

## MAIN PATHOLOGIES ASSOCIATED WITH *STAPHYLOCOCCUS AUREUS* INFECTIONS IN RABBITS: A REVIEW

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**ABSTRACT:** *Staphylococcus aureus* is a versatile opportunistic pathogen that causes a wide spectrum of pathologies. In rabbits, this bacterium infects dermal lesions causing suppurative dermatitis, and invades subcutaneous tissues, causing different well-known disease conditions such as mastitis, abscesses (subcutaneously or affecting internal organs) and pododermatitis. However, the lesions associated with *S. aureus* have rarely been described in detail in the literature. The aim of this paper is to update the knowledge on rabbit staphylococcosis by focusing mainly on the different pathologies that this organism produces in commercial rabbits.

**Key words:** *Staphylococcus aureus*, rabbit, infection, pathology.

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### INTRODUCTION

*Staphylococcus aureus* is a versatile opportunistic microorganism which is capable of persisting and multiplying in a variety of environments and causes a wide spectrum of diseases in both humans and animals (Cucarella *et al.*, 2004). In animals, staphylococcal infections lead to substantial economic losses in the livestock industry worldwide (Mork *et al.*, 2005). This bacterium affects rabbits of different ages, infects dermal lesions and invades subcutaneous tissues (Okerman *et al.*, 1984), resulting in different pathologies including suppurative dermatitis, mastitis, multisystemic abscessation and pododermatitis (Hagen, 1963; Segura *et al.*, 2007; Vancraeynest *et al.*, 2004). In suckling kits, the most typical lesion is exudative dermatitis (Okerman *et al.*, 1984; Holliman and Girvan, 1986). In broiler rabbits, abscesses lead to increased slaughterhouse condemnations. Sporadically, abscesses in lungs, liver and uterus are also observed, leading to poor production, infertility and death (Vancraeynest *et al.*, 2006a). Mastitis is the most important gross pathological cause of culling in adult rabbit does, followed by subcutaneous abscesses and pyometra (Segura *et al.*, 2007). Finally, staphylococcosis caused by *S. aureus* in rabbits may be characterised by fatal septicaemia or suppurative inflammations which may occur in practically any organ or site (Flatt, 1974).

It has been described that rabbits can be infected by two types of *S. aureus* strains: high- and low-virulence strains. Healthy animals may be carriers of highly virulent *S. aureus* strains and it is currently generally accepted that these microorganisms are usually introduced into a rabbit flock with no previous history

of the disease by such carrier rabbits (Devriese *et al.*, 1981). Colonisation may be found throughout the body (Hermans *et al.*, 1999). It has been reported that the carrier status may favour invasion of primary traumatic lesions (Okerman *et al.*, 1984). As neither isolation nor culling of diseased animals prevents the disease, the carriage of pathogenic strains by apparently healthy animals is of considerable importance in maintaining infection within the herd. In addition, the healthy kits of a diseased doe may transmit infection when fostered by an uninfected doe (Adlam *et al.*, 1976).

In most rabbitries, infection occurs sporadically and is only a minor cause of losses (Okerman *et al.*, 1984; Holliman and Girvan, 1986). These infections are caused by low-virulence (LV) *S. aureus* strains. Serious economic losses have however been reported in the USA (Hagen, 1963), England (Holliman and Girvan, 1986), Italy, France and Belgium (Okerman *et al.*, 1984). This special form of staphylococcal disease, associated with a high mortality rate in young rabbits, is assigned to specific high virulence (HV) *S. aureus* strains.

Although staphylococcosis in rabbits is a well-known disease, the pathological findings have rarely been described in detail in the literature. For this reason, the objective of this review is to update the knowledge on rabbit staphylococcosis by focusing mainly on the different disease conditions and lesions that this microorganism may cause in commercial rabbits.

## AETIOLOGY AND EPIDEMIOLOGY

Staphylococcosis in rabbits is caused by *S. aureus*. This species can be divided into several biotypes based on the biochemical properties of the isolates. Some biotypes are considered host-specific, while other may colonise different hosts. Human, bovine, ovine and poultry biotypes have been described (Devriese, 1984). Strains of these biotypes may occasionally be found in other host species.

