2005; Samuelson, 2007).

Sensory nerve endings are found in the dermis, just under the epidermis. They carry sensations of pressure, pain, heat, and cold. Motor nerves cause the sweat glands to secrete (Wakuri *et al*., 1995; Aughey and Frye, 2001; Scott, 2004; Wong, 2005; Samuelson, 2007).

The growth and shedding of hair coats follows a definite cyclical pattern. A heavier coat grows with shorter periods of daylight and when the weather turns colder. The thick coat is shed when days become longer and warmer and there are longer periods of light. There are health conditions that can affect this normal shedding cycle. For example, horses suffering from Cushing's disease often carry a heavy coat even during long-light, hot summer days (Wakuri *et al*., 1995; Aughey and Frye, 2001; Scott, 2004; Wong, 2005; Samuelson, 2007).

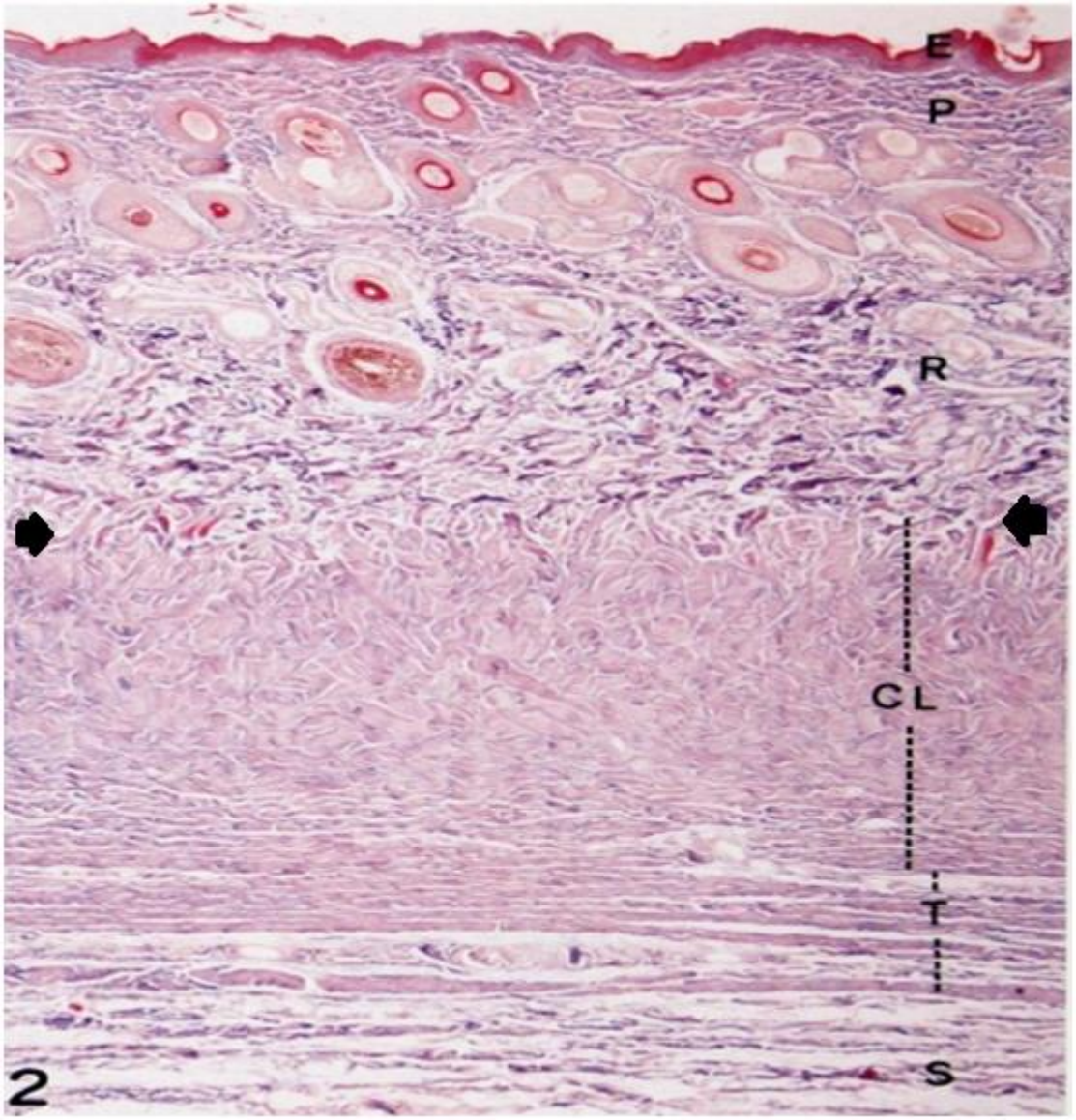

Plate 1: Vertical section of equine skin from the cranial abdominal region.
Epidermis (E), papillary layer (P), reticular layer (R), cordovan-leather tissue layer (main cordovan layer, CL; accessory cordovan layer, T) and subcutis (S). Notice the distinct separation of the reticular layer and cordovan-leather tissue layer of the dermis at the level of the black arrows. Masson's trichrome stain: x40. Sourced from Wakuri *et al*., 1995

2.6 Horse skin in health and disease

Horse skin consists of many layers and is tough and resilient, and generally heals quickly when injured. It is a common site of pathological masses and lesions. Some skin masses initially appear as minor lesions that can either remain as such, while others transform into major lesions if left unattended (Crabbe and Carter, 2007; Gore *et al*., 2008). Changes in the normal characteristics of the skin may indicate problems with nutrition, endoparasite infestation or an underlying disease (Edwards, 1991).

There is limited information on the occurrence of skin conditions of horses in Kenya. Many problems can affect a horse's skin and are easily identified since the skin is the most accessible and visible organ of the body. These lesions affect the aesthetics that compels the owners to request for their immediate management. Additionally, some of the lesions can indicate a more serious underlying disease that may be more detrimental. The lesions may range from inflammatory to neoplastic nodules and swellings (Edwards, 1991; Crabbe and Carter, 2007; Gore *et al*., 2008).

2.7 Approach to diagnosis of cutaneous nodular lesions and other surface swellings

Tentative clinical diagnosis of any nodular lesion or swelling of the skin is based on history taking and clinical examination. A more definitive diagnosis is obtained using various laboratory techniques, some of which can be applied in the field. Most of these techniques require examination of samples such as biopsies, impression smears, fine-needle aspirates, squash smears and faeces (for cutaneous lesions requiring faecal examination) and any other samples. The most commonly employed techniques include dermoscopy (surface microscopy or epiluminescence microscopy), cytology and

histopathology. Recently, immunohistochemistry has been gaining recognition in equine dermatology. Other techniques are available but have not been adapted into regular practice (Scott, 1988: Argenziano and Soyer, 2001; Muller *et al*., 2007; Scott and Miller, 2011). Dermoscopy is rarely used in Kenya.

2.7.1 Dermoscopy

Dermoscopy is a non-invasive technique used on the skin of the horse that employs the optical phenomenon of immersion oil or similar fluids such as alcohol and water. These fluids eliminate surface reflection making the cornified layer translucent such that the pigmented structures of the epidermis, the dermoepidermal junction, and superficial dermis can be examined by microscopy. This procedure also affords examination of the superficial vascular plexus of the skin. This method is fairly cheap to use, does not require any biopsies to be collected and enables diagnosis of pigmented skin lesions. The disadvantages are that it is limited to diagnosis of pigmented lesions and can only be performed by few trained personnel (Scott, 1988: Argenziano and Soyer, 2001; Scott and Miller, 2011).

2.7.2 Cytology

Cytology is a technique used to examine impression smears obtained from the cut surface of lesions or fine-needle aspirates of nodular lesions. These smears are fixed and stained before examination under a light microscope. The cellular characteristics are used to arrive at a diagnosis. This technique is fairly cheap and helps in distinguishing inflammatory lesions from neoplastic lesions. However, a correct

diagnosis may only be arrived at in 50% of the cases and it requires formal training in identification of the various cell types (Scott, 1988: Muller *et al*., 2007; Scott and Miller, 2011).

2.7.3 Histopathology

This technique involves the preparation of thin sections of tissue obtained through biopsy-taking, which are stained and examined under a light microscope. Histopathology allows the evaluation of the cellular and architectural alterations within the section. Advantages of this method include accurate analysis of the cell types and associated connective tissue. It enables differentiation of lesions and provides for accurate diagnosis. However, the method requires formal training; it may be subjective based on the experience of the user and can create confusion for lesions that show similar cellular characteristics (Scott, 1988: Muller *et al*., 2007; Scott and Miller, 2011).

2.7.4 Immunohistochemistry

Immunohistochemistry (IHC) is the process of detecting antigens *in situ* in tissue sections by exploiting the specificity of antibody-antigen reactions and a variety of microscopically dense markers (Leong *et al*., 2010). Immunohistochemical staining is widely used in the diagnosis of abnormal cells such as those found in neoplasms. Specific molecular markers are characteristic of particular cellular events such as proliferation or cell death. Immunohistochemistry is also widely used in basic research to understand the distribution and localization of biomarkers and differentially

expressed proteins in different parts of a biological tissue. Visualising an antibody-antigen interaction is accomplished in a number of ways. In the most common instance, an antibody is conjugated to an enzyme, such as peroxidase, that can catalyse a colour-producing reaction. Alternatively, the antibody can also be tagged to a fluorophore, such as fluorescein or rhodamine; or some particles, such as gold or silver, in a technique referred to as immunofluorescence (Leong *et al*., 2010).

Several studies have used immunohistochemistry in diagnosis of cutaneous conditions of horses. Thamm *et al*. (2008), Altamura *et al*. (2012), Finlay *et al*. (2012) and Farouk (2014) have used immunohistochemistry on various equine tumours, especially equine sarcoids with success. They have either used antibodies to general antigens such as cytokeratins, vimentin, S100 and Ki-67, or specific antigens such as cyclooxygenase-2, O6-methylguanine-DNA methyltransferase, Tumour protein p53, or antigens of aetiological agents.

2.7.4.1 Antibodies against Cytokeratins

Cytokeratins are a family of water-soluble proteins with molecular weights between 40-70 kDa that form the cytoskeleton of epithelial cells. At least 19 different cytokeratins have been identified and can be divided into two subfamilies. Subfamily A comprises relatively acidic cytokeratins (with a pH under 5.5) whereas members of subfamily B have a relatively basic pH of 6 or over. They are specific markers for epithelial cell lineage, and are specific to organs and tissues. The subtype expressed depends on the stage in the sequence of terminal differentiation and the stage of development. An initial screen includes ‘‘pancytokeratin’’ characterized by the

antibody AE1/AE3. Once the initial differentiation is made, more specific individual cytokeratins such as CK7 and CK20 can be used to better characterize an epithelial neoplasm. Cytokeratins are used to differentiate epithelial neoplasms from non-epithelial neoplasms (Eichner *et al*., 1984; Nelson *et al*., 1984; Pinkus *et al*., 1986; Listrom and Dalton, 1987).

2.7.4.2 Antibodies against Vimentin

Vimentin is a 57 kDa intermediate filament protein, which forms part of the cytoskeleton of vertebrate cells. Vimentin belongs to class III of the five classes of intermediate filaments, showing a high degree of specificity for cells of mesenchymal origin. Since there is co-expression of intermediate filaments, particularly vimentin and cytokeratins in a variety of normal tissues and in neoplastic lesions, the use of a panel of antibodies in differential neoplasm diagnosis is necessary. Vimentin generally differentiates between mesenchymal and non-mesenchymal neoplasms (Azumi and Battifora, 1987; Osborn *et al*., 1984).

2.7.4.3 Antibodies against S100

S100 is a multigene family of low molecular weight Ca^{2+}-binding proteins. The family compromises 19 members that are differentially expressed in a large number of cell types. Thus S100B (S100β) is most abundant in glial cells of the central and peripheral nervous system, in melanocytes, chondrocytes, and adipocytes, whereas S100A1 (S100A/S100α) is most abundant in cardiomyocytes, slow twitch skeletal muscle cells, salivary epithelial cells, and renal cells. Additionally, S100B is found in

neoplastic cells and subpopulations of neurons, while S100A1 has also been detected in hippocampal neurons. S100A6 is expressed by fibroblasts and smooth and heart muscle cells. Members of the S100 family have been implicated in Ca2+-dependent regulation of a variety of intracellular activities, for example, protein phosphorylation, cell proliferation including neoplastic transformation, and differentiation. For cutaneous lesions, S100 helps to identify neoplasms of melanocytic origin and cutaneous schwannomas (Vanstapel *et al*., 1986; Ilg *et al*., 1996; Bogaert *et al*., 2011).

2.7.4.4 Antibodies against Ki-67

The Ki-67 antigen is a nuclear protein, which is defined by its reactivity with monoclonal antibody from the Ki-67 clone. Two isoforms of 345 and 395 kDa have been identified. The Ki-67 antigen is preferentially expressed during all active phases of the cell cycle (G_1, S, G_2 and M-phases), but it is absent in resting cells (G_0-phase). During interphase, the antigen can be exclusively detected within the nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes. The antigen is rapidly degraded as the cell enters the non-proliferative state, and there appears to be no expression of Ki-67 during DNA repair processes. Hence Ki-67 can be used to differentiate between neoplastic and non-neoplastic cells (Gerdes *et al*., 1984; Scholzen and Gerdes, 2000).

2.7.4.5 Tumour protein p53

Tumour protein p53 is a tumour suppressor protein coded by the tumour protein p53 (TP53) gene. Its function is to regulate cell division and thus prevents uncontrolled growth and replication of cells. The protein is found in the nucleus of the cell where it attaches to the DNA. In the event of DNA damage, the protein plays a role in determining whether the cell undergoes apoptosis or the damaged DNA is repaired. In horses, it has been theorised that p53 is either mutated or inhibited when neoplasms such as sarcoids develop. These theories require further evaluation. The protein has been used for immunohistochemical studies for equine sarcoids and papillomas (Momand *et al*., 1992; Buchner *et al*., 1996; Nasir *et al*., 1999; Albaric *et al*., 2001; Postey *et al*., 2007; Finlay *et al*., 2012).

2.8 Common cutaneous nodular lesions and other surface swellings of the horse

Cutaneous nodular lesions and surface swellings can be categorised into inflammatory, neoplastic and miscellaneous conditions.

2.8.1 Inflammatory skin conditions

Inflammatory skin conditions are primarily characterised at histopathology by presence of morphological features consistent with the type and nature of inflammation. They range from hypersensitivity reactions to microbial and parasitic induced inflammatory lesions.

2.8.1.1 Cutaneous Hypersensitivity Reactions

The most frequently observed cutaneous hypersensitivity reactions in horses include insect bite hypersensitivity and urticaria. Others include aural polyps and pemphigus foliaceus.

2.8.1.1.1 Urticaria

Urticaria are raised and pruritic lesions on the skin resulting from an allergy, and are also referred to as hives or nettle rash. It is usually acute or peracute, developing soon after immunological challenge; and may occur once or twice in a life-time, resolving on their own or after treatment; or may persist or recur. Urticaria is a clinical manifestation of a disease and not a disease in itself (Siegal, 1996; Hayes and Knightbridge, 2002; Jubb *et al*., 2005; Crabbe and Carter, 2007; Gore *et al*., 2008; Ural and Ulutas, 2010; Scott and Miller, 2011; Littlewood, 2011; Wilson, 2011; Srivastava *et al*., 2013; Kehrli *et al*., 2015). Horses seem to develop urticaria more readily than other domesticated animals (Littlewood, 2011). There is no age or sex predilection but it has been observed that Arabian and Thoroughbred horses seem to be more prone to urticaria than other breeds (Srivastava *et al*., 2013). A study has shown that urticaria can occur concurrently with other hypersensitivity reactions such as insect bite hypersensitivity and recurrent airway obstruction also known as chronic obstructive pulmonary disorder (Kehrli *et al*., 2015).

There are many causes of urticaria which can be grouped as immunological and non-immunological agents. Immunological agents include drugs, feed and feed

additives, environmental allergens, transfusions, infections, insects and systemic diseases; while non-immunological agents include dermatographism, cholinergic urticaria, cold urticaria, exercise induced urticaria and pressure urticaria. Idiopathic urticaria has also been observed (Justus and Krook, 2006; Rodriguez-Palacios *et al*., 2007; Ural and Ulutas, 2010; Littlewood, 2011; Lorch *et al*., 2013; Srivastava *et al*., 2013).

Clinically, horses develop a fever, anorexia and dullness before developing cutaneous eruptions in the form of elevated, round to flat-topped wheals or plaques measuring 1 to 20 cm in diameter. These lesions are areas of localised dermal oedema which result in depressed centres or "pitting" with pressure. They are observed on any part of the body, but mainly on the back, flanks, neck, eyelids, and legs. In advanced cases, they may be found on the mucous membranes of the mouth, nose, conjunctiva, rectum, and vagina. The wheals may coalesce or progress to exudative forms. Other accompanying lesions that may be seen include scales, crusts, papules and alopecia (Siegal, 1996; Hayes and Knightbridge, 2002; Jubb *et al*., 2005; Crabbe and Carter, 2007; Gore *et al*., 2008; Ural and Ulutas, 2010; Scott and Miller, 2011; Littlewood, 2011; Wilson, 2011; Srivastava *et al*., 2013; Kehrli *et al*., 2015).

Histologically, lesions of urticaria show dermal oedema with perivascular or diffuse mixed cell inflammatory infiltration comprising eosinophils, T-lymphocytes, monocytes and neutrophils; with mast cells seen occasionally (Jubb *et al*., 2005; Hinden *et al*., 2012; Srivastava *et al*., 2013).

Diagnosis of urticaria is easily made from the history and clinical examination, however identifying the cause is much more difficult and requires intradermal testing or other forms of allergen testing (Siegal, 1996; Hayes and Knightbridge, 2002; Jubb *et al*., 2005; Crabbe and Carter, 2007; Gore *et al*., 2008; Ural and Ulutas, 2010; Scott and Miller, 2011; Littlewood, 2011; Wilson, 2011; Srivastava *et al*., 2013; Kehrli *et al*., 2015).

Management of urticaria is mainly chemotherapeutic with administration of anti-histaminic drugs and anti-inflammatory drugs for acute cases and administration of anti-IgE drugs and anti-inflammatory drugs for chronic and recurrent cases. For all cases of urticaria, removal of the allergen is indicated to prevent relapse. This involves identification of the allergen using food elimination and intradermal tests (Bindslev-Jensen and Skov, 2010; Ural and Ulutas, 2010; Littlewood, 2011; Srivastava *et al*., 2013). A study by Srivastava *et al*. (2013) with herbal remedies has shown some positive results in treatment of urticaria in horses using a polyherbal formulation.

2.8.1.1.2 Insect Bite Hypersensitivity

Insect bite hypersensitivity (IBH) is a seasonal dermatitis in horses as a result of a type I hypersensitivity reaction against the bites of midges (*Culicoides* spp.), black flies (*Simulium* spp.) and other insects (Anderson *et al*., 1988; Hellberg *et al*., 2009; Jónsdóttir, 2011; Schaffartzik *et al*., 2012). It can occur in any breed and is seasonal, occurring when the implicated insect populations are highest.

Common names for the condition include “sweet itch”, “summer eczema”, “Kasen”, “Queensland itch” and “*Culicoides* hypersensitivity”.

Clinically, IBH is characterised by formation of several papules, abnormal increase in sensitivity to stimuli and pruritus. This causes the horse to scratch leading to thickening of the skin, hair loss, skin abrasion with a serous exudate and secondary infections. In chronic cases, the skin appears scaly and folded. The lesions are usually found along the back and at the tail and mane base (Anderson *et al*., 1988; Jónsdóttir, 2011; Schaffartzik *et al*., 2012).

Histological examination shows presence of subepidermal oedema and perivascular-to-diffuse mixed inflammatory reaction comprising eosinophils, neutrophils and mononuclear cells. The overlying epidermis shows acanthosis and becomes parakeratotic. In chronic cases, fibroblasts deposit a large amount of collagen leading to fibrosis and the epidermis may show focal areas of necrosis (Anderson *et al*., 1988; Schaffartzik *et al*., 2012).

Diagnosis of IBH can be done made from the history and clinical signs. Intradermal tests or *in vitro* tests using whole body extracts from *Culicoides* and *Simulium* spp. has been used as a confirmatory test, although the sensitivity and specificity of these are low (Hellberg *et al*., 2009; Jónsdóttir, 2011; Schaffartzik *et al*., 2012).

Management is aimed at eliminating exposure to the insect allergens. This may be done by use of blankets as a mechanical means to prevent bites, housing in insect-proof buildings at times when the insects are actively feeding, and topical application of insect repellents. Symptomatic treatment is indicated in severe

pruritic cases where steroidal anti-inflammatory drugs are preferred (Anderson *et al*., 1988; Jónsdóttir, 2011; Schaffartzik *et al*., 2012).

2.8.1.1.3 Equine eosinophilic granuloma

Equine eosinophilic granuloma (EEG) is the most common non-neoplastic nodular cutaneous lesion diagnosed in the horse with no age, sex or breed predilections. Equine eosinophilic granuloma is also referred to as nodular necrobiosis or nodular collagenolytic granuloma (Bimbo, 1986; Slovis *et al*., 1999; Meierhenry, 2006; Yu, 2015).

The cause of EEG is not fully understood but studies and theories suggest that it is a result of hypersensitivity reactions to insect bites, trauma, atopy, silicone-coated hypodermic needles and food allergies (Slovis *et al*., 1999; Meierhenry, 2006; Yu, 2015).

Clinically, EEG begins as papules measuring up to 1 cm in diameter within 24 to 48 hours of immunological challenge and then progresses to single or multiple, round, firm nodular lesions measuring 1-10 cm in diameter, found on the neck, withers and back. The granulomas are usually haired, non-ulcerated and do not show abnormal pigmentation. Some lesions may ulcerate, or become cystic or plaque-like; while others may become calcified in their core (Meierhenry, 2006; Yu, 2015).

Microscopically, the nodules show a granulomatous inflammation comprising mixed populations of inflammatory cells including eosinophils, neutrophils, macrophages, lymphocytes, fibrocytes, multinucleated giant cells and epithelioid

cells. The inflammation surrounds a core of abnormally staining collagen fibre bundles with degranulated degenerating eosinophils. Chronic lesions may show calcifications (Slovis *et al*., 1999; Jubb *et al*., 2005; Meierhenry, 2006; Scott and Miller, 2011; Yu, 2015).

A tentative diagnosis can be based on history and clinical presentation, but a confirmatory diagnosis requires histopathology showing some of the characteristic pathology (Slovis *et al*., 1999; Jubb *et al*., 2005; Meierhenry, 2006; Scott and Miller, 2011; Yu, 2015).

Treatment of a single nodule can be done by surgical excision; however multiple nodules require steroidal anti-inflammatory drug therapy that has its own side effects. Management of the condition should also include identification and removal of possible causes through ectoparasite control or allergen removal (Meierhenry, 2006; Yu, 2015).

2.8.1.2 Infectious nodules

Infectious nodules may be caused by fungi, parasites or bacteria which may be zoonotic.