Low-virulence *S. aureus* strains causing infections in rabbits may belong to the human or poultry biotype (Devriese *et al.*, 1981; Devriese, 1984). Severe outbreaks of the disease in rabbitries are, however, usually caused by high-virulence strains belonging to another biotype, the “mixed CV-C” type (Devriese, 1984; Devriese *et al.*, 1996). This biotype differs from most human strains and from all poultry strains in its production of beta haemolysin. Furthermore, human strains produce staphylokinase, while poultry strains display a different growth type on tryptose agar containing crystal violet. Highly virulent rabbit *S. aureus* strains, which always belong to the “mixed CV-C” biotype, are typically beta haemolysin-positive, staphylokinase-negative and display a purple growth on crystal violet agar (Devriese *et al.*, 1981; 1996). Biotyping is not sufficient to distinguish between high- and low- virulence *S. aureus* strains in rabbits since the “mixed CV-C” biotype comprises both high- and low-virulence strains (Devriese *et al.*, 1981). Therefore, other typing methods are required to distinguish further between high- and low-virulence strains.

Phage typing provides a further subdivision of *S. aureus* strains through the identification of bacteriophages to which the bacterium is susceptible. Phage typing is accomplished using bacteriophages of the international typing set for human *S. aureus* strains (Parker, 1962). *S. aureus* isolates from rabbits are usually susceptible to several of these phages. Low-virulence strains may belong to a wide range of phage types (Devriese *et al.*, 1981; Hermans *et al.*, 1999). The strains isolated from epidemic outbreaks are susceptible to at least one of the phages of phage group II, comprising phages 3A, 3C, 55 and 71 (Vancraeynest *et al.*, 2006a). A highly virulent strain belonging to the “mixed CV-C” biotype, but with a different phage susceptibility pattern (phage type 29/79/42E/92/D11/HK2), has been described only once (Devriese *et al.*, 1996). In an experimental skin infection trial, LV strains and classic HV strains could clearly be distinguished based on the severity of the lesions (Meulemans *et al.*, 2007). Classic HV strains have been described as causing severe problems in many different European countries, including

Belgium, the United Kingdom, Germany, Ireland, Italy, Greece and Spain (Okerman *et al.*, 1984; Carolan, 1986; Holliman and Girvan, 1986; Rossi *et al.*, 1995; Hermans *et al.*, 2000; Vancraeynest *et al.*, 2006a), and their importance has not appreciably declined since their first description. Severe problems of disseminating staphylococcosis have also been described in the USA (Hagen, 1963), but the *S. aureus* strains responsible have not been further characterised.

Besides their phenotypic characterisation, *S. aureus* strains from rabbits have also been examined genotypically. Hermans *et al.* (2000) described classic HV strains belonging to a specific Randomly Amplified Polymorphic DNA (RAPD) type. Their studies suggested a single clonal origin of the typical HV strains (Hermans *et al.*, 2000; Hermans *et al.*, 2001). Vancraeynest *et al.* (2006a) confirmed this using Pulsed Field Gel Electrophoresis (PFGE) as all the HV strains showed the same PFGE type. Viana *et al.* (2007) used a different genotypic method based on three genetic markers (coagulase, staphylococcal protein A and clumping factor B genes). They found that the most prevalent type in the Province of Valencia on the Spanish Mediterranean coast was A1/III1/δ (*coa/spa/clfB* combination genotype).

Disseminating problems of staphylococcosis in domestic rabbits were first described in the USA in 1963 (Hagen, 1963). In Western Europe, these infections have been considered important since the 1980s, when outbreaks were frequently reported in several European countries (Devriese *et al.*, 1981; Okerman *et al.*, 1984; Carolan, 1986; Holliman and Girvan, 1986; Devriese *et al.*, 1987; Rossi *et al.*, 1995; Devriese *et al.*, 1996). This scenario was probably the result of a higher occupation density due to the appearance of large-scale commercial rabbitries. Because of increased demand, the traditional production of rabbits evolved to an industrial or semi-industrial level. High densities of animals increase the importance of infectious diseases since this phenomenon may favour the spread of pathogenic agents throughout the flock.

Disseminating staphylococcosis can be found in hygienic and less hygienic rabbitries (Hermans *et al.*, 1999), and might indicate that bacterium-host interactions rather than management factors determine the epidemic spread of the disease in the rabbitry. The capacity to colonise host epithelia has been shown to be higher in HV strains (Hermans *et al.*, 1999). Better colonisation may imply higher infection pressure, which may increase the risk of the expression of the disease in the flock.

Transmission of HV and LV *S. aureus* strains from humans to rabbits or between rabbits may be direct or indirect through cages, hair or food (Devriese *et al.*, 1987; Matthes, 1995; Rossi *et al.*, 1995). Direct transmission of *S. aureus* bacteria may occur between does and suckling kits (Devriese *et al.*, 1981; Matthes, 1995), between litter mates and between stable mates (Devriese *et al.*, 1981). Devriese *et al.* (1981; 1987) noticed that rabbitries infected with identical *S. aureus* strains were often in direct or indirect contact, and that the intake of new breeding rabbits in the flock was probably the most important source of infection. Sperm (even after artificial insemination) is also a potential risk of infection by HV *S. aureus* strains in rabbits (Rossi *et al.*, 1995).

## PATHOGENESIS AND LESIONS

Generally *S. aureus* infection gives rise to suppurative lesions or to abscess formation at the infection site (Hermans *et al.*, 2003). The lesions caused by *S. aureus* are thus mainly suppurative dermatitis, mastitis, abscesses (subcutaneous or affecting internal organs) and pododermatitis (Okerman *et al.*, 1984; Segura *et al.*, 2007).

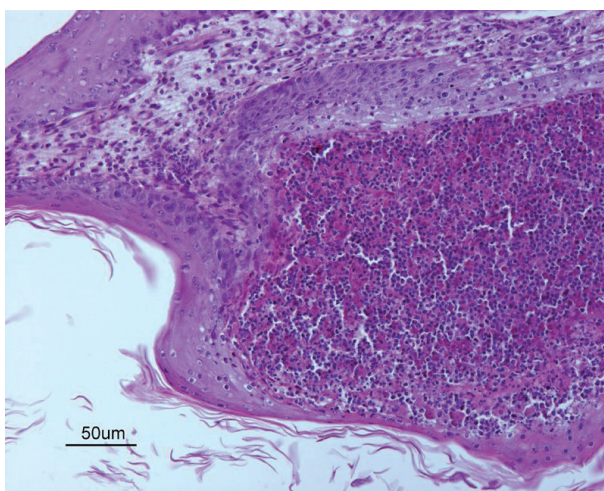
### Suppurative dermatitis

In Mediterranean countries, veterinarians, farmers and other professionals consider the term “staphylococcosis” to be a synonym of “cutaneous staphylococcosis” (Rosell, 2000). Although the first

reference to this condition dates back to 1903 (Catterina, 1903 cited by Rosell, 2000), the first detailed description was reported in 1963 (Hagen 1963). Several cases of this cutaneous form of staphylococcosis, associated with high mortality in kits, have been described (Hagen, 1963; Okerman *et al.*, 1984). In these outbreaks, different forms of disease were observed. Exudative dermatitis with small superficial pustules occurred in the youngest hairless animals. Losses caused by this form of lesion were high and mainly occurred in the second week, the whole litter usually being affected and dying. Different-sized subcutaneous abscesses were found in older animals, and purulent rhinitis and conjunctivitis were frequent signs in suckling rabbits (Okerman *et al.*, 1984). In such cases, where the rabbit-pathogenic phage type 3A/3C/55/71 was identified, the heaviest losses were associated with chronic mastitis, while high losses of kits were partly due to difficulties in suckling (Devriese *et al.*, 1996). It was during these outbreaks that the term “highly virulent” *S. aureus* strains was coined.