2.8.1.2.1 Fungal nodules

Fungal infections that form cutaneous nodules in horses include Mycetomas, Phaeohyphomycoses, Rhinosporidiosis, Pythiosis, Sporotrichosis, Zygomycoses, Histoplasmosis and Blastomycosis. The organisms usually gain entry into the skin

through an open wound. The infections can affect any age and sex of horse and usually involve distal parts of the limbs, head and neck but any part of the body can be affected (Miller and Campbell, 1984; Chaffin *et al*., 1995; Keegan *et al*., 1995; López-Sanromán J. *et al*., 2000; Leeming *et al*., 2007; Cafarchia *et al*., 2013; Sellon and Long, 2013; Seyedmousavi *et al*., 2013; Yu, 2015). Table 2 summarises the causes, pathology, diagnosis and treatment of these fungal infections in horses.

These fungal infections form granulomatous to pyogranulomatous nodules on the skin and subcutaneous tissues. Histopathology shows a granulomatous inflammation composed of macrophages, lymphocytes and other mononuclear cells along with fibroplasia that may surround fungal elements. Diagnosis usually involves cytology, histopathology, culture or a combination of the three. Treatment ranges from surgical excision of single nodules to systemic anti-fungal administration in cases of multiple nodules (Keegan *et al*., 1995; Cafarchia *et al*., 2013; Sellon and Long, 2013; Seyedmousavi *et al*., 2013; Yu, 2015).

Table 2: Summary of disease condition, organisms involved, pathology, diagnosis and treatment of some fungal infections forming cutaneous nodules in the horse. Adapted from Cafarchia *et al*. (2013).

DISEASE CONDITION	ORGANISM(S)	PATHOLOGY	DIAGNOSIS	TREATMENT
Mycetoma	*Aspergillus versicolor* *Curvularia verruculosa* *Madurella mycetomatis* *Pseusoallescheria boydii*	Subcutaneous masses often having a sinus tract with exudate containing black or white grains	Cytology on fine-needle aspirate from mass Culture of minced lesion Histopathology	Miconazole Thiabendazole Sodium or potassium iodide Surgical excision
Phaeohyphomycoses	*Alternaria alternata* *Bipolaris* spp. *Drechslera spicifera*	Black alopecic skin lesions 1-10 cm in diameter with small pustules or nodules that ulcerate and have fistulating tracts	PCR on fine-needle aspirate from mass or exudate Culture of minced lesion Histopathology	Fluconazole Potassium iodide Surgical excision
Rhinosporidiosis	*Rhinosporidium seeberi*	Single or multiple, polyp-like soft pink growth with a lobular roughened surface attached at a base or pedunculated	Histopathology	Surgical excision
Pythiosis	*Pythium insidiosum*	Single or multiple cutaneous or subcutaneous, tumour-like nodular masses with draining tracts and discharge with “kunkers”	PCR Culture of minced lesion Histopathology Serology	Co-administration of antifungal and antibiotic agents Surgical excision
Sporotrichosis	*Sporothrix schenckii*	Small, firm, red nodules that are not painful or pruritic which are dermal or subcutaneous measuring 1-5 cm in diameter that exude thin seropurulent fluid	Cytology on fine-needle aspirate or exudate Culture on fine-needle aspirate or exudate Histopathology	Systemic iodine therapy Griseofulvin

Table 2 continued

INFECTION	ORGANISM(S)	PATHOLOGY	DIAGNOSIS	TREATMENT
Zygomycosis	*Absidia corymbifera* *Basidiobolus ranarum* *Conidiobolus coronatus* *C. incongruous* *C.lamprauges* *Mucor* spp. *Rhizopus* spp. *Saksenaea* spp.	Ulcerated, extensive and pruritic granulomatous lesions	Culture Histopathology	Amphotericin B
Histoplasmosis	*Histoplasma capsulatum* var. *farciminosum*	Granulomatous wounds which tend to ulcerate	Cytology Culture Histopathology	Sodium iodide Amphotericin B
Blastomycosis	*Blastomyces dermatitidis*	Exudative cutaneous lesions	Cytology Histopathology Fungal culture	Surgical excision Fluconazole

Key:

spp. – Species

cm – centimetres

PCR – Polymerase chain reaction

2.8.1.2.2 Parasitic nodules

Cutaneous nodules caused by parasites include tick-bite granulomas, hypodermiasis, halicephalobiasis, parafilariasis and habronemiasis. Cutaneous habronemiasis is the most common of the parasitic conditions causing cutaneous nodules. Other parasitic conditions of concern are Leishmaniosis and Besnoitiosis (Koehler *et al*., 2002; Dubey *et al*., 2005; Onmaz *et al*., 2013; Pugh *et al*., 2014; Yu, 2015).

2.8.1.2.2.1 Cutaneous habronemiasis

Cutaneous habronemiasis, commonly referred to as summer sores, is associated with the larvae of the nematode parasites *Habronema muscae*, *H. majus* and *Draschia megastoma*, resulting from their unusual deposition in open wounds by their intermediate hosts the stable fly, *Stomoxys calcitrans* and the house fly, *Musca domestica*. Since the larvae are in an aberrant location, they die and provoke a granulomatous reaction. Predilection sites include limbs, ocular and periocular areas, commissure of the lips, prepuce, external genitalia and ventral abdomen (Drouin, 1986; Rebhun, 1996; Yarmut *et al*., 2008; Schuster *et al*., 2010; Onmaz *et al*., 2013; Pugh *et al*., 2014; Yu, 2015).

Clinically, cutaneous habronemiasis presents as pruritic proliferative granulation tissue which occasionally ulcerate. The lesions contain gritty plaques that are yellow to white in colour within a serosanguineous exudate (Rebhun, 1996; Pugh *et al*., 2014).

Histological analysis of cutaneous habronemiasis granulomas shows a core made up of degenerated larva surrounded by necrotic zones and an inflammatory reaction composed of a mixture of numerous eosinophils, few neutrophils and mononuclear cells along with granulation tissue. The overlying epidermis may become hyperplastic and hyperkeratotic with ulceration or become eroded with presence of numerous bacterial colonies signifying secondary bacterial infection (Rebhun, 1996; Yarmut *et al*., 2008; Pugh *et al*., 2014; Yu, 2015).

A tentative diagnosis can be made from history and clinical presentation; however, there are several other conditions that present similarly including equine sarcoids, squamous cell carcinoma, phycomycosis and onchocerciasis. A confirmatory diagnosis can be arrived upon using cytology, histopathology and semi-nested PCR (Traversa *et al*., 2004; Traversa *et al*., 2007; Yarmut *et al*., 2008; Pugh *et al*., 2014; Yu, 2015).

Treatment of cutaneous habronemiasis requires administration of anti-inflammatory drugs, anthelmintic drugs and antimicrobial drugs. If the nodules persist after chemotherapy, surgical excision is preferred. Prevention of the condition targets the intermediate host by use of blankets, insect-proof housing and topical application of insect repellant or insecticide (Drouin, 1986; Yarmut *et al*., 2008; Pugh *et al*., 2014; Yu, 2015).

2.8.1.2.2.2 Cutaneous Onchocerciasis

Cutaneous Onchocerciasis is caused by the microfilariae of the filarial nematode *Onchocerca cervicalis* and is thought to be a hypersensitivity reaction to the

presence of the larvae in the skin. The lesions are usually crusty, alopecic and pruritic; and are commonly found on the ventral abdomen, thorax, withers and face; and mainly occurs in adult horses. Nodules are not seen, but the lesion appears like a "bull's eye" as a result of epidermal acanthosis and hyperkeratosis with a central area of ulceration and necrosis. Within the dermal core of the lesion, microfilariae of *Onchocerca cervicalis* may be present surrounded by a mixed cellular inflammation comprising eosinophils, neutrophils and mononuclear cells. Thus the lesion can be classified as dermatitis rather than a granulomatous reaction. Diagnosis of Onchocerciasis can be made tentatively based on history and clinical presentation, and confirmed using histopathology and parastilogical techniques (Lees *et al*., 1983; Drouin, 1986; Onmaz *et al*., 2013; Yu, 2015).

2.8.1.2.2.3 Cutaneous leishmaniosis

Cutaneous leishmaniosis in equids has commonly been reported from South America, especially from Argentina, Venezuela and Brazil. The organism implicated was *Leishmania braziliensis*. However, Koehler *et al*. (2002) identified *L. infantum* in a case from a horse in southern Germany. The organism is transmitted by sandflies and produces a cutaneous nodule, measuring 1-5 cm in diameter, with ulcerations of the overlying skin. Histologically, a multifocal to diffuse granulomatous inflammatory reaction is seen comprising macrophages and lymphocytes with scattered epitheloid cells and multinucleated giant cells that contain numerous amastigotes. A tentative diagnosis can be based on history and

clinical presentation and confirmed using histopathology, electron microscopy, immunohistochemistry or PCR. Spontaneous resolution has been reported but anti-protozoal therapy can be used (Ramos-Vara *et al*., 1996; Koehler *et al*., 2002).

2.8.1.2.2.4 Cutaneous besnoitiosis

There have been limited reports of Besnoitiosis in horses and several from donkeys. The cause is the protozoan parasite *Besnoitia bennetti* which is transmitted mechanically by biting flies. Upon entry into circulation, the sporozoites of the parasite invade the endothelial cells of blood vessels of superficial tissues including the skin, where they undergo endodyogeny to form tachyzoites which are released from the cell and infect other endothelial cells and fibroblasts. Here, they produce large cysts that contain bradyzoites that lead to formation of a rough, thickened skin with scabs and hair loss. In other cases, small hairless nodules measuring up to 1 cm in diameter. Microscopically, the epidermis shows hyperkeratosis, parakeratosis, comedones, acanthosis and acantholysis. A multifocal inflammatory reaction comprising neutrophils and lymphocytes are seen in the epidermis and dermis and around blood vessels. Cysts can also be seen within the sections, either empty or filled with parasites (Terrell and Stookey, 1973; Dubey *et al*., 2005).

2.8.1.2.2.5 Parafilariasis

Parafilariasis is another filarial nematode-induced cutaneous disease of horses caused by *Parafilaria multipapillosa* which is transmitted by the stable fly, *Haematobia atripalpis*. The adults reside in subcutaneous nodules that fistulate to the skin through which bloody exudate is discharged, giving rise to the other names of the condition, namely “bloody sweat” or “summer bleeding”. The adults have a predilection for the trunk, shoulders and neck; where the lesions appear (Onmaz *et al*., 2013).

2.8.1.2.3 Viral nodules

Viral infections can lead to development of cutaneous nodules such as in papillomatosis and Uasin Gishu Disease (Horsepox). A granulomatous dermatitis caused by Equine Herpesvirus-2 (EHV-2) has been reported by Sledge *et al*. (2006).

2.8.1.2.3.1 Papillomatosis

Papillomas, or warts, are white to grey, firm, raised, cauliflower-like lesions caused by the Equine Papilloma Virus; and can measure up to 10 mm in diameter but may coalesce to form larger growths. They are common in horses up to 3 years of age occurring on the muzzle, neck and ears; commonly resolving within 1 to 9 months. Warts are harmless in almost all situations, except where pain is involved. They are spread by direct contact from animal to animal; and can also be spread when contaminated equipment, such as halters, are not cleaned properly

between animals (Cook and Olson, 1951; Farewell, 1986; Fairley and Haines, 1992; Hamada *et al*., 1992; Postey *et al*., 2007; Munday and Kiupel, 2010).

Microscopically, there is epidermal hyperplasia with elongation of rete pegs into the dermis, some cells of the stratum corneum may retain their nuclei, cells of the stratum spinosum show ballooning degeneration with basophilic intracytoplasmic inclusion bodies. The epidermis also shows hyperkeratosis and parakeratosis; while the epidermo-dermal junction may show inflammatory foci composed of polymorphonuclear cells, lymphocytes and histiocytes (Cook and Olson, 1951; Hamada *et al*., 1992; Meuten, 2002; Jubb *et al*., 2005).

Diagnosis is usually confirmed at histopathology, but confirmation of the aetiological agent may require immunohistochemistry or PCR. Small warts can be removed surgically, crushing, pinching off, and freezing (cryosurgery). Since the condition is spread through direct contact or through fomites, affected animals need to be isolated and all instruments and equipment cleaned and disinfected before being used on healthy horses (Cook and Olson, 1951; Farewell, 1986; Fairley and Haines, 1992; Hamada *et al*., 1992; Meuten, 2002; Jubb *et al*., 2005; Postey *et al*., 2007; Munday and Kiupel, 2010).

2.8.1.2.3.2 Poxviral lesions

Poxviral lesions in horses in Kenya, referred to as Uasin Gishu disease, is caused by a poorly described Orthopoxvirus first discovered by Kaminjolo *et al*. (1974a, b); and is thought to be related to the Horsepox Virus. Little work has been done on the disease and there are many aspects that are still not understood. However, it

is known that clinically, Uasin Gishu disease is characterised by the formation of generalised papillomatous lesions all over the body but are commonly found on the face, neck, back, flank and rump; and it tends to be a chronic condition. Lesions begin as haired nodules measuring up to 20 mm in diameter which are covered by white powdery scabs. The scabs detach leaving behind bleeding alopecic lesions that reveal a papilloma (Fenner *et al*., 1989; Fenner, 2000; Tulman *et al*., 2006; Mair and Scott, 2009; Mário *et al*., 2010).

Microscopically, the lesions show swelling and hyperplasia of the epidermis with cells of the stratum spinosum being the most affected showing ballooning degeneration and cytoplasmic inclusion bodies within which pox viral particles can be seen using an electron microscope (Kaminjolo and Winquist, 1975).

Diagnosis is confirmed through histopathology using light and electron microscopy. An effective treatment option is not available and lesions tend to resolve and recur spontaneously (Fenner *et al*., 1989; Mair and Scott, 2009; Mário *et al*., 2010).

2.8.1.2.4 Bacterial Nodules

Cutaneous bacterial infections in horses leading to nodular dermatoses include actinomycosis, nocardiosis, staphylococcosis, tuberculosis, glanders and *Corynebacterium pseudotuberculosis* infections. They usually form papules and cause folliculitis leading to alopecia.

C. pseudotuberculosis infections form single or multiple pyogranulomatous nodules in the pectoral region, face, neck, axilla, groin and limbs; that can

fistulate and progress to cellulitis. The bacterial agent may be transmitted by insect vectors such as stable flies, horn flies and houseflies. With time, the nodules rupture and ooze a purulent exudate. Single lesions can be hot fomented to induce maturity and rupture after which an antiseptic solution can be used for cleaning, while systemic antibiotics should be used for multiple lesions (Yu, 2015).

2.8.1.3 Pemphigus foliaceus

Pemphigus foliaceus is the most common autoimmune skin disease of the horse, which is directed against the desmosomes in the stratified squamous epithelium leading to changes such as loss of intracellular cohesion, acantholysis and blister formation within the epidermis. These lesions are initially seen on the face, belly, or limbs and can progress to involve the entire body. The primary lesions of pemphigus foliaceus are fragile vesicles, bullae and pustules, but most horses develop secondary lesions such as erosions, epidermal collarettes (a circular lesion with a circular rim of scales), crusts, alopecia, exudation and scaling. Histopathology reveals epidermal acantholysis and pustules within the epidermis which appear as lesions with a thin fibrous capsule containing numerous neutrophils and dead cells with or without bacteria, while the dermis shows a lymphocytic inflammatory reaction (Scott, 1989; von Tscharner *et al*., 2000; Zabel *et al*., 2005; Scott and Miller, 2011).

A definitive diagnosis can be made by a combination of tests including direct smears, skin biopsies, immunofluorescence or immunohistochemical tests, and by ruling out differential diagnoses such as dermatophytosis. These horses require high doses of

steroids to suppress the immune system in order to manage the condition (Scott, 1989; von Tscharner *et al*., 2000; Zabel *et al*., 2005; Scott and Miller, 2011).

2.8.2 Neoplastic Skin Conditions

The skin of the horse is the most common site for neoplasms in horses of all breeds and ages. However, the frequency of neoplasia in horses is relatively low compared to other domestic animals. Neoplasms in horses tend to be locally invasive and slow to metastasize. There are three common types of nodular skin neoplasms: sarcoids, melanomas, and squamous cell carcinomas. Many other types of neoplasms can also occur but are not as common. The causes of neoplasms vary based on the type; but generally, radiation, viral infections, trauma and inflammation are the common causes. Early recognition, accurate diagnosis, and early treatment are crucial to obtaining relatively good success rates. Delayed recognition and treatment increase the chances of metastasis or recurrence after treatment (Farewell, 1986; Foy *et al*., 2002; Meuten, 2002; Jubb *et al*., 2005; Meierhenry, 2008; Gomes, 2011). The incidence of cutaneous neoplasms in equine is higher than that of non-neoplastic conditions with regard to abnormal cutaneous swellings and growths (Valentine, 2006).

2.8.2.1 Equine Sarcoid

Equine sarcoids are fibroblastic neoplasms occurring in horses, donkeys and mules. They occur at sites of previous injury or scarring, predominantly on the head, ventrum, limbs, and around the genitalia. They appear as locally invasive sarcomas

and tend to recur after surgical excision (Knottenbelt and Matthews, 2001; Meuten, 2002; Jubb *et al*., 2005; Corteggio *et al*., 2012).

The cause of equine sarcoids is not exactly known, but it has long been suggested that the cause is the viral agent, Bovine Papillomavirus-1 and 2 (BPV-1 and 2), in combination with trauma and genetics (Amtmann *et al*., 1980; Knottenbelt and Matthews, 2001; Chambers *et al*., 2003; Borzacchiello *et al*., 2008).

Equine sarcoids are often classified in six different forms; occult sarcoid, verrucous sarcoid, nodular sarcoid, fibroblastic sarcoid, mixed sarcoid and malevolent form. Occult sarcoids produce alopecic scaly lesions that are limited to the superficial epidermal layers; usually occurring in the medial thigh, sheath, neck and face; and can be confused with dermatophytosis and lice infestation (pediculosis). Verrucous sarcoids appear as small or large, wart-like lesions having an irregular margin and a rough surface; usually occurring in the axillary or groin regions. Differential diagnoses of verrucous sarcoids include papillomatosis, hyperkeratosis from continuous irritation, equine sarcoidoisis, equine molluscum contagiosum, Horsepox and squamous cell carcinoma. Nodular sarcoids usually occur subcutaneously under grossly normal intact skin having a spherical shape and a fairly smooth surface; usually occurring in the groin or in the eyelid margins. Nodular sarcoids may ulcerate and progress to the fibroblastic form. Differential diagnoses of nodular sarcoids include fibroma, neurofibroma, melanoma, hypodermiasis, dermoid cyst, and allergic collagen necrosis. Fibroblastic sarcoids are the most aggressive type of sarcoids resembling true neoplasms. Fibroblastic sarcoids are large lobulated masses with irregular margins and ulcerated surfaces and may or may not be pedunculated.

Differential diagnoses of fibroblastic sarcoids include exuberant granulation tissue, cutaneous botryomycosis, fibrosarcoma, neurofibrosarcoma and squamous cell carcinoma. Mixed sarcoids are composed of two or more types of sarcoids usually found in the axillary and groin region. The malevolent form of sarcoid usually invades local lymphatics and may spread to regional lymph nodes (Knottenbelt *et al*., 1995; Knottenbelt and Matthews, 2001; Meuten, 2002; Jubb *et al*., 2005; Gomes, 2011; Corteggio *et al*., 2012).

A tentative diagnosis can often be made based on the appearance of the lesions, while a confirmatory diagnosis is based in histopathology of a biopsy after partial or complete surgical removal. However, care must be taken when performing partial surgical removal since verrucous sarcoids can change into more aggressive fibroblastic neoplasms when they are injured or traumatized. Immunohistochemistry using several antibodies especially those against p53 are essential to identifying mutated fibroblasts present in equine sarcoids. It has also been suggested by various authors that detection of BPV DNA in suspect lesions may also be a confirmatory test (Knottenbelt *et al*., 1995; Knottenbelt and Matthews, 2001; Meuten, 2002; Jubb *et al*., 2005; Gomes, 2011; Farouk, 2014).

Microscopically, sarcoids typically present as diphasic lesions having pathology in the epidermis and dermis. Within the dermis, bundles of fibroblasts are arranged in whorls, tangled bundles or herringbone patterns; with a variable amount of collagen. The fibroblasts are oval to spindle-shaped, with elongated nuclei and are well differentiated thus showing a low level of anaplasia. The rate of mitosis varies with higher rates in aggressively growing tumours or in areas where ulcerations are

present. The cells are locally infiltrating and hence the lesion is not well demarcated microscopically. The epidermis usually shows hyperkeratosis and hyperplasia and forms numerous elongated rete pegs in to the dermis, however, in some cases it may be thin or ulcerated (Meuten, 2002; Hallamaa *et al*., 2005; Jubb *et al*., 2005; Gomes, 2011; Corteggio *et al*., 2012; Farouk, 2014).

Treatment of sarcoids can be difficult due to the recurrence of the neoplasm. A successful treatment plan should incorporate a combination of two or more types of therapy and regular checks for recurrence. Various modes of treatment used by various authors with varied success rates include complete surgical excision, cryosurgery, laser surgery, radiofrequency-induced hyperthermia, immunotherapy, topical application of immune modulators, topical application of cytotoxic drugs, anti-neoplastic drug administration either locally or systemically, electrochemotherapy, photodynamic therapy, and radiotherapy (Knottenbelt *et al*., 1995; Martens *et al*., 2000; Martens *et al*., 2001; Hallamaa *et al*., 2005; Cemazar *et al*., 2008; Gomes, 2011).

Several studies have placed the occurrence of equine sarcoid at 35-90% of all dermatological neoplasms (Goodrich *et al*., 1998; Foy *et al*., 2002; Knottenbelt, 2009; Scott and Miller, 2011).

2.8.2.2 Melanoma and Melanosarcoma

Melanomas are most common in grey horses, with the incidence increasing past the age of 6 years, whereby 80% of grey horses over the age of 15 years will suffer from a melanoma. Melanomas result from over-active melanoblasts and are firm, round,

grey to dark, neoplasms that can occur individually or most commonly in multiple dark masses. These neoplasms can occur anywhere on the body, but are more common around the tail base, anus, vulva, limbs, penis, prepuce, udder, eyelids, and head. Melanomas are slow growing and do not metastasize, but if they occur in coloured horses such as bays and chestnuts, they are much more likely to be malignant. Melanosarcomas, on the other hand, are fast growing, malignant, and can spread to the lymph nodes, liver, lungs, and spleen; however, they rarely occur in horses (Meuten, 2002; Smith *et al*., 2002; Jubb *et al*., 2005; Rizk, 2012; Javanbakht *et al*., 2014).

The exact aetiology for melanoma is not known but several authors have made several theories on the aetiopathogenesis. One theory suggests that the initial cause is the altered metabolism of melanin in greying horses whereby melanin granules fail to incorporate into the hair follicles leading to hypertrophy and hyperplasia of melanoblasts. This eventually leads to hyperactivity and increased production of melanin leading to malignancy (Scott and Miller, 2011). Fleury *et al*. (2000) suggested that the tumours are associated with hairless, thin skin near mucocutaneous junctions. A study by Seltenhammer *et al*. (2010) suggested a heritable genetic cause.

Valentine (1995) identified four different patterns for melanoma; namely, melanocytic nevi which is benign usually occurring on the legs or trunk and involves the superficial dermis with or without the derma-epidermal junction; dermal melanoma which are solitary dermal nodular lesions with occasional metastasis; dermal melonomatosis which occur as infiltrative plaques with or without nodules

within the deep dermis which often metastasize; and anaplastic malignant melanoma which are large, locally invasive, poorly pigmented lesions which metastasize quickly.

A melanoma can be easily identified at histopathology; they are asymmetrical and poorly circumscribed lesions composed of neoplastic melanocytes which are spindloid to stellate in shape with large, round to ovoid euchromatic nuclei and distinct nucleoli. The cells infiltrate locally by superimposing the epidermis or may be found within lymphovascular spaces. Associated with the neoplastic melanocytes are phagocytic mononuclear cells such as epitheloid cells referred to as melanophages which contain numerous engulfed melanin granules. Mitotic figures are variably seen in amelanotic growths or in bleached biopsies (MacGillivray *et al*., 2002; Meuten, 2002; Smith *et al*., 2002; Seltenhammer *et al*., 2004; Jubb *et al*., 2005; Javanbakht *et al*., 2014).

Melanomas that are found in older animals are often left untreated unless they interfere with normal body functions (urination, defecation). Neoplasms that are judged to be aggressive or are causing complications are removed by surgical excision, cryotherapy, radiofrequency ablation, radiotherapy or electrochemotherapy ensuring that the entire neoplasm is removed. Prolonged administration of high doses of the H_2-receptor antagonist, Cimetidine, may be required to prevent occurrence of additional neoplasms and to limit the growth of pre-existing neoplasms (Spugnini *et al*., 2011; Rizk, 2012).

Valentine (2006) reported that cutaneous and mucocutaneous melanomas are the second most commonly reported skin neoplasm of horses, accounting for up to 18%

of all cutaneous neoplasms. Fleury *et al*. (2000) reported that in a study of 83 grey-skinned horses, 93.9% of cutaneous melanomas occurred around the tail base while a rare 3.8% occurred in the vulva. MacGillivray *et al*. (2002) reported that around 14% of cutaneous melanomas can metastasize to other organs such as regional lymph nodes, lungs, spleen, liver, blood vessels, serosal surfaces, skeletal muscles, salivary glands, bone or bone marrow, adrenal glands, kidneys, diaphragm, heart, guttural pouches, mammary gland, uterus, pancreas, larynx and pharynx.

2.8.2.3 Squamous Cell Carcinoma

Squamous cell carcinoma (SCC) is one of the most commonly diagnosed skin neoplasm of horses, accounting for 18-30% of all skin and mucocutaneous neoplasms in adult to geriatric horses of any breed, especially breeds with non-pigmented skin such as Paint horses and Appaloosas (Bastianello, 1983; Farewell, 1986; Valentine, 2006; Meierhenry, 2008; Scott and Miller, 2011). Squamous cell carcinomas are commonly found at the mucocutaneous junctions on the skin, ocular and peri-ocular areas, and the external genitalia; other locations include the face, ear pinna, perianal area and extremities (Meuten, 2002; Jubb *et al*., 2005; Bukar *et al*., 2007; Taylor and Haldorson, 2013).

Aetiological risk factors include chronic ultraviolet radiation exposure and chronic keratosis. Vanderstraeten *et al*. (2011), Kainzbauer *et al*. (2012) and Knight *et al*. (2012) have reported identification of *Equus caballus* Papillomavirus 2 (EcPV-2) in samples of various types of SCC in the horse suggesting a role played by the virus in development of the neoplasm.

Clinical presentation varies depending on the stage of the neoplasm whereby they may appear as a small sore or a red raised bump or a granulomatous cauliflower-like growth. The SCCs may develop from existing papillomas or independently depending on the actual cause. Histopathology shows islands and cords of neoplastic epithelial cells at varying stages of squamous differentiation infiltrating the dermis from the overlying epidermis frequently forming the characteristic "keratin pearls" in the centre of the increasingly prominent epidermal rete. The epidermis also shows hyperplasia, hyperkeratosis, parakeratosis and acanthosis. The individual neoplastic cells are large and ovoid having a vesicular nucleus and a prominent nucleolus and abundant cytoplasm that varies in staining from pale to eosinophilic; with distinct cell borders and variable number of mitotic figures (Meuten, 2002; Jubb *et al*., 2005; Bukar *et al*., 2007; Taylor and Haldorson, 2013).

A tentative diagnosis of SCC can be made based on clinical presentation and confirmed by histological analysis of skin biopsies obtained from complete surgical excision of suspected masses. Other confirmatory diagnostic methods include cytology on fine-needle aspirates, cytology on superficial scrapings and impression smear examination. Differential diagnoses of SCC include sarcoids, papilloma, melanoma, mast cell tumour, habronemiasis, cutaneous melanoma, proud flesh and pythiosis or other phycomycoses (Foy *et al*., 2002; Meuten, 2002; Jubb *et al*., 2005; Taylor and Haldorson, 2013).

When a SCC is identified and treated early, a higher success rate is achieved when compared to treatment done on late stage SCC. Treatment options like surgical excision, cryosurgery, hyperthermia, radiotherapy, chemotherapy and photodynamic

therapy have been used and have varied success rates (Farewell, 1986; King *et al*., 1991; Chahory *et al*., 2002; Foy *et al*., 2002; Bukar *et al*., 2007; Ota *et al*., 2008; Barnes *et al*., 2009; Hazen *et al*., 2009; Taylor and Haldorson, 2013).

Squamous cell carcinoma is the most common neoplasm of the equine eye and ocular adnexa and the second most common neoplasm of the horse overall (Meuten, 2002; Valentine, 2006). According to MacFadden and Pace (1991), SCCs account for 20% of the cutaneous neoplasms seen in horses. They may occur in sun-exposed areas such as the nares or eyelids, or in sun-exposed mucocutaneous junctions. They may also occur in the male or female genitalia, accounting for 45% of the neoplasms seen in the male genitalia and 12% in the female perineum. Squamous cell carcinomas are more common on non-pigmented skin areas and are seen primarily in light-skinned horses, and all breeds may be affected. King *et al*. (1991) reported that the most frequent site for ocular involvement of SCC is the nictitating membrane and conjunctiva. According to Chahory *et al*. (2002), the cornea, sclera, and the eyelids may also be involved with multiple and bilateral lesions occurring infrequently.

2.8.2.4 Lymphoma

Lymphomas originate from organs of the lymphoid system such as lymph nodes, spleen, thymus and other lymphoid tissues within other body systems. They are the most common haematopoietic neoplasm in horses, though this category of neoplasms are rare in horses (Rebhun and Del Piero, 1998; Meuten, 2002; Jubb *et al*., 2005; Meyer *et al*., 2006; Meierhenry, 2008). They are usually malignant in horses giving rise to lymphosarcoma and four different forms have been described which include

the generalised or multicentric lymphoma where nodular neoplasms occur in various organs and systems at the same time; intestinal lymphoma where neoplasms occur within the intestinal wall of the small intestines; mediastinal lymphoma where neoplasms occur in the mediastinum with or without involvement of the mediastinal lymph nodes; and cutaneous lymphoma where neoplasms occur in the skin or subcutaneously in superficial regional lymph nodes (Meuten, 2002; Jubb *et al*., 2005; Meierhenry, 2008; Sugiyama *et al*., 2008; Taintor and Schleis, 2011).

The cause of lymphoma in horses is not known but several authors have suggested a range of causes including a retrovirus, herpesvirus and infection by *Corynebacterium* spp. (McKercher *et al*., 1963; Marayama *et al*., 1970; Tomlinson *et al*., 1979; Sheahan *et al*., 1980; Detilleux *et al*., 1989). There are no age, breed or sex predilections for lymphoma in horses; though studies relating to this are limited (Meyer *et al*., 2006). Cutaneous lymphoma is more commonly diagnosed than other forms since they are more readily observed clinically compared to the internal forms which are usually incidental findings during exploratory laparotomy or necropsy. Lesions are commonly encountered on the head, limbs, trunk and perineum; but can occur anywhere on the body. Cutaneous lymphoma may be a manifestation of metastasis of internal forms of lymphoma and thus there is need for a thorough examination in order to prognosticate the condition accurately (de Bruijn *et al*., 2007; Meierhenry, 2008; Taintor and Schleis, 2011).

Cutaneous lymphomas can be divided into epitheliotropic and non-epitheliotropic cutaneous lymphoma. Epitheliotropic cutaneous lymphoma (ECL), also referred to as mycosis fungoides, is characterised by a generalised scaling of the skin forming

patches and plaques with or without alopecia; showing infiltration of the epidermis and adnexal structures by pleiomorphic lymphoid cells of T-cell origin at histopathology and immunohistology. Non-epitheliotropic cutaneous lymphoma (NECL) is much more common than ECL and is characterised by the development of single to multiple subcutaneous nodules showing pleiomorphic neoplastic B-cells containing an irregular nucleus and small nucleolus and occasional mitotic figures along with numerous non-neoplastic t-cells at histopathology and immunohistology (Meyer *et al*., 2006; de Bruijn *et al*., 2007; Taintor and Schleis, 2011; Durham *et al*., 2012).

A tentative diagnosis of cutaneous lymphoma is based on clinical presentation and ancillary tests such as haematology, serum chemistry, ultrasonography and radiology. A confirmatory diagnosis requires cytology on fine-needle aspirates of nodular lesions or histopathology on excisional biopsies combined with immunohistochemistry. Differential diagnoses include urticaria, cutaneous hypodermiasis, cutaneous amyloidosis, cutaneous pseudolymphoma and eosinophilic granuloma (D'Agostino and Brightman, 2004; Meierhenry, 2008; Taintor and Schleis, 2011).

Treatment of cutaneous lymphoma should be considered if it is not accompanied by internal lymphomas, as the internal lymphomas have a poor prognosis. Treatment options include surgical excision of individual nodules, radiotherapy or chemotherapy (Meierhenry, 2008; Taintor and Schleis, 2011).

2.8.2.5 Mastocytosis/Mast cell tumour

Mast cell tumours in horses are less common than in dogs with few cases being reported in literature; and occur in horses of any age, breed and sex. They usually occur as single benign firm dermal or subcutaneous nodules on the head, neck, trunk and limbs with overlying skin that may be alopecic and ulcerated; but can occur on any cutaneous or mucocutaneous areas. A systemic form has been reported in horses which appeared and regressed spontaneously. Individual mast cell tumours have rarely been reported to metastasise. The cause of mast cell tumour in horses is not known (Cheville *et al*., 1972; Farewell, 1986; Wenger and Caron, 1988; Richardson *et al*., 1994; Reppas and Canfield, 1996; Cole *et al*., 2007; Millward *et al*., 2010; Yu, 2015).

Microscopically, mast cell tumours are composed of sheets or clumps of mast cells admixed with eosinophils along with degenerate collagen and foci of necrosis; the cellular infiltrates may be walled off by fibrous tissue in some instances (Cheville *et al*., 1972; Altera and Clark, 1970; Millward *et al*., 2010; Yu, 2015).

Diagnosis is usually confirmed by histopathology and immunohistochemistry to identify mast cells in section. Differential diagnoses include eosinophilic granulomas, cutaneous habronemiasis, cutaneous onchocerciasis and calcinosis circumscripta (Cheville *et al*., 1972; Cole *et al*., 2007; Millward *et al*., 2010; Yu, 2015).

Treatment options include complete surgical excision, radiotherapy, intralesional steroidal anti-inflammatory drug injections or systemic anti-histamines. Prognosis is

usually good since recurrence is rare in horses (Farewell, 1986; Cole *et al*., 2007; Yu, 2015).

2.8.2.6 Fibroma and Fibrosarcoma

Fibromas and fibrosarcomas are mesenchymal tumours of fibrous connective tissue; where fibromas are benign while fibrosarcomas are malignant. Fibromas are rare in horses while cutaneous fibromas are even rarer. Cutaneous fibromas usually occur in adult horses of any sex or breed; with a predilection for distal and lateral parts of the limbs, lateral part of the chest, prepuce, penis and solar surface or frog of the foot. Other locations where fibromas have been identified include the guttural pouch, orbit and periorbital areas, mandible, nasal cavity and paranasal sinuses and the abdomen. It has been reported that fibrosarcomas grow aggressively with quick infiltration, but metastasize in about 25% of the cases (Merriam, 1972; Farewell, 1986; Morse *et al*., 1988; Baker, 1999; Dixon and Head, 1999; Colitz *et al*., 2000; Meuten, 2002; Valentine, 2006).

Clinically, fibromas may appear as single firm wart-like or cauliflower-like cutaneous growths or as firm subcutaneous nodules; which on histopathology show bundles of proliferating, well-differentiated fibrocytes arranged in interlacing patterns embedded in a mucinous, vacuolated, basophilic stromal mass. The neoplastic cells usually have oval euchromatic nuclei and the cell borders are indistinct as they appear to blend with the surrounding stroma. The lesion itself is well demarcated and rarely encapsulated; however fibrosarcomas do show local infiltration into the surrounding tissue. There are two forms of fibromas: the soft

fibroma (fibroma molle) consisting of many loosely connected cells and less fibrous tissue, and the hard fibroma (fibroma durum) consisting of a lot of fibrous tissue and few cells (Farewell, 1986; Meuten, 2002; Jahromi *et al*., 2008).

Diagnosis is confirmed by histopathology on biopsies; however it can be confused with equine sarcoids. Other clinical differential diagnoses include squamous cell carcinoma, exuberant granulation tissue, melanomas and mastocytoma (Farewell, 1986; Meuten, 2002; Jahromi *et al*., 2008).

Treatment of fibromas is usually successful with complete surgical excision with rare cases of recurrence having been reported; other treatment options include radiotherapy and chemotherapy (Whitford, 1950; Farewell, 1986; Hewes and Green, 2007; Jahromi *et al*., 2008).

2.8.3 Miscellaneous Skin Conditions

Miscellaneous skin conditions are grouped as non-inflammatory non-neoplastic conditions that produce nodules or subcutaneous swellings which include exuberant granulation tissue and cysts (Hillyer *et al*., 2003; Wilmink and Van Weeren, 2004; Wilmink and Van Weeren, 2005; Muñoz *et al*., 2007).

2.8.3.1 Exuberant Granulation Tissue

Exuberant granulation tissue (EGT), commonly referred to as proud flesh, usually occurs on distal limb wounds. Granulation is a normal stage in wound healing, but in horses, there is a tendency for this stage to occur quickly and to be prolonged

especially on wounds that are in areas of high tension. Other contributing factors include poor perfusion, tissue hypoxia, chronic inflammation, wound infection, poor wound care, motion, bandages and casts, and the size of the horse. The excessive granulation tissue impedes wound healing and requires multiple excisions in order to promote wound healing (Bertone, 1989; Wilmink and Van Weeren, 2004; Wilmink and Van Weeren, 2005).

Clinically, exuberant granulation tissue appears as a cauliflower like tissue above the surface of the skin over an open wound. Microscopically, proud flesh appears as granulation tissue with macrophages and neutrophils embedded in a matrix of collagen produced by fibroblasts along with neocapillarization (Bertone, 1989; Wilmink and Van Weeren, 2004; Wilmink and Van Weeren, 2005).

Diagnosis can be made tentatively based on the clinical appearance and confirmed at histopathology. Clinical differential diagnoses include equine sarcoids, eosinophilic granuloma and squamous cell carcinoma (Bertone, 1989; Meuten, 2002; Wilmink and Van Weeren, 2004; Jubb *et al*., 2005; Wilmink and Van Weeren, 2005).

Treatment of EGT is usually successful with surgical excision and proper wound management, although several sessions of surgical excision may be required. Once excised, corticosteroid cream or ointment can be applied to the wound to reduce chances of recurrence. A preventive measure that could be employed would be the application of a silicone-based gel on wounds before bandaging or casting (Bertone, 1989; Wilmink and Van Weeren, 2004; Wilmink and Van Weeren, 2005).

2.8.3.2 Cutaneous Cysts

Cutaneous cysts in horses are rare and include epidermal cysts and dermoid cysts. Epidermal cysts also referred to as infundibular cysts or epidermal inclusion cysts; are usually acquired and often develop in the false nostril; usually seen in yearlings and adults. They are single or multiple, dermal or subcutaneous, circumscribed lesions measuring up to 1 cm in diameter which enlarge with time. Dermoid cysts are congenital abnormalities usually found in younger animals; appearing as single or multiple, raised, dermal or subcutaneous spherical nodules that usually occur at the base of the ear and in other areas such as the distal limbs, ventral thorax and the dorsum. A rare retrobulbar dermoid cyst has been reported (Weiss and Frese, 1974; Hillyer *et al*., 2003; Muñoz *et al*., 2007).

Microscopically, epidermal cysts are lined by a stratified squamous epithelium resembling the layers of the epidermis with loose packing of the cornified cells, along with hairs. The lumen contains highly eosinophilic laminated material resembling keratin, which on rare occasions can become infected or the cyst may transform into a squamous cell carcinoma. Dermoid cysts are lined by a stratified squamous epithelium resembling the epidermis with rete pegs along with adnexal structures such as hair follicles and sebaceous glands in the dermis that surrounds the cyst. The lumen contains keratin, sebaceous material, cholesterol crystals and coiled hairs. The dermoid cysts may communicate to the overlying epidermis by means of a tract. Diagnosis of epidermal and dermoid cysts is confirmed through histopathology. Treatment is usually successful by complete surgical excision (Weiss and Frese, 1974; Hillyer *et al*., 2003; Muñoz *et al*., 2007).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study site

The study was conducted in close consultation with the equine practitioners in Kenya, specifically those that are recognised by the Horse Association of Kenya and the Jockey Club of Kenya, in order to obtain the clinical background and the gross features of the cutaneous masses in the horse in the prospective study. Histopathology and immunohistochemistry of the cutaneous masses were conducted at the Department of Veterinary Pathology, Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Nairobi.

3.2 Study design

3.2.1 Sample size determination

Based on **Kothari (2004)**, the formula to determine the sample size from a finite population where the prevalence rate is known is as follows:

Sample size $$n = \frac{z^2 \cdot p \cdot q \cdot N}{e^2 (N-1) + z^2 \cdot p \cdot q}$$

Where: n = sample size

z = value of standard variate at confidence level

p = sample proportion (prevalence)

q = 1 – p

N = population size

e = confidence interval

A pilot study had been undertaken by the author (Shah, unpublished work) and the prevalence of cutaneous nodular lesions and swellings in horses was calculated to be 0.1 (p=0.1). The population size (N) was estimated at 4000. The confidence interval (e) was 0.05, thus z = 1.96 from the Normal Distribution

Thus, the sample size was:

$$n = \frac{1.96^2 * 0.1 * 0.9 * 4000}{0.05^2 (4000-1) + 1.96^2 * 0.1 * 0.9}$$

$$= 133.7081480433025$$

$$\approx 134$$

3.2.2 Retrospective study

The study involved a retrospective data collection from archive records at the Department of Veterinary Pathology, Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Nairobi from January 1st 1967 to December 31st 2011. A data clean-up exercise was performed to select relevant cases for the study. The paraffin-wax embedded tissue blocks of the identified cases were retrieved and sectioned afresh at 4-5 µm using a microtome (two slides per block), stained with haematoxylin and eosin, and examined using an Olympus® Biological Microscope Model CX22.

3.2.3 Prospective study

Tissue specimens of cutaneous nodular growths and other surface swellings from horses were processed, stained and examined histologically from January 1st 2012 to 31st December 2014. A close liaison with equine practitioners in Kenya was established to ensure that a thorough clinical record of the case and gross description of the sample was made and that a representative sample was obtained from each animal observed such that only a few nodules or swellings were sampled. The relevant cases were actively pursued and consent from the owner was obtained before a biopsy was excised surgically by the practitioner. The relevant data was entered in a data capture form (Appendix 1). All biopsies were photographed before excision (*in situ*). In most instances, the investigator participated in the surgical procedures for biopsy sampling. The practitioner sedated and anaesthetised the animal to allow for surgical excision. Once excised, the biopsy was cut to a preferred size of 1 cubic centimetre ensuring both pathological and normal tissues were involved. This was then fixed in 10% buffered formalin for a minimum period of 48 hours. Following fixation, the biopsy was trimmed to a thickness of 2-4 mm ensuring both pathological and normal tissues were included. These were then processed for histopathology using a modification to the method described by Carson and Hladik (2009). Briefly, this involved dehydration of tissues using graded alcohols in increasing concentrations, clearing of alcohol using xylene, infiltration with paraffin wax (Paraplast® plus) and then embedding in paraffin wax using paper boats or tissue-teks. Once the wax solidified, the paraffin-embedded tissue blocks were sectioned using a microtome to obtain tissue slices of 4 µm thickness. These were placed on clean glass slides which had been pre-treated with an adhesive (egg

albumin). The slides were kept upright in an oven at 36°C overnight to dry and melt the excess wax around the tissue and enhance the adhesion of the tissue to the glass slide. Sections were then deparaffinised using xylene, rehydrated using graded alcohols in decreasing concentration, and then washed and stained with haematoxylin and eosin.

3.2.4 Immunohistochemistry

Immunohistochemical staining was performed to confirm histological cases that showed close similarity in histological appearance making it difficult to derive the diagnosis at histology. Reagents for immunohistochemistry were obtained from Dako™, a global leader based in Denmark with over 40 years of experience in the field of immunohistochemistry. The company had local representation in Kenya under the name of Labulax Supplies Limited. The choice of antibody to be used was made at histology. Cases that had more than one probable diagnosis, or a challenging diagnosis where there was morphological similarity with another, were selected for immunohistochemistry. Antibodies compatible with antigens in lesions identified at histological diagnosis were selected for immunohistochemistry. For example, a case of an inflammatory nodule with extensive fibrous tissue reaction which can mimic an equine sarcoid (mesenchymal tumour) over-run with inflammation warranted the use of antibodies against Ki-67 (nuclear antigen in rapidly replicating cells) and vimentin (antigen in neoplastic cells of mesenchymal origin). On the other hand, cases of amelanotic melanomas which could be confused with other epithelial neoplasms

warranted the use of antibodies against Ki-67 (nuclear antigen in rapidly replicating cells) and S100 (antigen in melanocytes).

All commercial antibodies (Dako™) relevant to the study (Anti-Cytokeratin AE1/AE3, Anti-Vimentin, Anti-S100 and Anti-Ki-67) were based on human antigens and had cross-reactivity against horse antigens. All specific histological cases were sectioned from the paraffin-embedded tissue block as described earlier by the modification to the method described by Carson and Hladik (2009). The protocol for immunohistochemistry supplied by Dako (2009) has been summarized in Appendix 2. All antibodies chosen for the study followed the same procedure.

3.3 Data collection

3.3.1 Histopathology

Haematoxylin and eosin-stained tissues were examined using the Olympus® Biological Microscope Model CX22 at low magnification objective (4x and 10x) and then at higher magnification (40x) to identify the major histological lesions. Fifty (50) randomly chosen fields of view were examined at 40x objective to characterise the prominent histological changes. The frequency of the changes were recorded in a data capture form (Appendix 3) and evaluated in order to formulate the diagnosis.

3.3.2 Immunohistochemistry

The slides stained with the specific antibodies were examined for the presence of targeted antigen, its frequency, intensity and distribution. This was done at 40x objective in 50 randomly chosen fields. The extent of histological lesion was correlated with the occurrence, distribution and intensity of immunohistological features and recorded as described for histopathology.

3.4 Analysis of Data

The data collected was coded and input into Microsoft Office Excel 2013. Descriptive statistics was derived to obtain the most frequent type of lesion (modal type of lesion), the most frequently occurring diagnosis (modal diagnosis) and the part of the body most commonly affected (modal part of body). The results generated helped in identifying the most commonly encountered diagnosis of cutaneous lesions of the horse since 1967.