It is likely that the portal of entry is either the umbilical stump or skin abrasions, or both. Infection probably occurs on the day of birth, as it has been reported that healthy kits that had been transferred to infected litters during the first week after birth did not develop abscesses (Hagen, 1963).

Cutaneous staphylococcosis is characterized by a suppurative dermatitis, which is mainly observed in kits. Animals develop numerous small white subcutaneous abscesses scattered over the lower abdomen, the inner aspects of the forelegs and the lower jaw. When rabbits are 3 to 5 d old, these appear in the form of pinpoint lesions that develop into firm caseous pustules. Once the rabbits are 10 d old, lesions are obscured by fur (Hagen, 1963). Septicaemia is a common consequence with abscesses in the heart, lungs, and sometimes in the brain and kidneys (Okerman *et al.*, 1984). Microscopically, the epidermis shows dystrophic alterations, the dermal connective tissue is infiltrated with leucocytes (Figure 1), and some bacterial colonies can be seen (Marcato and Rosmini, 1986). When septicaemia develops, scattered foci of necrotic areas may be detected in other organs, such as the lungs and heart. In these cases, myocardic muscle cells undergo pronounced degenerative changes, and inflammatory cells are not detected (Hagen, 1963).



**Figure 1:** Cutaneous staphylococcosis. Dermal connective tissue infiltrated with abundant heterophils. 200×. Hematoxylin-Eosin.

## Mastitis

The most adverse effect of a staphylococcal infection in breeding does is the frequent occurrence of mastitis. In fact, chronic purulent mastitis has been reported to be the main gross cause of culling in farms, and *S. aureus* was isolated from 78.6% of the animals with mastitis (Segura *et al.*, 2007).

Mastitis may occur at any time during lactation: whereas one animal can contract the disease by suckling its first litter, another can produce several litters before becoming infected. Does which recover are often reinfected during a subsequent lactation (Adlam *et al.*, 1976). Animals with a history of repeated infections frequently develop ulcerated hocks. Occasionally, arthritis is also observed. Most infected animals nevertheless recover slowly and can eventually be returned to the herd (Adlam *et al.*, 1976).

When mastitis is contracted early in lactation, litters are usually lost. Does recovering from the disease frequently refuse to mate or to suckle new kits. In such cases, the economic losses stemming from the disease are significant. There have been reports that the number of does with active disease at any time on the farm ranges between 4 and 19 percent of a total of approximately 200 animals in lactation (Adlam *et al.*, 1976).

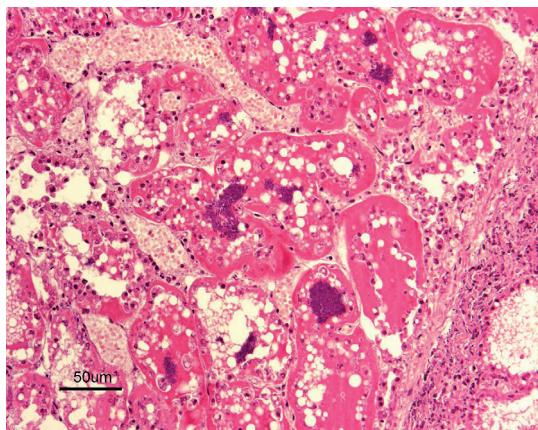
Clearly two main kinds of staphylococcal mammary infection have been described as occurring naturally in rabbits: acute or gangrenous, and chronic or purulent mastitis. In the acute or gangrenous form, one or more mammary glands become warm, reddened and swollen, and tend to become cyanotic at a later stage during the course of the infection. This lesion is therefore called “blue breast”. The mammary tissue becomes oedematous and haemorrhagic (Figure 2). This type of mastitis can spread rapidly through a rabbitry, and litters of affected does usually die of starvation (Zumpt, 1976; Adlam *et al.*, 1977; Holliman and Girvan, 1986); the doe can die within hours, or survive with chronic mammary gland changes (Zumpt, 1976). The chronic or purulent form is characterized by a thickening or induration of the mammary tissue around or near one or more teats. Infected animals develop abscesses in the mammary tissue of 2 cm to 10 cm in diameter over a period of 2 to 3 weeks (Figure 3). These abscesses discharge pus or develop into more chronic lesions which frequently contain sterile caseous material (autosterilization is a frequent consequence of chronic purulent lesions). Rabbit does look lethargic and are sometimes unable to suckle their kits (Adlam *et al.*, 1976).