CHAPTER FOUR: RESULTS

4.1 General Results

A total of 141 samples comprising 100 retrospective records from cutaneous biopsies submitted directly to the Department or sampled during necropsy, and 41 prospective samples were analysed. At histopathology, both neoplastic and non-neoplastic lesions were diagnosed. Most of the cutaneous nodular lesions and surface swellings encountered from the horse in the period of study were neoplastic (64.5% - 91/141); with inflammatory nodules and swellings accounting for 31.9% (45/141) and miscellaneous conditions accounting for 3.5% (5/141) as shown in Figure 1 and Table 3. The most common diagnoses were squamous cell carcinoma (22.7% - 32/141), granulomas (14.9% - 21/141) and equine sarcoids (13.4% - 19/141) as summarised in Figure 2 and Table 4.

Majority of the neoplasms were squamous cell carcinoma (35.2% - 32/91), while sarcoids accounted for 20.9% (19/90) of the neoplasms as shown in Figure 3 and Table 5. Granulomas were the most common inflammatory lesions (46.7% - 21/45) as summarised in Figure 4 and Table 6. There were eleven cases which comprised biopsies from different areas with the same lesion or different lesions, while others had different lesions from the same area. Seven such cases had mixed types of lesions (a combination of neoplastic, inflammatory and miscellaneous lesions).

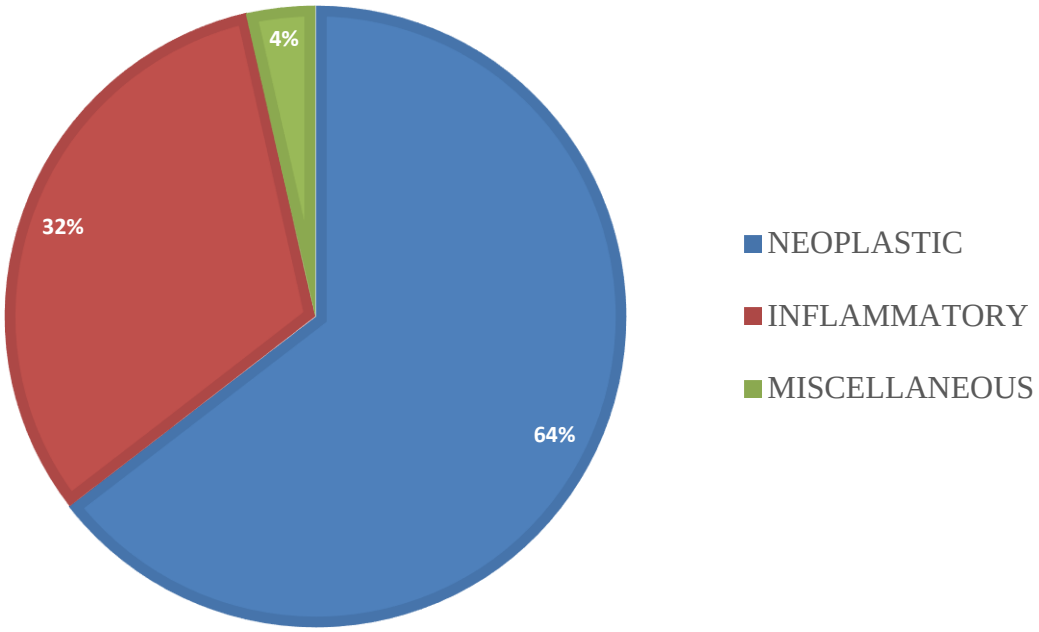

Figure 1: The distribution of the different types of cutaneous nodular lesions and surface swellings of horses in Kenya between 1967 and 2014

Table 3: The number of different types of cutaneous nodular lesions and surface swellings of horses in Kenya between 1967 and 2014

TYPE OF CUTANEOUS LESION	NUMBER OF CASES
NEOPLASTIC	91
INFLAMMATORY	45
MISCELLANEOUS	5
TOTAL	**141**

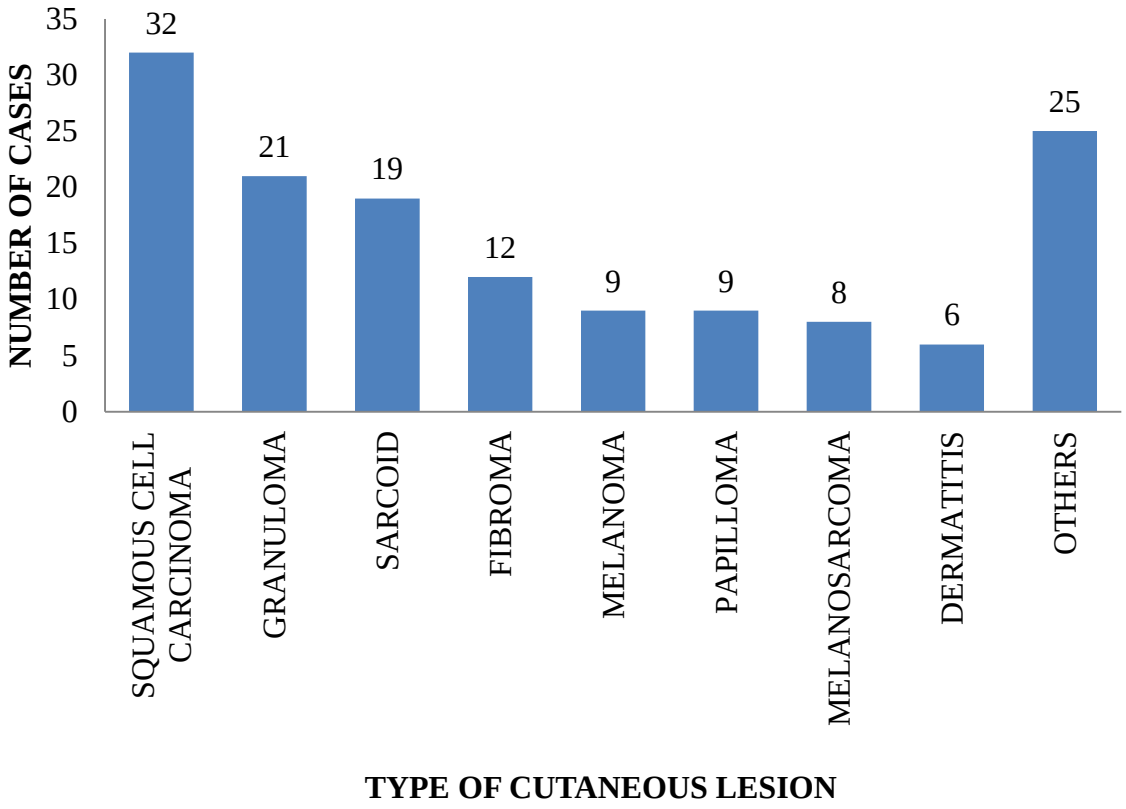

Figure 2: The distribution of the most common diagnosis of cutaneous nodular lesions and surface swellings of horses in Kenya between 1967 and 2014

Table 4: The number of cases for each diagnosis of cutaneous nodular lesions and surface swellings of horses in Kenya between 1967 and 2014

TYPE OF CUTANEOUS LESION	NUMBER OF CASES DIAGNOSED
SQUAMOUS CELL CARCINOMA	32
GRANULOMA	21
SARCOID	19
FIBROMA	12
MELANOMA	9
PAPILLOMA	9
MELANOSARCOMA	8
DERMATITIS	6
CONJUNCTIVITIS	4
ADENOMA	2
INFLAMMATORY NODULE	2
PARAKERATOSIS	2
ADENOCARCINOMA	1
ADENOMATOUS HYPERPLASIA	1
BASAL CELL TUMOUR	1
DERMOID CYST	1
EPIDERMOID CYST	1
EXUBERANT GRANULATION TISSUE	1
FIBROSARCOMA	1
HAEMANGIOSARCOMA	1
LYMPHANGITIS	1
LYMPHOID HYPERPLASIA	1
LYMPHOMA	1
LYMPHOSARCOMA	1
MALIGNANT SCHWANNOMA	1
ODONTOMA	1
OSTEOCHONDROMA	1
TOTAL	**141**

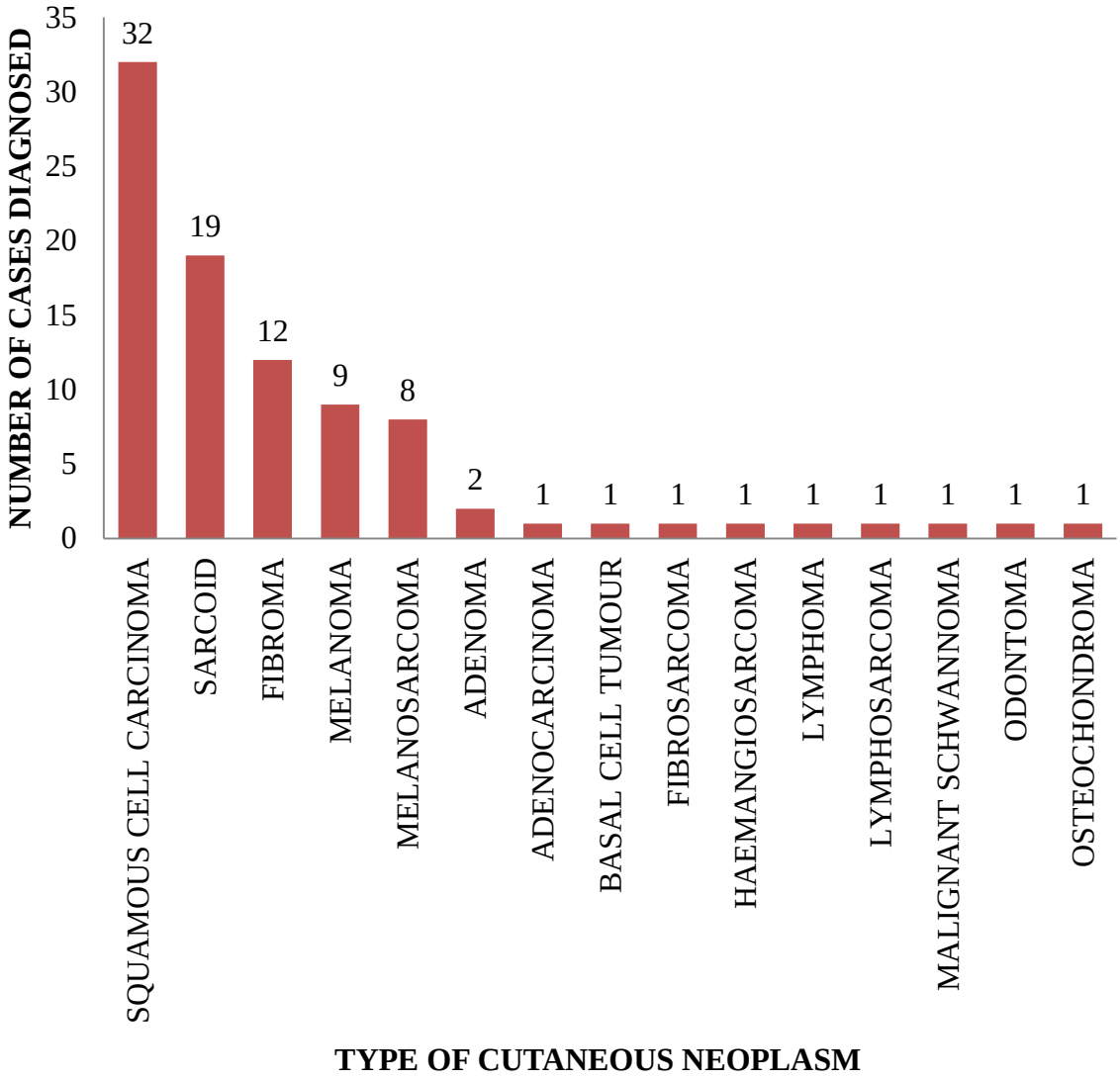

Figure 3: The distribution of the neoplastic cutaneous nodular lesions and surface swellings of horses in Kenya between 1967 and 2014

Table 5: The number of cases of different types of neoplastic cutaneous nodular lesions and surface swellings of horses in Kenya between 1967 and 2014

TYPE OF CUTANEOUS NEOPLASM	NUMBER OF CASES DIAGNOSED
SQUAMOUS CELL CARCINOMA	32
SARCOID	19
FIBROMA	12
MELANOMA	9
MELANOSARCOMA	8
ADENOMA	2
ADENOCARCINOMA	1
BASAL CELL TUMOUR	1
FIBROSARCOMA	1
HAEMANGIOSARCOMA	1
LYMPHOMA	1
LYMPHOSARCOMA	1
MALIGNANT SCHWANNOMA	1
ODONTOMA	1
OSTEOCHONDROMA	1
TOTAL	**91**

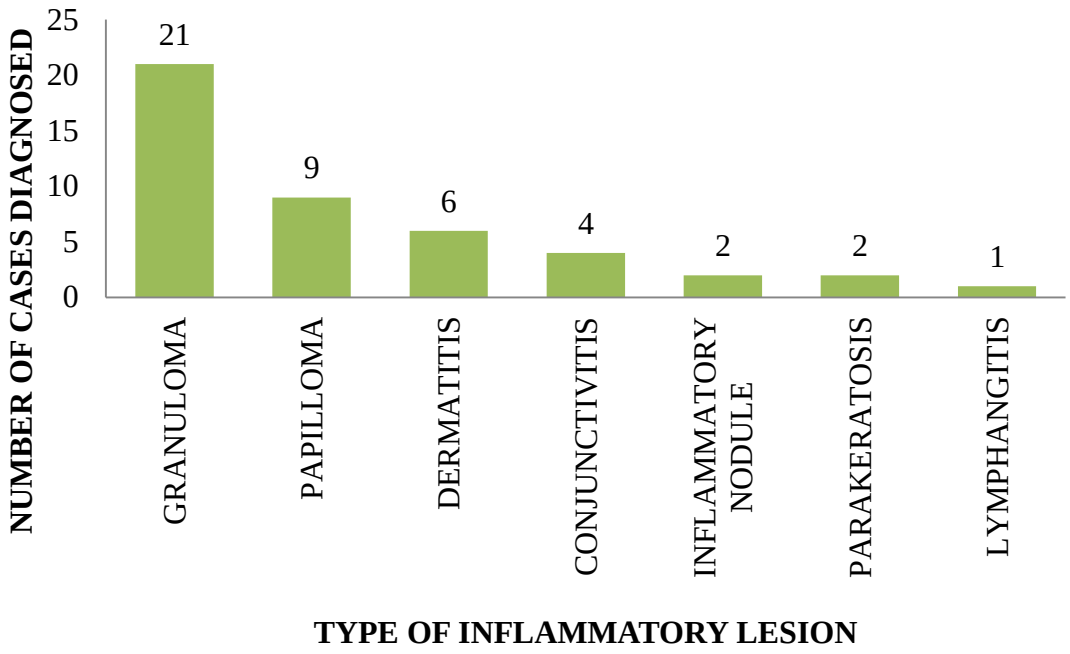

Figure 4: The distribution of the inflammatory cutaneous nodular lesions and surface swellings of horses in Kenya between 1967 and 2014

Table 6: The number of cases of different types of inflammatory cutaneous nodular lesions and surface swellings of horses in Kenya between 1967 and 2014

TYPE OF INFLAMMATORY LESION	NUMBER OF CASES DIAGNOSED
GRANULOMA	21
PAPILLOMA	9
DERMATITIS	6
CONJUNCTIVITIS	4
INFLAMMATORY NODULE	2
PARAKERATOSIS	2
LYMPHANGITIS	1
TOTAL	**45**

4.2 Histopathology Results

The most common histopathological diagnosis of cutaneous nodular lesions and other surface swellings encountered during the period of study was squamous cell carcinoma (22.7% - 32/141), followed by granulomas (14.9% - 21/141). Sarcoids were the third most common diagnosis at 13.5% (19/141). Other diagnoses include melanomas and melanosarcomas (12.1% - 17/141), adenomas and adenocarcinomas (2.1% - 3/141), cutaneous besnoitiosis (1.4% - 2/141), and basal cell tumour (0.7% - 1/141).

Squamous cell carcinomas were mostly encountered in ocular and periocular tissues (75.0% - 24/32), mostly in the third eyelid (43.8% - 14/32) as shown in Table 7. Archived records for two cases of squamous cell carcinoma did not indicate the exact organ affected. Histopathology of the cases of squamous cell carcinoma generally revealed presence of immature epithelial cells that formed clusters and elongated cords leading to enlargement of the rete pegs. At the core of some of these, the cells tend to form concentric structures (epithelial pearls). Some of the cells at the core tended to undergo necrosis and cornification leading to the deposition of keratin in concentric rings (keratin pearls). Mitotic figures occurred moderately. These are shown in Plate 3.

Granulomas were the most common non-neoplastic lesion (42.0% - 21/50). At histopathology, most of the granulomas were consistent with those described for *Habronema* spp. and *Onchocerca* spp. origin. Table 8 summarises the various anatomical areas where granulomas were encountered during the period of the study. In all the granulomatous nodules and swellings, the general observations at histopathology included presence of a mixed inflammatory reaction comprising neutrophils and macrophages, and occasionally lymphocytes, plasma cells and giant cells. Granulomas

consistent with parasitic origin revealed conspicuous presence of eosinophils among other inflammatory cells. The inflammatory granulomas also showed vascular hyperaemia/congestion and fibrous tissue proliferation, which at times were exuberant and tended to be confused with fibroma during diagnosis. These are shown in Plate 4.

Sarcoids were relatively equally distributed in various anatomical regions (Table 9). Histopathologically, they were characterised by irregular arrangement of well differentiated fibroblasts at different stages of maturity in parallel, criss-crossing, whorl and herring bone patterns. Inflammatory foci were invariably present. These are shown in Plate 6. Melanomas and melanosarcomas presented as neoplasms of round to elongated cells with or without the melanin pigment granules that had an irregular arrangement; a lymphocytic inflammatory reaction was variably found. This is shown in Plate 8.

Adenomas showed uniform glandular structures with each glandular unit comprising conical cells with basal nuclei and abundant cytoplasm containing clear flocculated material. These features are shown in Plate 9. On the other hand, a subcutaneous adenocarcinoma showing more immature cells with large hypochromatic nuclei and irregular glandular structures was confirmed from a subcutaneous swelling. Mitotic figures were much more common. These features are shown in Plate 10.

Some of the rare diagnoses encountered include a dermatitis caused by *Besnoitia* spp. and a third eyelid basal cell tumour. The case of cutaneous besnoitiosis manifested a mononuclear dermatitis mostly around blood vessels containing the *Besnoitia* cyst which was at first instance confused with a thrombus. This is shown in Plate 11. The basal cell tumour showed immature epithelial cells, with large hypochromatic nuclei, originating

from the stratum basale and extending into the dermis and forming islands separated from each other by a fibrous tissue wall. This is shown in Plate 12.

The poxviral lesions of Uasin Gishu disease manifested as epidermal hyperplasia due to hyperplasia and ballooning degeneration of the cells in the stratum spinosum and stratum granulosum as shown in Plate 14.

Table 7: The number of cases of squamous cell carcinoma based on anatomical region affected in horses in Kenya between 1967 and 2014

ANATOMICAL REGION AFFECTED BY SQUAMOUS CELL CARCINOMA		**NUMBER OF CASES DIAGNOSED**
Ocular and Peri-ocular	Third Eyelid	14
	Limbus	4
	Eyelid	3
	Sclera	2
	Medial Canthus	1
Non-ocular	Vulva	3
	Prepuce	1
	Penis	1
	Ventrum	1
Unspecified		2
TOTAL		**32**

Table 8: The number of cases of granulomas based on anatomical region affected in horses in Kenya between 1967 and 2014

ANATOMICAL REGION AFFECTED BY GRANULOMA	NUMBER OF CASES DIAGNOSED
NECK	3
EAR-PINNA	2
LIMB-ELBOW	2
LIP COMMISSURE	2
VENTRUM	2
EAR-BASE	1
EYE-LIMBUS	1
FACE	1
LIMB-KNEE	1
NOSTRIL	1
THIGH	1
PREPUCE	1
BACK	1
UNSPECIFIED	2
TOTAL	**21**

Table 9: The number of cases of equine sarcoids based on anatomical region affected in horses in horses in Kenya between 1967 and 2014

ANATOMICAL REGION AFFECTED BY EQUINE SARCOID	NUMBER OF CASES DIAGNOSED
EAR-PINNA	5
LIP COMMISSURE	2
NECK	2
THIGH	2
AXILLA	1
EAR-BASE	1
LIMB-CARPUS	1
LIMB-FETLOCK	1
LIMB-KNEE	1
LIMB-METATARSUS	1
VENTRUM	1
UNSPECIFIED	1
TOTAL	**19**

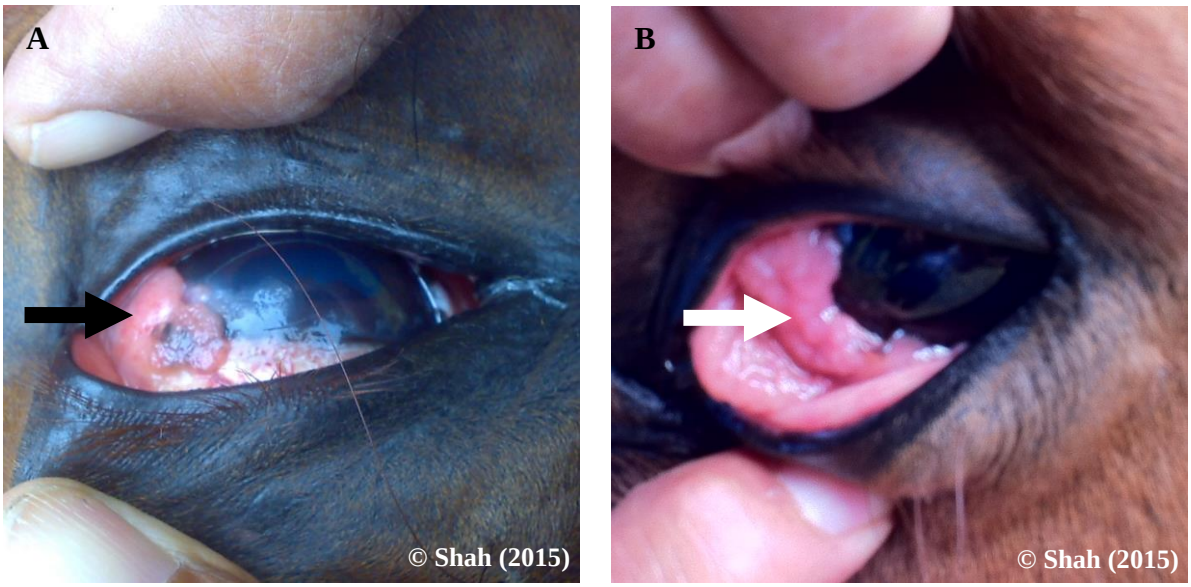

Plate 2: Gross appearance of squamous cell carcinoma: A - Squamous cell carcinoma 9Case Number 262/2011) on the corneoscleral junction of the left eye of an adult male Pony (arrow); B - Squamous cell carcinoma (Case Number 321/2012) on the third eyelid of the left eye of a 6-year-old female Thoroughbred (arrow).

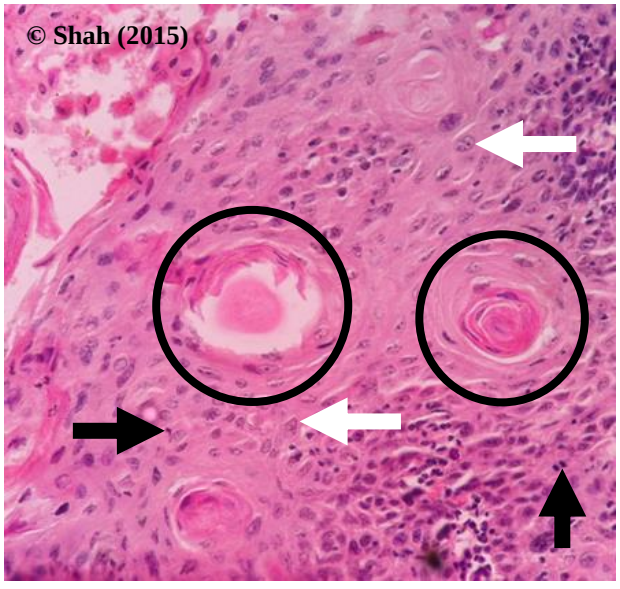

Plate 3: Photomicrograph of a limbic biopsy of equine eye showing squamous cell carcinoma (Case Number 219/2013). Keratin pearls (black circles), immature epithelial cells (white arrows) and mitotic figures (black arrows) are observed. [Haematoxylin and Eosin-stained, paraffin-embedded tissue section, 400x magnification]

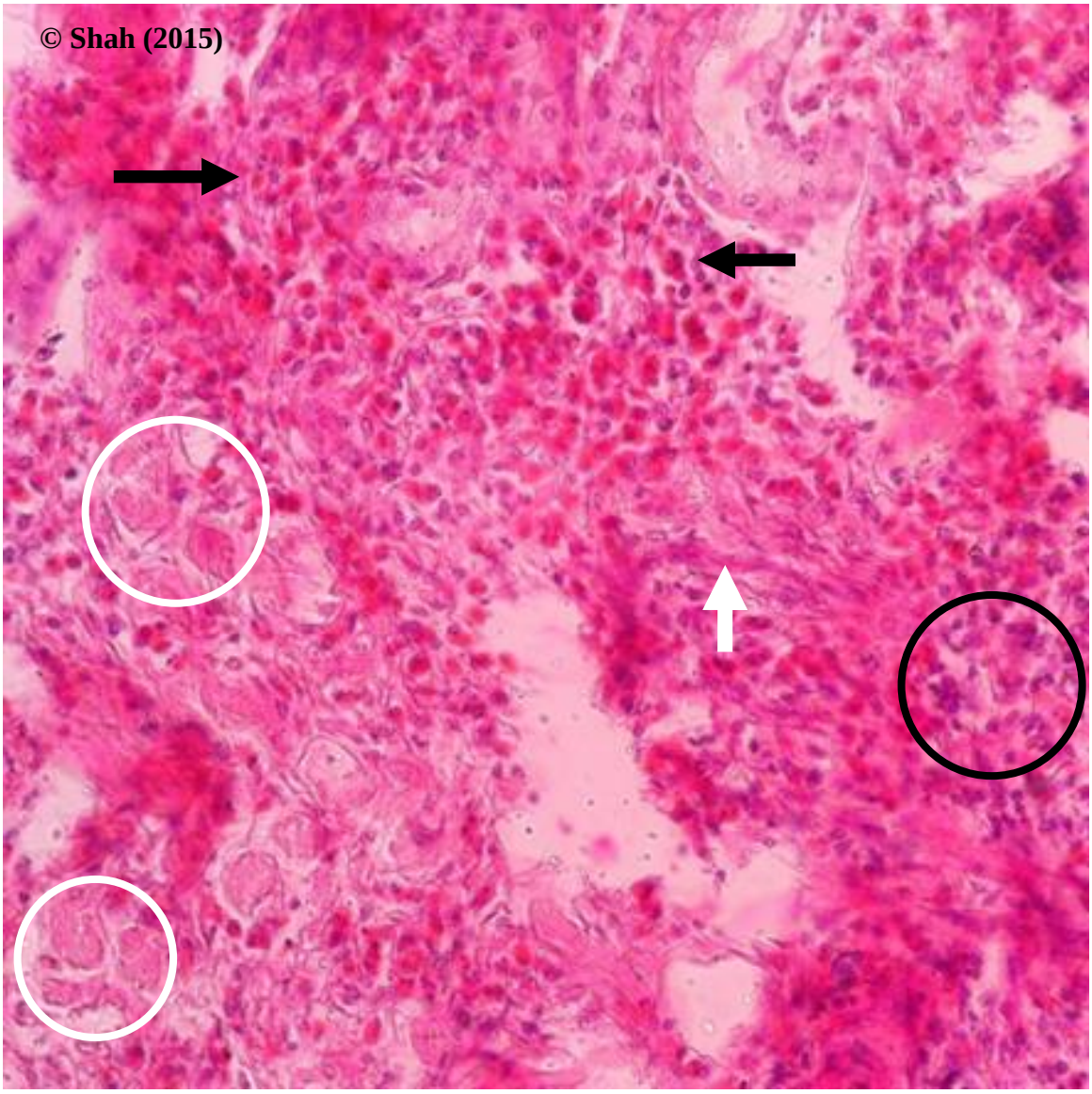

Plate 4: Photomicrograph of a horse biopsy from the skin of the neck showing an eosinophilic granuloma (Case Number 31/2012). Presence of numerous eosinophils in pools of various sizes (black arrows) with foci of mononuclear inflammation (black circle), embedded on a collagen-rich matrix of fibrous tissue proliferation (white arrow) and clumps of amorphous, pink-staining material (white circles) due to the parasitic cause of the lesion. *[Hematoxylin and Eosin-stained, paraffin-embedded tissue section, 400x magnification]*

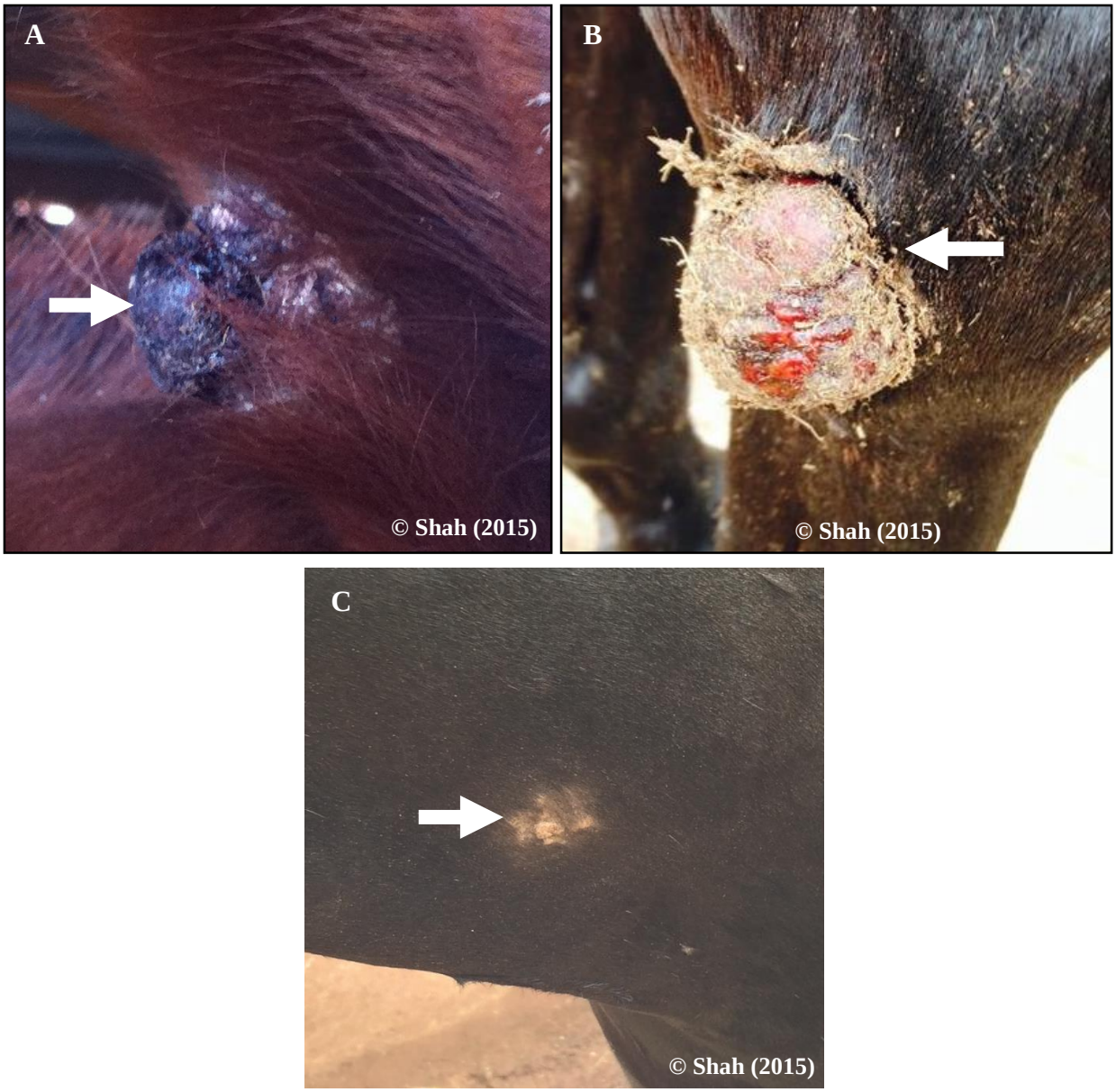

Plate 5: Equine sarcoids classified according to their gross appearance. A: Nodular sarcoid on the lateral margin of the pinna of the right ear of a 10-year-old female Thoroughbred (arrow) [Case Number 146/2014]; B: Fibroblastic sarcoid on the dorsal aspect of the left carpus of a 4-year-old male Thoroughbred (arrow) [Case Number 249/2014); C: Verrucous sarcoid on the left lateral aspect of the neck of 8-year-old male Warmblood (arrow) [Case Number 380/2014].

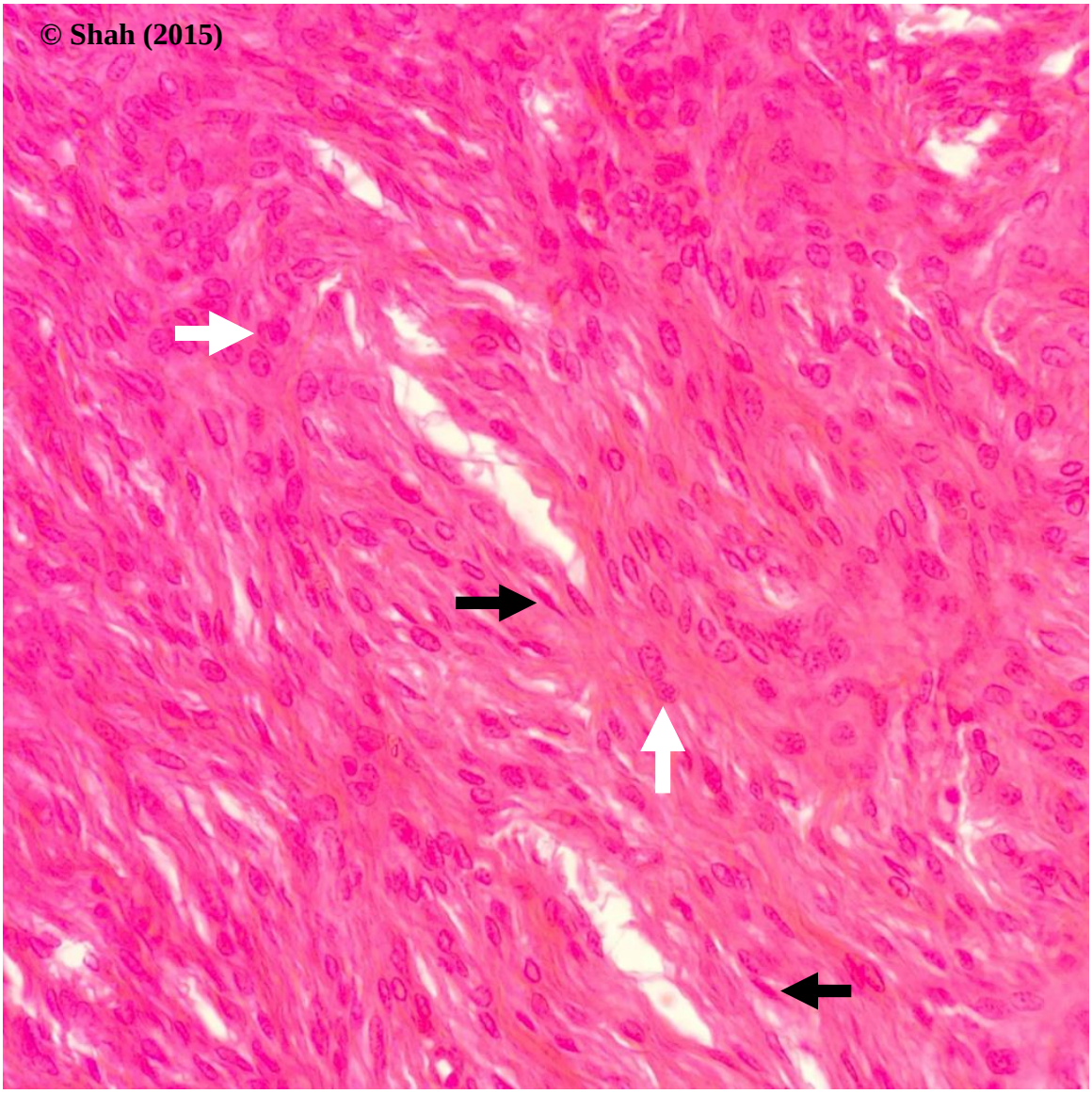

Plate 6: Photomicrograph of a biopsy from the ear pinna of a horse showing the features of a sarcoid (Case Number 401/2014): Irregular arrangement of fibroblasts at different stages of maturity creating parallel, herring bone and criss-crossing patterns (black and white arrows). *[Haematoxylin and Eosin-stained, paraffin-embedded tissue section, 400x magnification]*

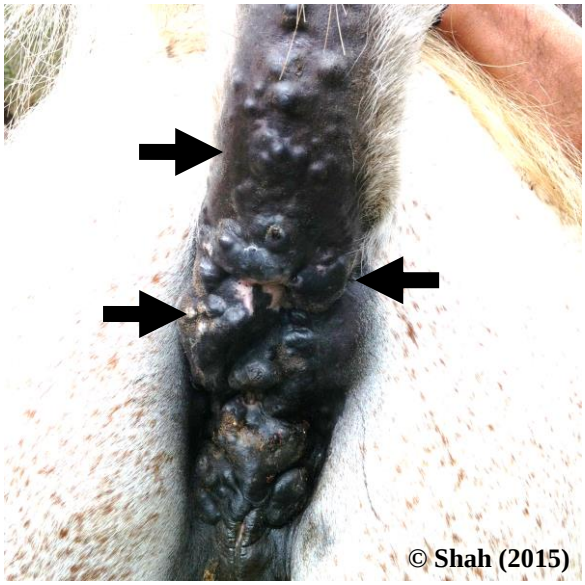

Plate 7: Several melanomas in the perineal region and on the ventral aspect of the tail of a 13-year-old female gray Thoroughbred (black arrows) [Case Number 351/2011].

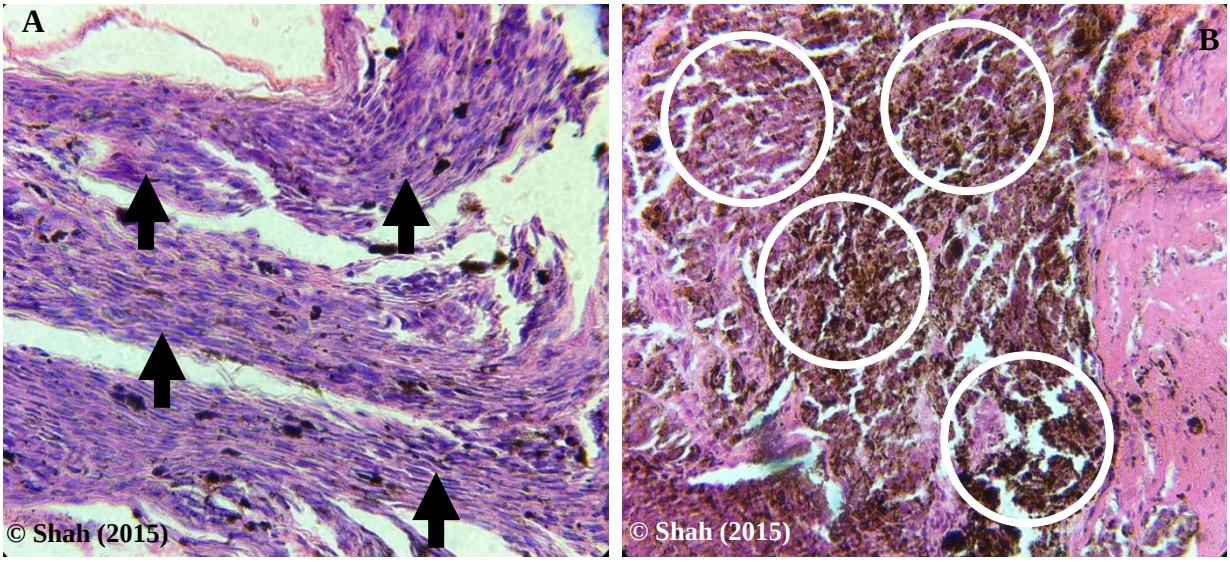

Plate 8: Photomicrographs of a melanoma of the equine eye - A: A limbic biopsy showing a melanoma (Case Number 351/2011) with less melanin and elongated melanocytes (black arrows). *[Haematoxylin and Eosin-stained, paraffin-embedded tissue section, 400x magnification]*; B: A limbic biopsy showing the features of a melanoma (Case Number 298/1992) with abundant melanin and melanoblasts (white circles). *[Haematoxylin and Eosin-stained, paraffin-embedded tissue section, 400x magnification]*

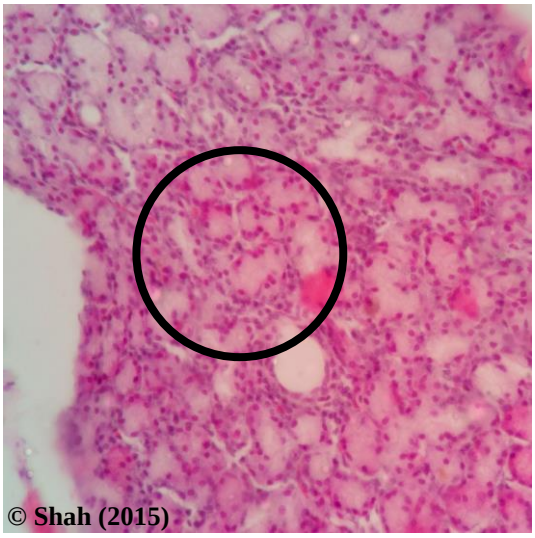

Plate 9: Photomicrograph of a biopsy from the equine third eyelid showing an adenoma (Case Number 109/2005): Increased number (hyperplasia) of glandular units made of conical cells with basally located, hyperchromatic nuclei and abundant cytoplasm (black circle). *[Haematoxylin and Eosin-stained, paraffin-embedded tissue section, 400x magnification]*

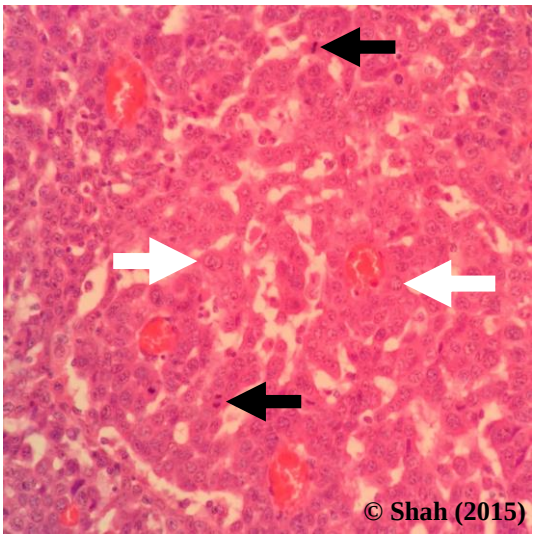

Plate 10: Photomicrograph of a biopsy from a subcutaneous swelling of the horse in the region of the parotid salivary gland showing the features of an adenocarcinoma (Case Number 499/1968): Irregular glandular structures with each glandular unit made of immature cells with a large hypochromatic nucleus (white arrows) and numerous mitotic figures (black arrows). *[Haematoxylin and Eosin-stained, paraffin-embedded tissue section, 400x magnification]*

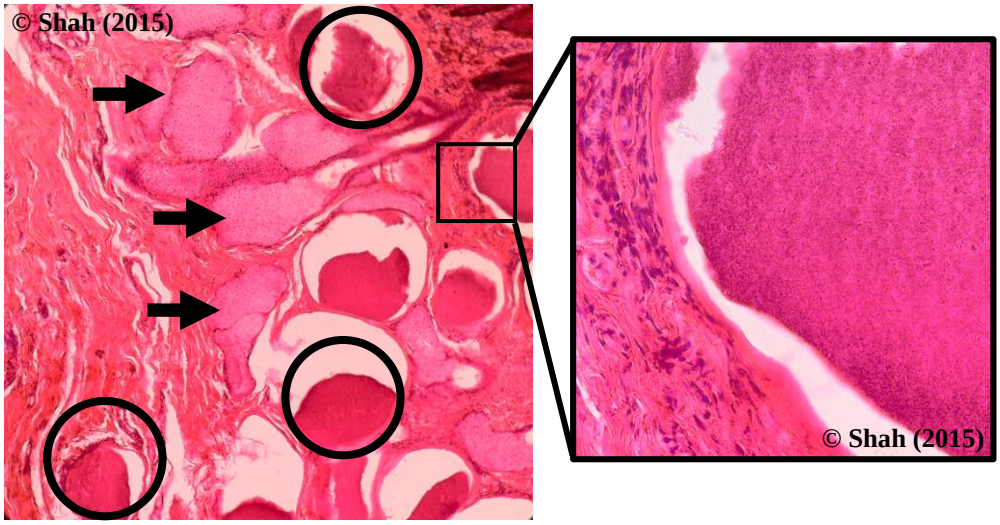

Plate 11: Photomicrograph of a biopsy from the horse skin on the muzzle showing cutaneous besnoitiosis (Case Number 135/2013): Presence of *Besnoitia* cysts in blood vessels wall (black circles), many of which are surrounded by a mononuclear inflammatory reaction (outset - *Haematoxylin and Eosin-stained, paraffin-embedded tissue section, 400x magnification*). There is a hyperplastic reaction by sebaceous glands in response to the dermatitis (black arrows). *[Haematoxylin and Eosin-stained, paraffin-embedded tissue section, 100x magnification]*

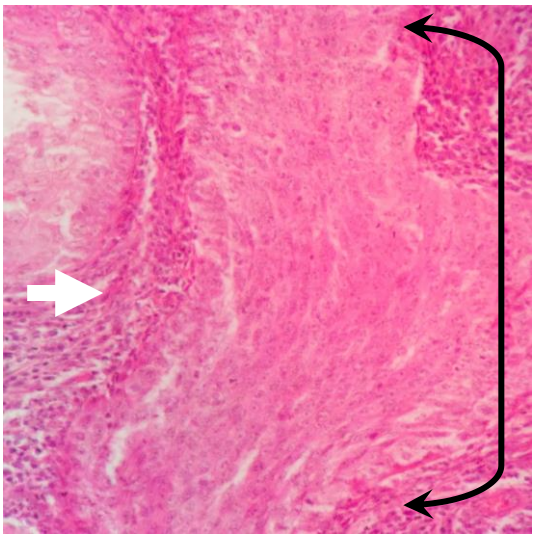

Plate 12: Photomicrograph of a biopsy from the third eyelid showing a basal cell tumour (Case Number 337/2012): Presence of immature epithelial cells with large hypochromatic nuclei irregularly arranged in an island (black arrow) separated from other islands by a fibrous tissue wall (white arrow). *[Haematoxylin and Eosin-stained, paraffin-embedded tissue section, 400x magnification]*

Plate 13: Papillomatous lesions of Uasin Gishu Disease on the neck of an 8-year-old female Thoroughbred. The lesions appear as haired warts with white powdery scales (white circles) which easily peel leaving behind bleeding lesions (black circles) [Case Number 343/2014].

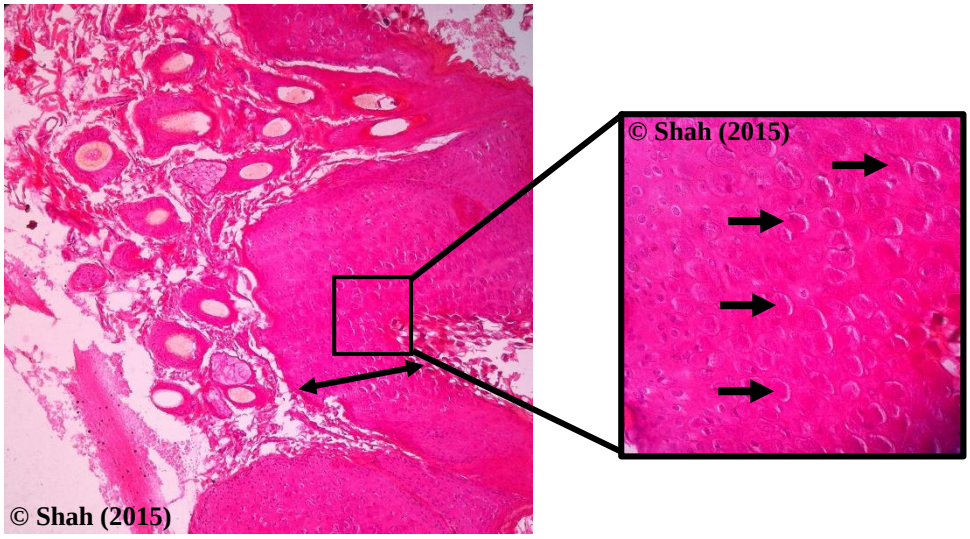

Plate 14: Photomicrograph of an equine biopsy from the skin on the neck showing Uasin Gishu disease (Case Number 343/2014): Epidermal hyperplasia (black arrow) with cells in the stratum spinosum and stratum granulosum showing ballooning degenration (arrows in outset - *Haematoxylin and Eosin-stained, paraffin-embedded tissue section, 400x magnification*). *[Haematoxylin and Eosin-stained, paraffin-embedded tissue section, 100x magnification]*

4.3 Immunohistochemistry Results

In general, 20 cases were selected for immunohistochemistry, with 90% (18/20) of the cases confirming the histopathological diagnosis. A total of 15 cases were selected for IHC with anti-vimentin antibodies. All sections selected from the cases were strongly positive for the presence of vimentin. The intensity of staining varied with the type of lesion. Fibromas and fibrosarcomas were strongly positive while sarcoids varied between moderately positive to strongly positive to anti-vimentin antibodies. Granulomas and inflammatory nodules were weakly positive. Exuberant granulation tissue appeared moderately positive. The rate of concurrence of the histopathological diagnosis and immunohistochemistry results from the randomly selected cases was 100% (15/15). Plates 15 – 19 show the distribution of the anti-vimentin antibodies and these were compared with a haematoxylin & eosin stained slide of the same tissue section.

A case of an inflammatory polyp being confused for an epithelial neoplasm was confirmed through IHC by using anti-vimentin antibodies and anti-cytokeratin antibodies. The tissue was anti-vimentin positive and anti-cytokeratin negative as demonstrated in Plate 20. In total, 5 cases were stained with anti-cytokeratin antibodies, with 40% (2/5) showing lack of staining.

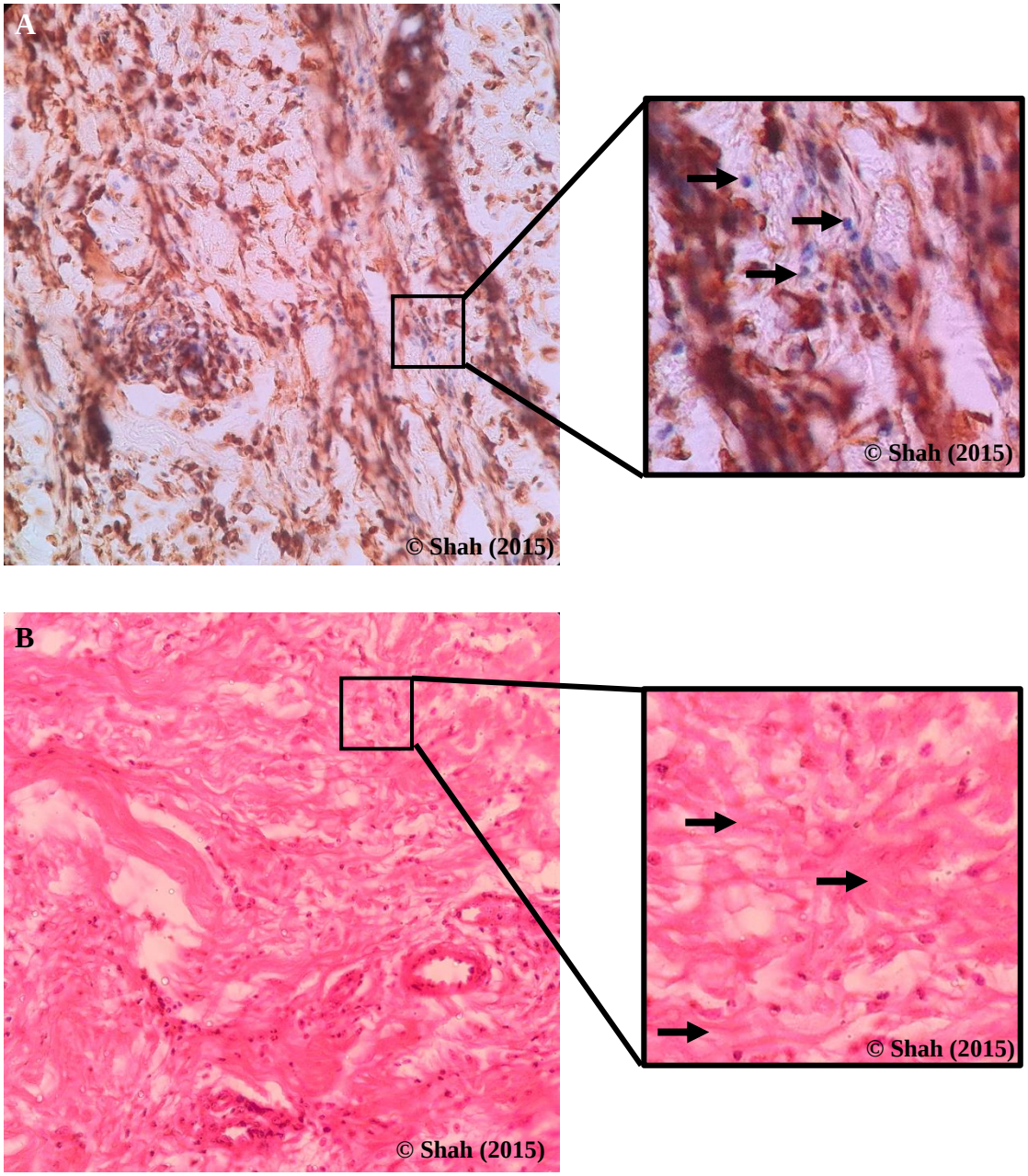

Plate 15: Photomicrographs of an inflammatory nodule of the horse skin (Case Number 134/1995): A - An inflammatory nodule stained with immunohistochemistry using anti-vimentin antibodies showing low intensity staining and loose packing of fibrocytes admixed with inflammatory cells which are devoid of the chromogen (arrows in outset) *[400x magnification]*; B - An inflammatory nodule stained with haematoxylin and eosin showing densely packed fibrous connective tissue in the lesion (arrows in outset) *[400x magnification]*

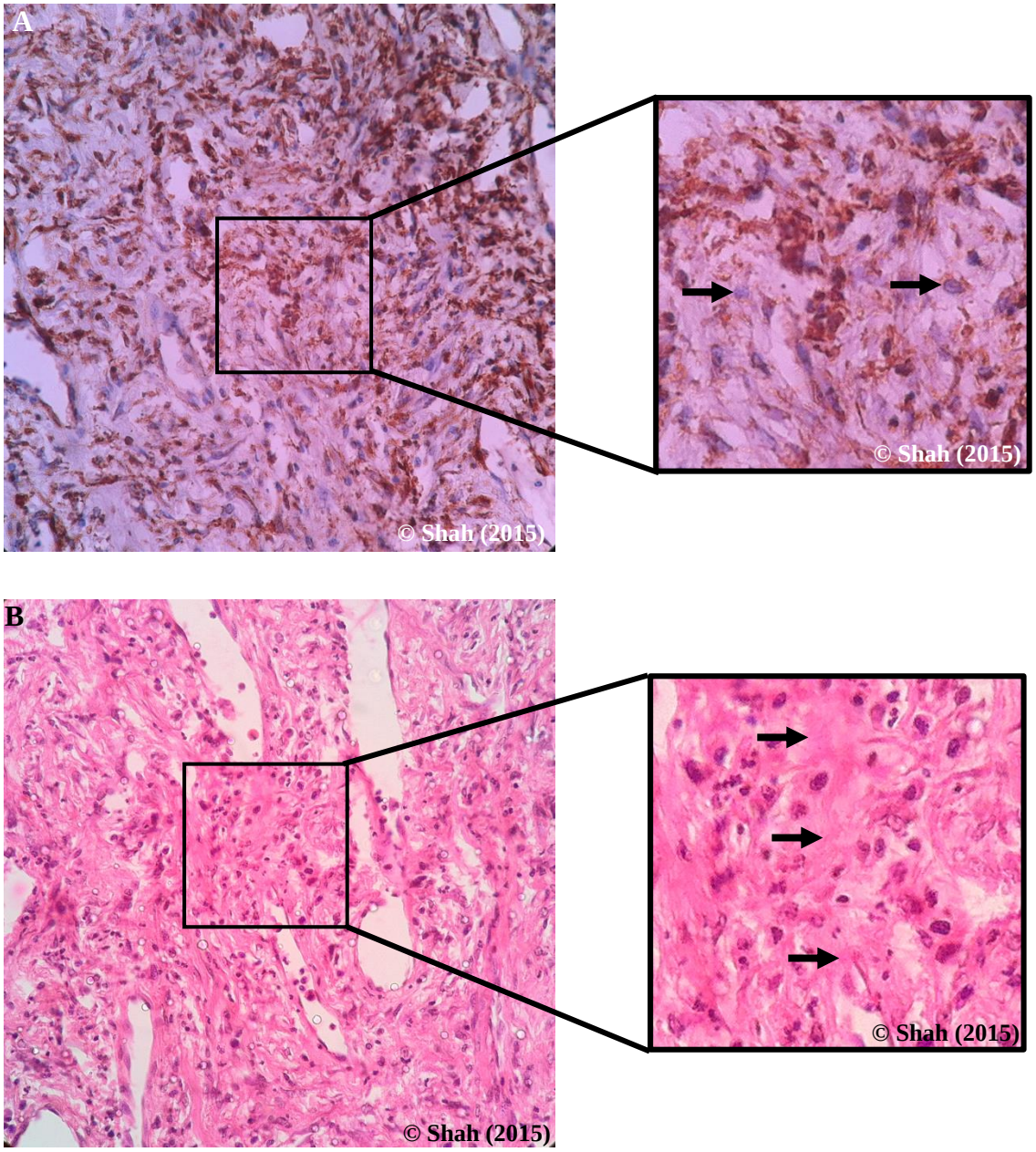

Plate 16: Photomicrographs of a granuloma of the horse skin (Case Number 65/1976): A – An equine cutaneous granuloma stained with immunohistochemistry using anti-vimentin showing low intensity staining and loose packing of fibrocytes admixed with inflammatory cells which do not take up the chromogen (arrows in outset) *[400x magnification]*; B – An equine cutaneous granuloma stained with haematoxylin and eosin showing heavy presence of fibrous tissue (arrows in outset) *[400x magnification]*

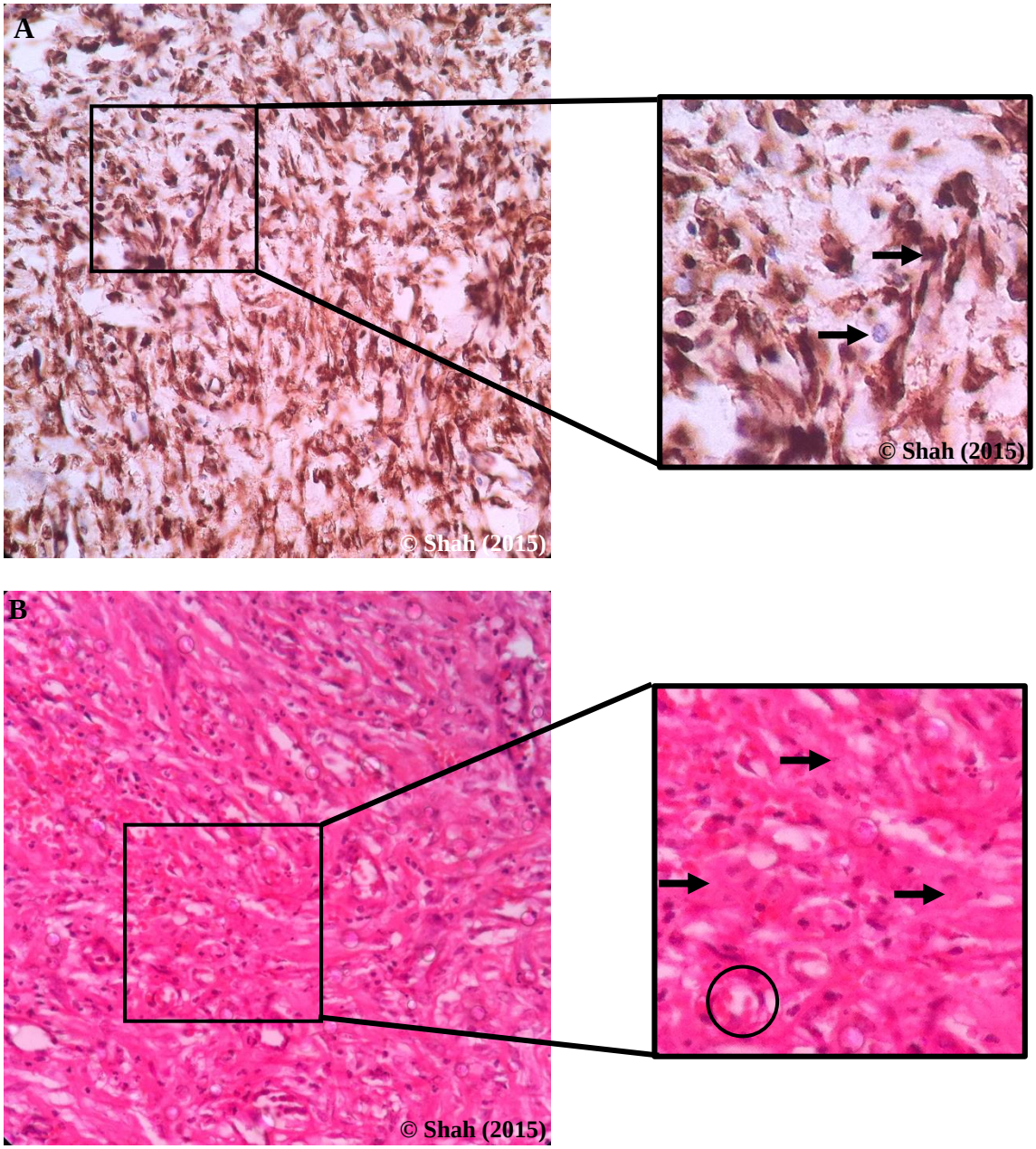

Plate 17: Photomicrographs of exuberant granulation tissue of the horse skin (Case Number 559/1974): A - Exuberant granulation tissue stained with immunohistochemistry using anti-vimentin showing moderate staining of fibrocytes cytoplasm and loose packing of fibrocytes admixed with inflammatory cells (arrows in outset) *[400x magnification]*; B: Exuberant granulation tissue stained with haematoxylin and eosin showing proliferation of fibrous tissue admixed with inflammation and neocapillarization (arrows and circle in outset) *[400x magnification]*

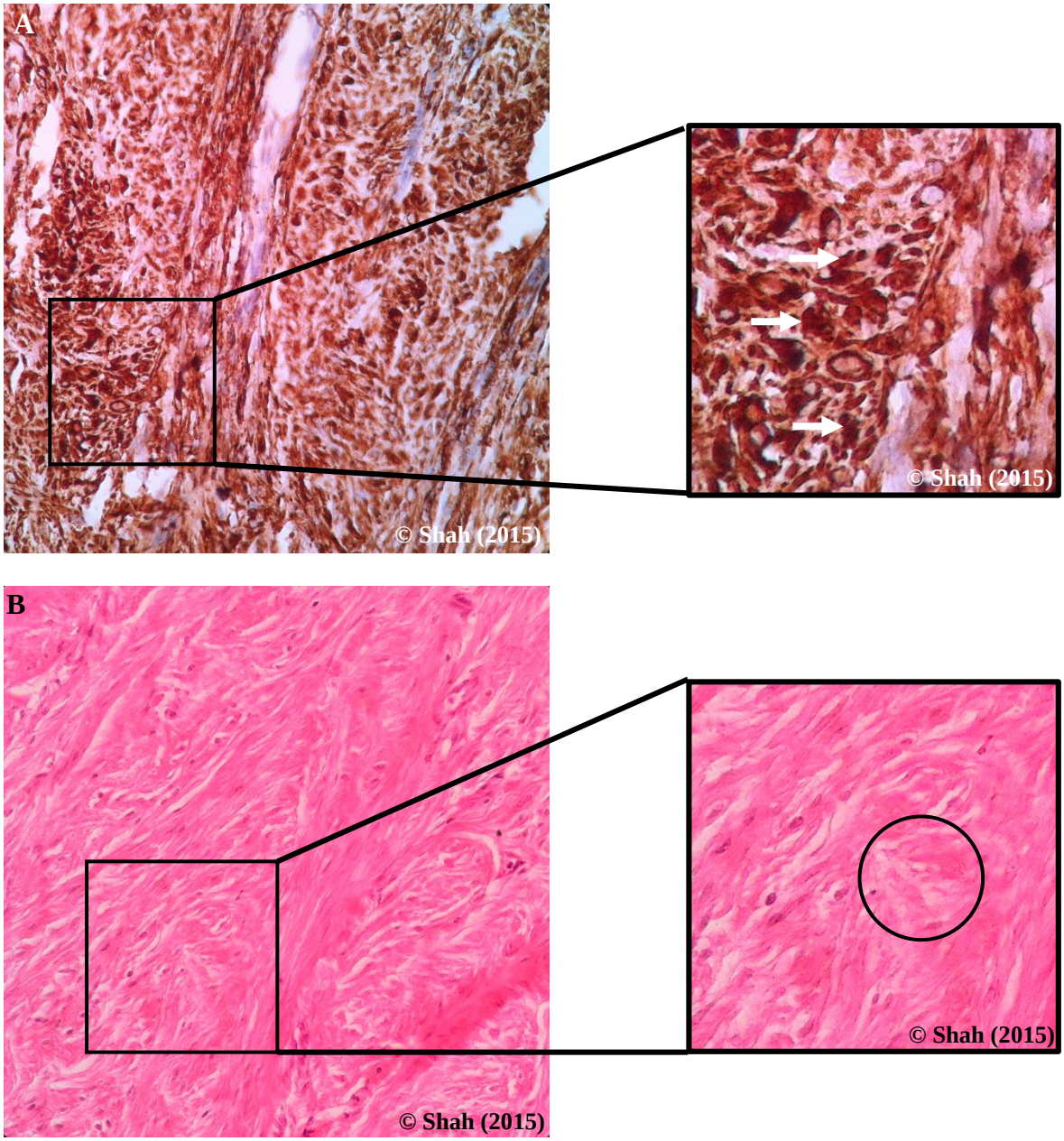

Plate 18: Photomicrographs of an equine sarcoid of the horse skin (Case Number 29/1976): A - Equine sarcoid stained with immunohistochemistry using anti-vimentin showing intense staining of fibroblast cytoplasm (arrows in outset) *[400x magnification]*; B - Equine sarcoid stained with haematoxylin and eosin showing tightly packed fibroblasts and criss-crossing fibrous tissue (circle in outset) *[400x magnification]*

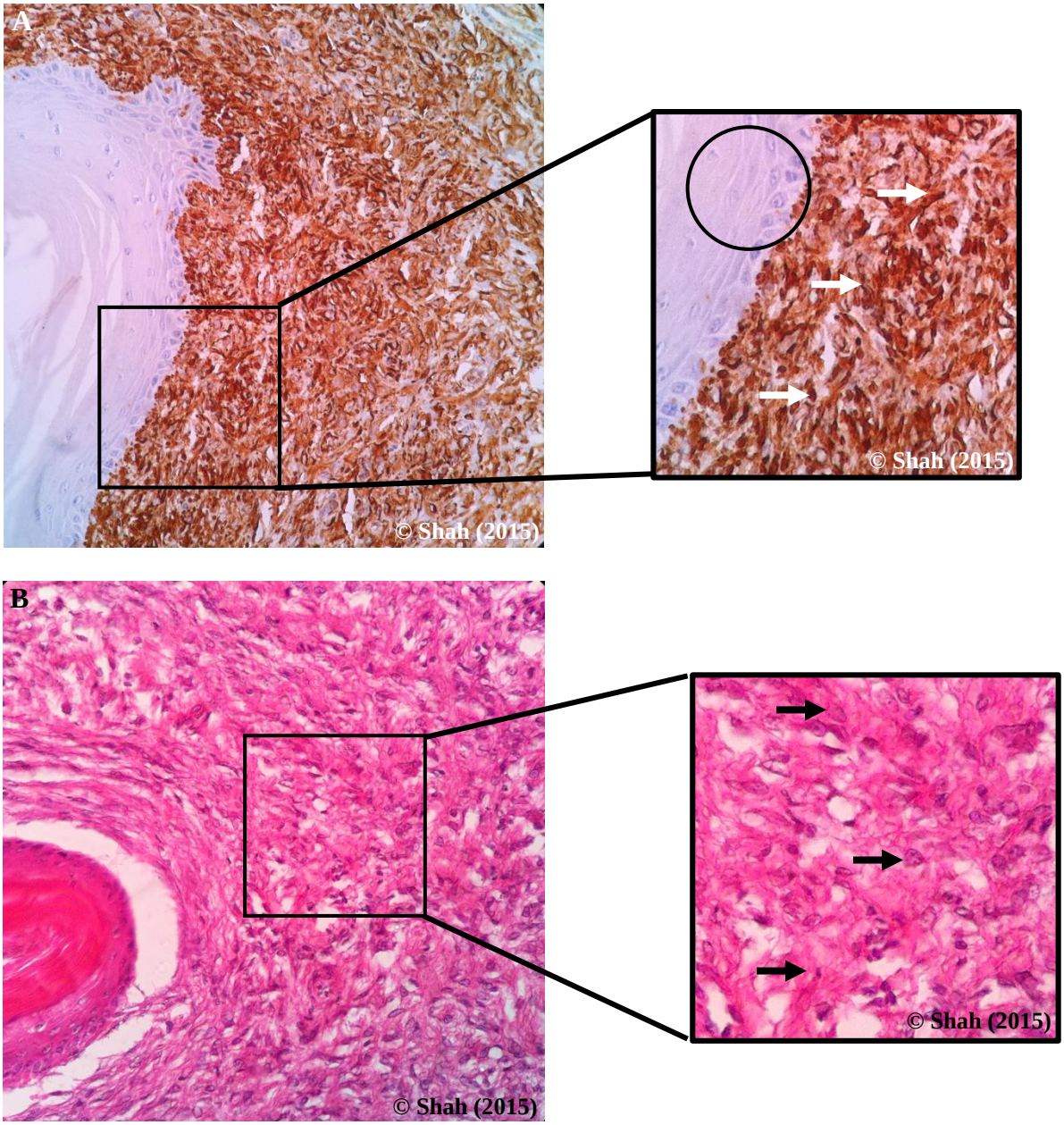

Plate 19: Photomicrographs of a fibrosarcoma of the horse skin (Case Number 198/1971): A - Fibrosarcoma stained with immunohistochemistry using anti-vimentin showing intense staining and tight packing of fibrocytes and lack of staining of the epithelial cells of the hair follicle on the left (arrows and circle in outset) *[400x magnification]*; B - Fibrosarcoma stained with haematoxylin and eosin showing numerous fibroblasts with moderate deposition of collagen (arrows in outset) *[400x magnification]*

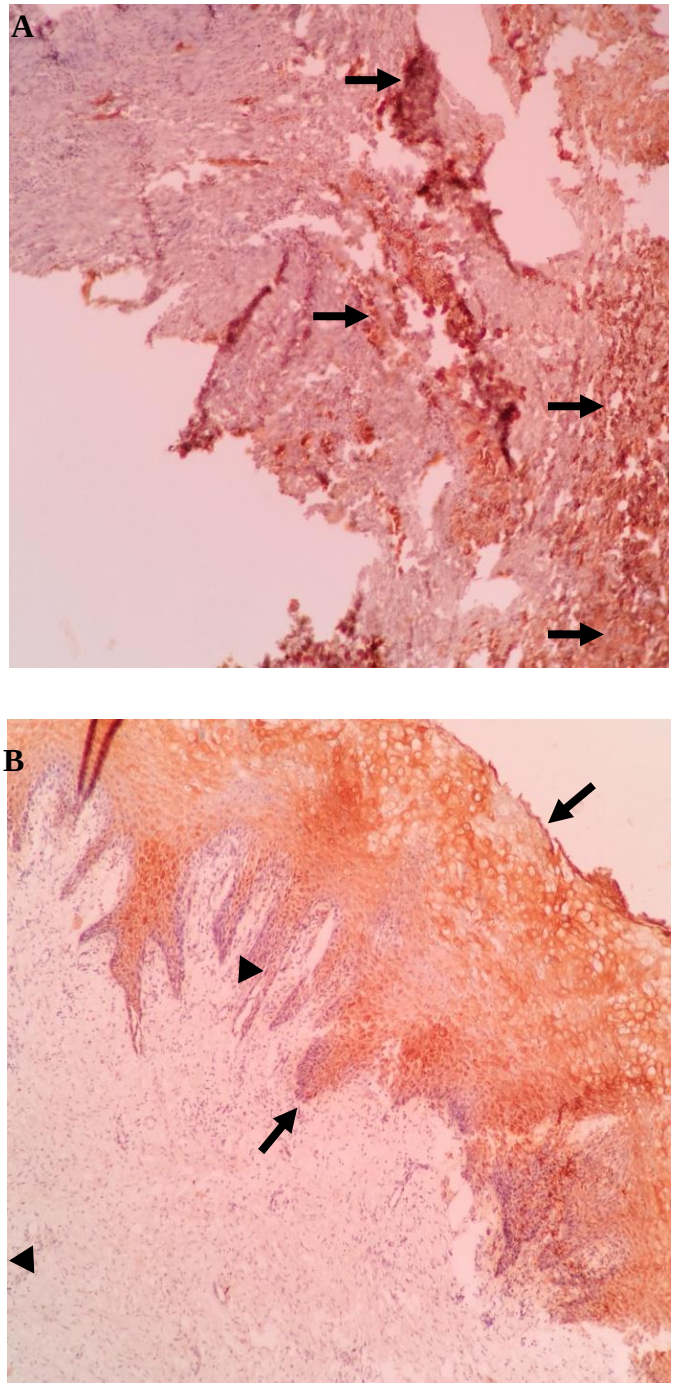

Plate 20: Photomicrographs of an inflammatory polyp of the skin of the horse (Case Number 134/1995) showing comparison in staining between different antibodies: A - Inflammatory polyp stained with immunohistochemistry using anti-vimentin showing staining of connective tissue within the dermis and subcutis (in between arrows) *[100x magnification]*; B - Inflammatory polyp stained with immunohistochemistry using anti-cytokeratin showing mild staining of the epithelial cells of the epidermis (between arrows) and no staining in the dermis and subcutis (in between arrowheads) *[100x magnification]*

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

According to this study, neoplastic lesions are more common than non-neoplastic as reported by Meuten (2002) and Valentine (2006). This study has revealed that squamous cell carcinomas are the most common in horses in Kenya, occurring mostly in ocular and peri-ocular tissue as reported by MacFadden and Pace (1991), Chahory *et al*. (2002), Valentine (2006) and Taylor and Haldorson (2013). Most squamous cell carcinomas occurred in the ocular and peri-ocular areas. The basic microscopic appearance of the squamous cell carcinomas correlated well with descriptions given by Meuten (2002), Jubb *et al*. (2005), Bukar *et al*. (2007) and Taylor and Haldorson (2013). The intensity of the lesions varied between the different masses examined. This could have been as a result of varying stages of the neoplasm in the different instances.

Unlike in the retrospective study performed by Valentine (2006) and the review by Foy *et al*. (2002), where sarcoids were encountered at a significantly higher level, this study showed that the occurrence of sarcoids was significantly lower than that of squamous cell carcinoma, occurring at 13.4% versus 22.7% for squamous cell carcinoma. A likely reason for the higher incidence of squamous cell carcinoma could be the higher rate of exposure of horses to ultra-violet (UV) radiation since Kenya is a tropical country compared to the temperate countries where such studies and reviews were performed (United States). Microscopic lesions of sarcoids obtained in this study matched with those described by Meuten (2002), Hallamaa *et al*. (2005), Jubb *et al*. (2005), Gomes (2011), Corteggio *et al*. (2012) and Farouk (2014). A few cases lacked recognizable inflammatory foci and other features, and could be confused

with a fibromatous neoplasm such as a fibroma or a fibrosarcoma. In such instances, immunohistochemistry using antibodies against p53 or BPV could have been useful in confirming the diagnosis; this was not attempted due to cost implications.

A comparatively low incidence of melanomas and melanosarcomas was observed amongst the neoplasms in this study, as reported by Valentine (2006) where the incidence of these tumours was 18%. It should be noted that most equine practitioners prefer not to interfere with melanomas on horses unless they affect normal functions such as defecation; this is because of the high probability of the melanomas becoming malignant and metastasizing. Therefore, the true incidence of melanomas and melanosarcomas might be much higher than reported in this study since most cases are not submitted for histopathology. Many of the masses diagnosed as melanomas and melanosarcomas in this study were obtained from carcasses at the post-mortem room in the Department, and most of the cases of melanosarcoma showed metastasis to several internal organs which included regional lymph nodes, skeletal muscles and the heart. Statistical analysis on the involvement of other systems in metastatic melanosarcomas was not performed as they were not fulfilling the criteria of the study. A review by MacGillivray *et al*. (2002) indicated that metastasis of melanosarcoma was seen in lymph nodes, liver, lungs and spleen. Classification of melanomas and melanosarcomas has been well described by Valentine (1995).

Very few cases of cutaneous lymphoma or lymphosarcoma were observed in this study; 2.2% of the neoplasms and 1.4% of all submissions. This study did not attempt to classify cutaneous lymphoma according to epitheliotropic and non-epitheliotropic cutaneous lymphoma as described by Meyer *et al*. (2006) and de Bruijn *et al*. (2007) due to the few cases encountered.

A rare case of cutaneous besnoitiosis was observed from a horse with diffuse minute subcutaneous nodules on the muzzle, face, trunk, back and rump. Further, a fibroblastic growth was observed on the pinna of the same horse which showed similar microscopic lesions, with the exception of extensive fibrous tissue proliferation. Reports of equine cutaneous besnoitiosis have not been made in Kenya, but have been made from donkeys, cattle and reindeer in other countries (Terrell and Stookey, 1973; Ayroud *et al*., 1995; Dubey *et al*., 2005; Igbokwe *et al*., 2009).

Based on interviews with equine practitioners, poxviral lesions of Uasin Gishu disease are very common in horses especially in the southern parts of Kenya. Unfortunately, these were not submitted regularly for diagnosis due to self-resolution or as a result of lack of consent from the owners for their removal. Most of the cases of this disease were submitted in the 1970s and 1980s and were extensively studied by Kaminjolo *et al*. (1974a, b) and Kaminjolo and Winquist (1975). The microscopic lesions of poxviral infection (Uasin Gishu disease) obtained through this study matched those from the older studies; however, electron microscopy was not available to identify virion particles within affected cells.

Basal cell tumours are rarely encountered in the horse, and this study came across one such case. Reports on basal cell tumour in the horse from Kenya do not exist and there are few reports globally; however, Baril (1973) reported one in Canada. The microscopic appearance of neoplastic cells was similar to the report by Baril (1973), however additional pathology such as oedema and haemorrhage were not observed in the single case of basal cell tumour from the eyelid in this study.

Cases of cutaneous hypersensitivity reactions were not encountered in the study due to the likely reason of quick and easy treatment leading to resolution (Anderson *et al*., 1988;

Bindslev-Jensen and Skov, 2010; Ural and Ulutas, 2010; Jónsdóttir, 2011; Littlewood, 2011; Schaffartzik *et al*., 2012; Srivastava *et al*., 2013). Mast cell tumours were not encountered in the study supporting the rarity of the lesion as reported by Cole *et al*. (2007), Millward *et al*. (2010) and Yu (2015).

Aetiological classification of granulomas was not attempted since it was beyond the scope of the study. However, most granulomas were attributed to parasitic causes based on the presence of eosinophils within most of the sections. A few fungal granulomas had also been suspected. Aetiology could have been derived after special staining techniques, such as Periodic acid-Schiff and Gram staining, or specific immunohistochemistry. This can be pursued in further studies.

Immunohistochemistry performed according to the manufacturer's (Dako®) protocol resulted in adequate staining of the antigen on selected cases. The commercial anti-human Vimentin antibody does show strong cross-reactivity with horse antigens and can be used to mark horse mesenchymal cells. For an accurate diagnosis, histopathology can be combined with general and specific immunohistochemistry. Sarcoids have been confirmed through the use of histopathology and immunohistochemistry targeted towards the presence of the BPV antigen, which has been suggested to be an aetiological agent for equine sarcoids, in the section or towards the absence of O6-methylguanine-DNA methyltransferase (MGMT) in section (Thamm *et al*., 2008; Altamura *et al*., 2012; Finlay *et al*., 2012; Farouk, 2014). There was concurrence between histological diagnosis and immunohistochemistry in most of the cases studied (90%). A few discrepancies were encountered between the histopathological diagnosis and the results of immunohistochemistry for the two cases suspected to be a subcutaneous adenoma (10%). Possible reasons for these could be the epithelial ballooning degeneration

being mistaken for glandular cells or low cross-reactivity between the anti-Ki-67 antibodies with horse antigen.

Some paraffin-embedded tissue blocks were not available for sectioning and hence were not included for histopathology, but were included for general results. Difficulties were encountered in correlating demographic data with clinical and pathological parameters due to insufficient records, especially for retrospective cases. Therefore, demographic analysis for the lesions was not possible due to inadequate records.

5.2 Conclusions

1. This study is the first to document the cutaneous nodular lesions and surface swellings of horses in Kenya and describe their histological and immunohistochemical characteristics.
2. Submissions of biopsies from horses are low even though accurate diagnosis of the lesion determine the type of treatment and eventual outcome of the case.
3. The study has established that commercially available standardized immunohistochemical kits for various antigens can be used effortlessly to distinguish between various morphologically related lesions.
4. The agreement in histopathology and immunohistochemistry diagnosis in 90% of cases shows that histopathology can appreciably be relied upon in confirmatory diagnosis of most tumours in the Department.
5. This study sets the stage and forms a basis for application of immunohistochemistry in routine veterinary diagnostics of animal tumours in Kenya.

5.3 Recommendations

1. It is recommended that adequate biodata and history be collected during the time of biopsy submission so as to maintain a complete record for the archives.
2. Equine practitioners should be encouraged to submit more biopsies for diagnosis since accurate diagnosis of the lesion will enable them to administer appropriate treatment and determine the eventual outcome of the case.
3. Immunohistochemistry should be adopted as a routine practice for difficult cases where microscopic lesions observed would relate to more than one morphologically related diagnosis. This would especially be helpful in differentiating inflammatory lesions from neoplastic lesions which could help clinicians in initiating the correct treatment options.
4. Further immunohistochemical studies with more specific antibodies are suggested especially for sarcoids where antibodies to p53 or BPV can be employed.
5. Further detailed studies to classify granulomas in horses based on aetiology are recommended.
6. Further comprehensive studies are suggested to describe and classify the types of tumours in other body systems of the horse.

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APPENDICES

7.1 Appendix 1: Biopsy submission form of cutaneous nodules and other surface swellings in the horse

STUDY OF EQUINE CUTANEOUS NODULAR LESIONS AND SURFACE SWELLINGS
M. Sc. PROJECT – DR. SHAH

BIOPSY SUBMISSION FORM FOR HISTOLOGY

Date collected*: _____ **Date submitted:** _____
Clinician's Name*: _____ **Address/Location *:** _____
Telephone/Mobile *: _____ **E-mail*:** _____
Patient Details
Species: _____ **Breed*:** _____
Sex*: _____ **Age (Years/Months)*:** _____
Name/Identification*: _____
Hair coat/Skin Colour*: _____
Owner's Details
Name*: _____ **Address/Location*:** _____
Telephone/Mobile *: _____ **E-mail*:** _____
Clinical History
No. in Herd*: _____ **No. affected*:** _____
History (Date first observed, duration, progression, recurrence)*: _____
_____
_____
_____
_____
Description of Lesion*
Colour of Lesion*: _____
Shape of Lesion* (e.g., circular, regular or irregular margins, etc.): _____
_____
Approximate Size (cm) of Lesion* (diameter or length & width): _____
Texture of Lesion* (smooth or rough, dry or moist): _____
_____
Consistency of Lesion* (soft or firm, fluctuant, crepitant): _____
_____
Location of Lesion* (anatomical location e.g. medial or lateral canthus of eye, medial side of right elbow, etc.): _____
_____
_____
Appearance of cut surface* (dry or moist, colour of cut surface, any visible pattern – lobulated, variegated, streaked): _____
_____
_____
Other Notes* (e.g. bleeding, ulcerated, exudate – type, colour etc.): _____
_____
_____
Clinical Diagnosis: _____

*** Important details to be filled**

7.2 Appendix 2: Dako® immunohistochemical staining protocol of cutaneous nodules and other surface swellings in the horse

A. Specimen Collection and Preparation

1. Formalin-fixed, paraffin-embedded tissue sections were mounted on the special Silanised Microscope Slides (FLEX IHC Slides) as flat and wrinkle-free as possible from a pre-heated water bath containing distilled or deionised water to ensure there were no additives such as gelatin or poly-L-lysine in the water which could interfere with staining.
2. To ensure proper adherence of sections to the slides, excess water beneath the sections was allowed to flow off by gravitation by placing the slides in an inclined upright position on a slide drying rack.
3. The sections were dried by placing in a hot air oven maintained at 60°C for up to 60 minutes.

B. Pretreatment procedure using Coplin jars - HIER

The following steps were meant to deparaffinize, rehydrate and subject the sections to heat-induced epitope retrieval (HIER):

1. The slides were placed in a xylene bath and incubated for 5 (±1) minutes. This was repeated once more in another xylene bath. Excess liquid was tapped off.
2. The slides were placed in absolute ethanol for 3 (±1) minutes. This was repeated once more in another absolute ethanol bath. Excess liquid was tapped off.

3. The slides were placed in 95% ethanol for 3 (±1) minutes. This was repeated once more in another 95% ethanol bath. Excess liquid was tapped off.
4. The slides were placed in distilled water for a minimum of 30 seconds.
5. A working solution was prepared by diluting the EnVision™ FLEX Target Retrieval Solution (50x) concentrate 1:50 in distilled or deionized water. Staining jars containing this working solution were covered with a lid and placed in a water bath and heated to 95-99 °C.
6. The room temperature sections in distilled water were removed and placed in the preheated EnVision™ FLEX Target Retrieval Solution (working solution) in the staining jars and incubated at 95-99 °C for 20 (±1) minutes.
7. The staining jars with sections were removed from the water bath and the entire jar with its contents was allowed to cool for 20 (±1) minutes at room temperature. The working solution was decanted and the sections rinsed thrice with room temperature EnVision™ FLEX Wash Buffer (20x) before incubating in the same for 1–5 minutes.

NOTE: All reagents were used only once and discarded.

C. Staining Procedure

1. The slides were not allowed to dry out during any stage of the process of HIER or staining. A working solution was prepared by diluting the EnVision™ FLEX Wash Buffer (20x) concentrate 1:20 in distilled or deionized water. The slides were prepared for manual staining by drying the back side and sides around the tissue with blotting paper. The Dako® pen was used around the tissue to create a hydrophobic border around the tissue which keeps the reagents on the tissue.

2. 100µl EnVision™ FLEX Peroxidase-Blocking Reagent Ready-to-use (RTU) was added to the tissue and incubated for 5 (±1) minutes. The slides were washed thoroughly with the diluted wash buffer solution.
3. 100µl of the antibody was added and incubated for 20 (±1) minutes. The slides were washed thoroughly with the diluted wash buffer solution.
4. 100µl EnVision™ FLEX/HRP (RTU) was added and incubated for 20 (±1) minutes. The slides were washed thoroughly with the diluted wash buffer solution and incubated in fresh wash buffer solution for 5 (±1) minutes.
5. EnVision™ FLEX Substrate Buffer was prepared by thoroughly mixing 1 drop EnVision™ FLEX DAB+ Chromogen with 1 mL EnVision™ FLEX Substrate Buffer. This was a photosensitive solution that was stored in the dark at 2-8°C and used within 5 days.
6. 100µl EnVision™ FLEX Substrate Working Solution was added to the tissue and incubated for 5 (±1) minutes. The slides were washed thoroughly with the diluted wash buffer solution.
7. 100µl Haematoxylin was added and incubated for 5 (±1) minutes. The slides were washed thoroughly with the diluted wash buffer solution and incubated in fresh wash buffer solution for 5 (±1) minutes.
8. The slides were then washed thoroughly with distilled or deionised water. The slides were then dehydrated with two changes of 95% ethanol and two changes of absolute ethanol, cleared of alcohol using two changes of xylene and cover-slip mounted using Di-N-Butylphthalate in Xylene (DPX).

D. Precautions

1. All waste reagents were collected in a leak-proof liquid waste disposal container since all the products supplied contained sodium azide (NaN3), a chemical highly toxic in pure form. NaN3 could react with lead and copper plumbing to form highly explosive build-ups of metal azides. The waste container was capped tightly and made leak-proof before disposing into the Departmental disposal pit as per National Environment Management Authority (NEMA) protocols.
2. Personal Protective Equipment was worn to avoid contact of reagents with eyes and skin.

7.3 Appendix 3: Data capture form for histopathology and immunohistochemistry of cutaneous nodules and other surface swellings in the horse

STUDY OF EQUINE CUTANEOUS NODULAR LESIONS AND SURFACE SWELLINGS
M. Sc. PROJECT – DR. SHAH

HISTOPATHOLOGY/IHC DATA CAPTURE FORM

Pathology Case Number: _____ **Date submitted:** _____
Species: _____ **Breed*:** _____
Sex*: _____ **Age (Years/Months)*:** _____
Name/Identification*: _____
Hair coat/Skin Colour*: _____
History (Date first observed, duration, progression, recurrence)*: _____
_____
_____
_____
_____
Clinical Diagnosis: _____
Gross Lesion(s) at Trimming: _____
_____
_____
_____
Histopathology:

Lesion Description	Tally	Frequency/50 fields

Histopathological Diagnosis: _____

Immunohistochemistry:

Antibody	Distribution	Tally	Frequency/50 fields

Immunohistochemistry Diagnosis: _____

7.4 Raw data of cutaneous nodules and other surface swellings in the horse

SN NO.	CASE #	NAME	BREED	SEX	AGE	SOURCE	AREA AFFECTED	CLINICAL DIAGNOSIS	FORMER HISTOPATHOLOGICAL DIAGNOSIS	CURRENT HISTOPATHOLOGICAL DIAGNOSIS	TYPE
1	1967_1388	UNSPECIFIED	UNSPECIFIED	M	17	C	PERINEUM	MELANOMA	MELANOSARCOMA	MELANOSARCOMA	N
2	1968_062	UNSPECIFIED	UNSPECIFIED	F	U	C	PERINEUM	UNSPECIFIED	MELANOSARCOMA	MISSING	N
3	1968_499	UNSPECIFIED	UNSPECIFIED	F	17	C	CHEEK	FIBROSARCOMA	ADENOCARCINOMA	ADENOCARCINOMA	N
4	1969_225	UNSPECIFIED	UNSPECIFIED	U	U	B	EYE-LIMBUS	UNSPECIFIED	SCC	SCC	N
5	1969_562	UNSPECIFIED	UNSPECIFIED	F	19	B	CHEEK	UNSPECIFIED	MALIGNANT SCHWANNOMA	MISSING	N
6	1970_244	UNSPECIFIED	PONY	F	20	B	EYE-MEDIAL CANTHUS	UNSPECIFIED	CONJUNCTIVITIS	CONJUNCTIVITIS	I
7	1970_244	UNSPECIFIED	PONY	F	20	B	EYE-MEDIAL CANTHUS	UNSPECIFIED	MELANOMA	MELANOMA	N
8	1970_306	UNSPECIFIED	UNSPECIFIED	M	3	B	BACK	UNSPECIFIED	DERMATITIS	DERMATITIS	I
9	1970_361	UNSPECIFIED	PONY	F	7	B	EYE-THIRD EYELID	SCC	SCC	SCC	N
10	1970_465	UNSPECIFIED	UNSPECIFIED	F	5	B	CHEEK	SARCOID	FIBROMA	MISSING	N
11	1971_096	UNSPECIFIED	PONY	M	30	C	PERINEUM	UNSPECIFIED	MELANOSARCOMA	MELANOSARCOMA	N
12	1971_198	UNSPECIFIED	UNSPECIFIED	M	2	B	VENTRUM	UNSPECIFIED	FIBROSARCOMA	FIBROSARCOMA	N

Appendix 7.4 continued

SN NO.	CASE #	NAME	BREED	SEX	AGE	SOURCE	AREA AFFECTED	CLINICAL DIAGNOSIS	FORMER HISTOPATHOLOGICAL DIAGNOSIS	CURRENT HISTOPATHOLOGICAL DIAGNOSIS	TYPE
13	1971_207	UNSPECIFIED	UNSPECIFIED	F	3.5	B	BACK	FIBROMA	GRANULOMA	MISSING	I
14	1971_467	UNSPECIFIED	UNSPECIFIED	F	8	B	NECK	DERMATITIS	GRANULOMA	GRANULOMA	I
15	1971_493	UNSPECIFIED	UNSPECIFIED	M	1.5	B	THIGH	UNSPECIFIED	SARCOID	SARCOID	N
16	1971_512	UNSPECIFIED	UNSPECIFIED	U	U	B	UNSPECIFIED	MICROFILARIASIS	GRANULOMA	GRANULOMA	I
17	1972_0081	UNSPECIFIED	UNSPECIFIED	M	3	B	MUZZLE	UNSPECIFIED	PAPILLOMA	PAPILLOMA	I
18	1972_0225	UNSPECIFIED	UNSPECIFIED	F	15	B	UNSPECIFIED	UNSPECIFIED	PAPILLOMA	PAPILLOMA	I
19	1972_0245	UNSPECIFIED	UNSPECIFIED	U	U	B	UNSPECIFIED	UNSPECIFIED	PARAKERATOSIS	MISSING	I
20	1972_0498	UNSPECIFIED	UNSPECIFIED	M	U	B	EYE-LIMBUS	SCC	SCC	MISSING	N
21	1972_0718	UNSPECIFIED	UNSPECIFIED	M	15	C	PENIS	UNSPECIFIED	MELANOSARCOMA	MELANOSARCOMA	N
22	1972_0718	UNSPECIFIED	UNSPECIFIED	M	15	C	PENIS	UNSPECIFIED	PAPILLOMA	PAPILLOMA	I
23	1972_0718	UNSPECIFIED	UNSPECIFIED	M	15	C	PENIS	UNSPECIFIED	SCC	SCC	N
24	1972_1086	UNSPECIFIED	PONY	U	U	B	NECK	UNSPECIFIED	PAPILLOMA	PAPILLOMA	I
25	1972_1126	UNSPECIFIED	UNSPECIFIED	M	U	B	BRISKET	UNSPECIFIED	DERMATITIS	MISSING	I
26	1973_701	UNSPECIFIED	UNSPECIFIED	M	23	C	SUBCUTANEOUS	MELANOSARCOMA	MELANOSARCOMA	MELANOSARCOMA	N
27	1974_435	UNSPECIFIED	UNSPECIFIED	M	U	B	EYE-EYELID	UNSPECIFIED	CONJUNCTIVITIS	CONJUNCTIVITIS	I

Appendix 7.4 continued

SN NO.	CASE #	NAME	BREED	SEX	AGE	SOURCE	AREA AFFECTED	CLINICAL DIAGNOSIS	FORMER HISTOPATHOLOGICAL DIAGNOSIS	CURRENT HISTOPATHOLOGICAL DIAGNOSIS	TYPE
28	1974_435	UNSPECIFIED	UNSPECIFIED	M	U	B	EYE-THIRD EYELID	UNSPECIFIED	CONJUNCTIVITIS	CONJUNCTIVITIS	I
29	1974_541	UNSPECIFIED	UNSPECIFIED	M	18	B	UNSPECIFIED	MELANOMA	MELANOSARCOMA	MELANOSARCOMA	N
30	1974_559	UNSPECIFIED	UNSPECIFIED	F	7	B	LIMB-METATARSUS	UNSPECIFIED	EGT	EGT	M
31	1974_605	UNSPECIFIED	UNSPECIFIED	M	U	B	LIP	UNSPECIFIED	FIBROMA	FIBROMA	N
32	1975_007	UNSPECIFIED	UNSPECIFIED	M	10	B	PENIS	UNSPECIFIED	PAPILLOMA	MISSING	I
33	1975_504	UNSPECIFIED	UNSPECIFIED	U	U	B	UNSPECIFIED	UNSPECIFIED	SCC	SCC	N
34	1975_582	UNSPECIFIED	TB	F	12	B	LIMB-FROG	UNSPECIFIED	PAPILLOMA	PAPILLOMA	I
35	1975_831	UNSPECIFIED	TB	M	13	C	CHEEK	UNSPECIFIED	MELANOMA	MELANOMA	N
36	1975_831	UNSPECIFIED	TB	M	13	C	THIGH	UNSPECIFIED	MELANOMA	MELANOMA	N
37	1975_934	UNSPECIFIED	UNSPECIFIED	M	U	B	EYE-THIRD EYELID	UNSPECIFIED	SCC	SCC	N
38	1975_941	UNSPECIFIED	UNSPECIFIED	M	16	B	PREPUCE	UNSPECIFIED	SCC	SCC	N
39	1976_029	UNSPECIFIED	UNSPECIFIED	M	2	B	LIMB-FETLOCK	SARCOID	SARCOID	SARCOID	N
40	1976_065	UNSPECIFIED	UNSPECIFIED	U	U	B	UNSPECIFIED	UNSPECIFIED	GRANULATION TISSUE	GRANULOMA	I
41	1976_867	UNSPECIFIED	PONY	M	20	C	TAIL BASE	UNSPECIFIED	MELANOSARCOMA	MELANOSARCOMA	N
42	1977_300	UNSPECIFIED	UNSPECIFIED	F	14	B	SUBCUTANEOUS-FOREHEAD	FIBROSARCOMA	OSTEOCHONDROMA	MISSING	N

Appendix 7.4 continued

SN NO.	CASE #	NAME	BREED	SEX	AGE	SOURCE	AREA AFFECTED	CLINICAL DIAGNOSIS	FORMER HISTOPATHOLOGICAL DIAGNOSIS	CURRENT HISTOPATHOLOGICAL DIAGNOSIS	TYPE
43	1977_395	UNSPECIFIED	UNSPECIFIED	F	3	B	FACE	UNSPECIFIED	GRANULOMA	GRANULOMA	I
44	1977_500	UNSPECIFIED	UNSPECIFIED	M	4	B	LIP COMMISSURE	UNSPECIFIED	SARCOID	SARCOID	N
45	1977_780	UNSPECIFIED	UNSPECIFIED	M	6	B	BACK	UNSPECIFIED	MELANOMA	MELANOMA	N
46	1977_781	UNSPECIFIED	UNSPECIFIED	M	7	B	LIP COMMISSURE	UNSPECIFIED	DERMATITIS	GRANULOMA	I
47	1977_878	UNSPECIFIED	UNSPECIFIED	F	U	B	VULVA	UNSPECIFIED	SCC	SCC	N
48	1978_064	UNSPECIFIED	UNSPECIFIED	F	U	B	EYE-MEDIAL CANTHUS	UNSPECIFIED	SCC	SCC	N
49	1978_192	UNSPECIFIED	UNSPECIFIED	F	3	B	EYE-THIRD EYELID	UNSPECIFIED	SCC	SCC	N
50	1978_301	UNSPECIFIED	UNSPECIFIED	F	U	B	PERINEUM	UNSPECIFIED	FIBROMA	FIBROMA	N
51	1980_019	UNSPECIFIED	UNSPECIFIED	F	7	B	EYE-THIRD EYELID	UNSPECIFIED	FIBROMA	FIBROMA	N
52	1980_028	UNSPECIFIED	UNSPECIFIED	M	30	B	PENIS	UNSPECIFIED	PAPILLOMA	PAPILLOMA	I
53	1980_239	UNSPECIFIED	UNSPECIFIED	F	U	B	VENTRUM	UNSPECIFIED	SCC	SCC	N
54	1987_024	UNSPECIFIED	UNSPECIFIED	F	2	B	MAMMARY GLAND	UNSPECIFIED	MELANOSARCOMA	MELANOSARCOMA	N
55	1989_290	UNSPECIFIED	UNSPECIFIED	M	14	B	UNSPECIFIED	UNSPECIFIED	FIBROMA	FIBROMA	N
56	1989_426	UNSPECIFIED	UNSPECIFIED	F	U	B	BACK	UNSPECIFIED	DERMOID CYST	DERMOID CYST	M
57	1991_090	UNSPECIFIED	UNSPECIFIED	F	8	B	EYE-THIRD EYELID	UNSPECIFIED	SCC	SCC	N

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SN NO.	CASE #	NAME	BREED	SEX	AGE	SOURCE	AREA AFFECTED	CLINICAL DIAGNOSIS	FORMER HISTOPATHOLOGICAL DIAGNOSIS	CURRENT HISTOPATHOLOGICAL DIAGNOSIS	TYPE
58	1991_371	UNSPECIFIED	UNSPECIFIED	F	U	B	LIMB-KNEE	GRANULOMA	GRANULOMA	GRANULOMA	I
59	1992_139	UNSPECIFIED	UNSPECIFIED	U	U	B	UNSPECIFIED	UNSPECIFIED	SARCOID	SARCOID	N
60	1992_155	UNSPECIFIED	UNSPECIFIED	U	U	B	EYE-EYELID	UNSPECIFIED	SCC	SCC	N
61	1992_179	UNSPECIFIED	UNSPECIFIED	U	U	B	BACK	UNSPECIFIED	DERMATITIS	DERMATITIS	I
62	1992_277	UNSPECIFIED	UNSPECIFIED	F	2	B	CHEEK	UNSPECIFIED	ODONTOMA	MISSING	N
63	1992_277	UNSPECIFIED	UNSPECIFIED	F	2	B	UNSPECIFIED	UNSPECIFIED	FIBROMA	MISSING	N
64	1992_298	UNSPECIFIED	UNSPECIFIED	U	U	B	EYE-SCLERA	UNSPECIFIED	MELANOMA	MELANOMA	N
65	1992_310	UNSPECIFIED	UNSPECIFIED	M	U	B	EAR-BASE	UNSPECIFIED	FIBROMA	FIBROMA	N
66	1995_078	UNSPECIFIED	UNSPECIFIED	F	2	B	LIP COMMISSURE	FIBROMA	GRANULOMA	GRANULOMA	I
67	1995_079	UNSPECIFIED	UNSPECIFIED	F	12	C	MAMMARY GLAND	LYPHOSARCOMA	LYMPHOSARCOMA	LYMPHOSARCOMA	N
68	1995_134	UNSPECIFIED	UNSPECIFIED	U	U	B	VENTRUM	UNSPECIFIED	INFLAMMATORY NODULE	GRANULOMA	I
69	1995_142	UNSPECIFIED	UNSPECIFIED	F	6	B	LIP COMMISSURE	UNSPECIFIED	FIBROMA	FIBROMA	N
70	1995_150	UNSPECIFIED	UNSPECIFIED	M	4	B	LIMB-METATARSUS	FIBROMA	SARCOID	SARCOID	N
71	1995_155	UNSPECIFIED	UNSPECIFIED	F	1.5	B	EYE-THIRD EYELID	UNSPECIFIED	LYMPHOID HYPERPLASIA	LYMPHOID HYPERPLASIA	M
72	1995_156	UNSPECIFIED	UNSPECIFIED	F	5	B	EYE-THIRD EYELID	UNSPECIFIED	ADENOMATOUS HYPERPLASIA	ADENOMATOUS HYPERPLASIA	M

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SN NO.	CASE #	NAME	BREED	SEX	AGE	SOURCE	AREA AFFECTED	CLINICAL DIAGNOSIS	FORMER HISTOPATHOLOGICAL DIAGNOSIS	CURRENT HISTOPATHOLOGICAL DIAGNOSIS	TYPE
73	1995_156	UNSPECIFIED	UNSPECIFIED	F	5	B	EYE-THIRD EYELID	UNSPECIFIED	SCC	SCC	N
74	1995_217	UNSPECIFIED	UNSPECIFIED	U	U	B	NECK	UNSPECIFIED	HAEMANGIOSARCOMA	MISSING	N
75	1995_244	UNSPECIFIED	UNSPECIFIED	F	U	B	EYE-EYELID	SCC	SCC	SCC	N
76	1995_311	UNSPECIFIED	UNSPECIFIED	M	3	B	EYE-THIRD EYELID	UNSPECIFIED	LYMPHOMA	LYMPHOMA	N
77	1996_198	UNSPECIFIED	UNSPECIFIED	F	U	B	UNSPECIFIED	UNSPECIFIED	MELANOMA	MELANOMA	N
78	1996_271	UNSPECIFIED	UNSPECIFIED	M	U	B	EYE-THIRD EYELID	UNSPECIFIED	SCC	SCC	N
79	1996_276	UNSPECIFIED	UNSPECIFIED	U	U	B	UNSPECIFIED	UNSPECIFIED	SCC	SCC	N
80	1997_220	UNSPECIFIED	UNSPECIFIED	F	12	B	LIMB-ELBOW	UNSPECIFIED	GRANULOMA	GRANULOMA	I
81	1997_220	UNSPECIFIED	UNSPECIFIED	F	12	B	NECK	UNSPECIFIED	SARCOID	SARCOID	N
82	1997_243	UNSPECIFIED	UNSPECIFIED	M	7	B	LIMB-KNEE	SARCOID	SARCOID	SARCOID	N
83	1998_289	UNSPECIFIED	EACB	F	5	B	PERINEUM	UNSPECIFIED	MELANOMA	MELANOMA	N
84	1998_333	UNSPECIFIED	UNSPECIFIED	U	U	B	EYE-THIRD EYELID	UNSPECIFIED	SCC	MISSING	N
85	2005_109	UNSPECIFIED	PONY	M	17	B	EYE-THIRD EYELID	SCC	ADENOMA	ADENOMA	N
86	2005_109	UNSPECIFIED	PONY	M	17	B	NECK	SARCOID	GRANULOMA	GRANULOMA	I
87	2007_310	UNSPECIFIED	TB	M	6	B	EAR-PINNA	SARCOID	SARCOID	SARCOID	N

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SN NO.	CASE #	NAME	BREED	SEX	AGE	SOURCE	AREA AFFECTED	CLINICAL DIAGNOSIS	FORMER HISTOPATHOLOGICAL DIAGNOSIS	CURRENT HISTOPATHOLOGICAL DIAGNOSIS	TYPE
88	2007_311	UNSPECIFIED	TB	M	18	B	EYE-LIMBUS	MELANOMA	MELANOMA	MELANOMA	N
89	2008_010	IVAN	UNSPECIFIED	U	U	B	EYE-SCLERA	MELANOMA	SCC	SCC	N
90	2008_126	UNSPECIFIED	UNSPECIFIED	U	U	B	PREPUCE	UNSPECIFIED	GRANULOMA	MISSING	I
91	2008_344	POLE STAR	TB	M	U	B	EYE-THIRD EYELID	SCC	SCC	SCC	N
92	2009_159	STEP-IN-THYME	CROSS	F	22	B	NOSTRIL	UNSPECIFIED	GRANULOMA	GRANULOMA	I
93	2010_292	ZWELI	EACB	M	U	B	EAR-PINNA	PAPILLOMA	PAPILLOMA	PAPILLOMA	I
94	2010_476	UNSPECIFIED	APPALOOSA	M	U	B	EYE-THIRD EYELID	SCC	SCC	SCC	N
95	2011_262	UNSPECIFIED	PONY	M	U	B	EYE-SCLERA	SCC	SCC	SCC	N
96	2011_351	UNSPECIFIED	TB	M	9	B	EYE-LIMBUS	UNSPECIFIED	MELANOMA	MELANOMA	N
97	2011_358	UNSPECIFIED	TB	F	6	B	LIMB-ELBOW	UNSPECIFIED	FIBROMA	FIBROMA	N
98	2011_404	SHEILA	EACB	F	18	B	EAR-BASE	SARCOID	SARCOID	SARCOID	N
99	2011_405	YOURESOVAIN	SATB	F	3	B	THIGH	SARCOID	SARCOID	SARCOID	N
100	2011_437	CASSIDY	APPALOOSA	M	9	B	LIMB-ELBOW	SARCOID	GRANULOMA	GRANULOMA	I
101	2012_031	TRES CHIC	UNSPECIFIED	F	U	B	NECK	GRANULOMA	GRANULOMA	GRANULOMA	I
102	2012_204	DIAMOND	ZaTB	F	22	B	THIGH	SARCOID	GRANULOMA	GRANULOMA	I

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SN NO.	CASE #	NAME	BREED	SEX	AGE	SOURCE	AREA AFFECTED	CLINICAL DIAGNOSIS	FORMER HISTOPATHOLOGICAL DIAGNOSIS	CURRENT HISTOPATHOLOGICAL DIAGNOSIS	TYPE
103	2012_283	PEACE	SATB	F	12	B	EYE-LIMBUS	SCC	GRANULOMA	GRANULOMA	I
104	2012_321	OHARA	TB	F	6	B	EYE-THIRD EYELID	SCC	SCC	SCC	N
105	2012_322	PC	EACB	F	9	B	EYE-THIRD EYELID	SCC	SCC	SCC	N
106	2012_331	EUPHORIA	PONY	M	8	B	EYE-EYELID	SCC	SCC	SCC	N
107	2012_337	JESSIE	ETHIOPIAN CROSS	F	10	B	EYE-THIRD EYELID	SCC	BASAL CELL TUMOUR	BASAL CELL TUMOUR	N
108	2012_389	U	UNSPECIFIED	M	18	B	EYE-THIRD EYELID	SCC	SCC	SCC	N
109	2012_400	DREAM	UNSPECIFIED	F	8	B	VENTRUM	FIBROMA	SARCOID	SARCOID	N
110	2013_005	LORD THOMAS	ZbTB	M	5	B	VENTRUM	SARCOID	GRANULOMA	GRANULOMA	I
111	2013_014	JAZZ	ZbTB	M	3	B	LIP COMMISSURE	SARCOID	SARCOID	SARCOID	N
112	2013_036	ARIZONA	TB	F	9	B	EAR-PINNA	SARCOID	FIBROMA	FIBROMA	N
113	2013_135	JCB	TB	M	3	B	LIP COMMISSURE	DERMATITIS	DERMATITIS	DERMATITIS	I
114	2013_136	JCB	TB	M	3	B	EAR-PINNA	DERMATITIS	DERMATITIS	DERMATITIS	I
115	2013_176	CHACHA	ETHIOPIAN PONY	M	6	B	EAR-PINNA	SARCOID	GRANULOMA	GRANULOMA	I
116	2013_187	PC	EACB	F	12	B	EYE-THIRD EYELID	SCC	CONJUNCTIVITIS	CONJUNCTIVITIS	I
117	2013_187	PC	EACB	F	12	B	EYE-THIRD EYELID	SCC	SCC	SCC	N

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SN NO.	CASE #	NAME	BREED	SEX	AGE	SOURCE	AREA AFFECTED	CLINICAL DIAGNOSIS	FORMER HISTOPATHOLOGICAL DIAGNOSIS	CURRENT HISTOPATHOLOGICAL DIAGNOSIS	TYPE
118	2013_188	DARLING HARBOUR	TB	F	13	B	LIMB-HEEL	SARCOID	FIBROMA	FIBROMA	N
119	2013_209	NORTHERN FRONTIER	SATB	M	2	B	PENIS	UNSPECIFIED	FIBROMA	FIBROMA	N
120	2013_219	GOLDIE	PONY	F	20	B	EYE-LIMBUS	SCC	SCC	SCC	N
121	2013_224	INKERMAN	TB	M	22	B	EAR-BASE	GRANULATION TISSUE	GRANULOMA	GRANULOMA	I
122	2013_289	CIELO	ZbTB	F	21	B	EAR-PINNA	SARCOID	SARCOID	SARCOID	N
123	2013_314	SPRITE	SATB	F	7	B	NECK	LYMPHANGITIS	LYMPHANGITIS	LYMPHANGITIS	I
124	2013_315	OFF THE HOOK	UNSPECIFIED	F	9	B	EYE-THIRD EYELID	UNSPECIFIED	ADENOMA	ADENOMA	N
125	2014_026	RAVEN'S CLIFF	UNSPECIFIED	M	16	B	EAR-PINNA	UNSPECIFIED	INFLAMMATORY NODULE	INFLAMMATORY NODULE	I
126	2014_107	TAKEWAY	SA PONY	M	9	B	AXILLA	SARCOID	SARCOID	SARCOID	N
127	2014_108	DIAMANTE	TB	M	23	B	EAR-PINNA	SARCOID	GRANULOMA	GRANULOMA	I
128	2014_119	CIELO	ZbTB	F	22	B	EAR-PINNA	SARCOID	SARCOID	SARCOID	N
129	2014_146	BARONESSA	TB	F	7	B	EAR-PINNA	SARCOID	SARCOID	SARCOID	N
130	2014_195	LEVENDISA	UNSPECIFIED	F	12	B	VULVA	CYST	SCC	SCC	N
131	2014_227	MARA NELLA	TB	F	15	B	EAR-CANAL	MELANOMA	INFLAMMATORY NODULE	INFLAMMATORY NODULE	I
132	2014_249	SHAKARA MAN	SATB	M	3	B	EYE-MEDIAL CANTHUS	SARCOID	EPIDERMOID CYST	EPIDERMOID CYST	M

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SN NO.	CASE #	NAME	BREED	SEX	AGE	SOURCE	AREA AFFECTED	CLINICAL DIAGNOSIS	FORMER HISTOPATHOLOGICAL DIAGNOSIS	CURRENT HISTOPATHOLOGICAL DIAGNOSIS	TYPE
133	2014_249	SHAKARA MAN	SATB	M	3	B	VENTRUM	SARCOID	PAPILLOMA	PAPILLOMA	I
134	2014_249	SHAKARA MAN	SATB	M	3	B	LIMB-CARPUS	SARCOID	SARCOID	SARCOID	N
135	2014_343	CARA MIA	TB	F	8	B	NECK	POX	PARAKERATOSIS	PARAKERATOSIS	I
136	2014_344	THISTLE	UNSPECIFIED	F	A	B	LIMB-SCAPULA	GRANULOMA	DERMATITIS	DERMATITIS	I
137	2014_369	LEVENDISA	UNSPECIFIED	F	A	B	VULVA	SCC	SCC	SCC	N
138	2014_380	WOW FACTOR	WARMBLOOD	M	5	B	NECK	SARCOID	SARCOID	SARCOID	N
139	2014_401	ARIZONA	TB	F	10	B	EAR-PINNA	SARCOID	SARCOID	SARCOID	N
140	2014_402	MEGA STAR	TB	M	8	B	EYE -THIRD EYELID	SCC	SCC	SCC	N
141	2014_454	SHEFFIELD	SATB	F	8	B	EYE-LIMBUS	SCC	SCC	SCC	N

Key:-

Breed:	TB – Thoroughbred	EACB – East African Countrybred	SATB – South African Thoroughbred
	ZaTB – Zambian Thoroughbred	ZbTB – Zimbabwean Thoroughbred	SA Pony – South African Pony
Sex and Age:	M – Male	F – Female	U - Unspecified
Source:	B – Biopsy	C – Carcass	
Diagnosis:	SCC – Squamous Cell Carcinoma	EGT – Exuberant Granulation Tissue	
Type:	N – Neoplastic	I – Inflammatory	M – Miscellaneous