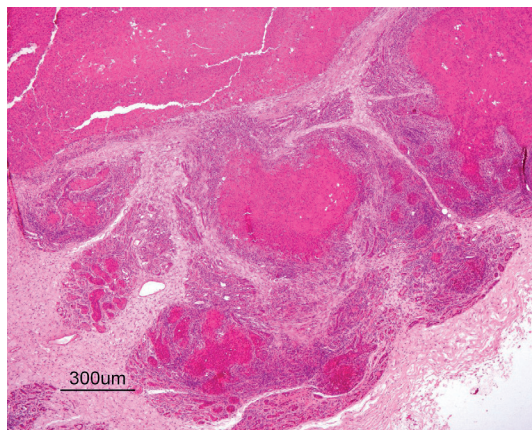
**Figure 2:** Acute mastitis (“blue breast”). The mammary glands are reddened and swollen. Subcutaneously, the mammary tissue is oedematous and haemorrhagic



**Figure 3:** Chronic mastitis. The mammary glands show a thickening and induration of the mammary tissue and an abscess discharging abundant white-yellowish pus.



**Figure 4:** Mammary tissue experimentally infected (48 h after infection). Clusters of *S. aureus* present within the alveoli with a low or no cellular response. 200×. Haematoxylin and eosin.



**Figure 5:** Mammary tissue chronically infected, showing a large number of heterophils within the alveolar spaces. The alveoli are packed with these cells. 40×. Haematoxylin and eosin.

Additionally, a special type of mastitis has been described which is not usually of the “blue breast” type, but of a more superficial kind with an exudation developing into a suppurative inflammation of the skin next to the teats. Inflammation of the deeper tissue is only seen in a few post mortem examinations, but attendants might not observe this variety as easily as the superficial form (Okerman *et al.*, 1984).

There are no reports describing the histological characteristics of acute naturally infected occurring staphylococcal mastitis. Does experimentally inoculated with *S. aureus* may develop acute gangrenous lesions. In this case, sections taken 48 h after inoculation either show organisms present in clusters within the alveoli with no cellular response or, more commonly, an involution of alveoli lined with mononuclear cells and desquamating epithelial cells (Figure 4). Sections taken at 4 d show alveoli lined by epithelial cells that are compressed due to the proliferation of polymorphonuclear leucocytes (PMN, also named heterophils in rabbits). Unlike the “abscess” type lesions, the majority of these cells are not present within the alveoli, but appear to infiltrate between them (Adlam *et al.*, 1976). In contrast to this, sections of chronically and mildly infected mammary tissue present a large number of PMN within the alveolar spaces (Figure 5). Some alveoli are packed with these cells; others appear to be normal or contain only a few cells. In old established lesions, large areas of the gland become degenerated and large clumps of organisms are visible (Adlam *et al.*, 1976).

Samples taken from gangrenous tissues at some distance from the experimental injection site are frequently sterile, suggesting that lesions are produced by toxin spreading rather than by organism proliferation (Adlam *et al.*, 1976). In fact, alpha-toxin has been reported to play a role in the pathogenesis of the haemorrhagic form of rabbit mastitis, “blue-breast”, seen in natural outbreaks. High serum anti-alpha-toxin titers have been associated with protection against this lethal form of mastitis, but only modify the clinical picture to the less severe and chronic abscess condition (Adlam *et al.*, 1977). In addition, and in contrast to the abscess lesion, PMN are not seen in tissue sections (Adlam *et al.*, 1976). Apparently if a staphylococcal strain is able to produce sufficient alpha-toxin to destroy PMN, the disease becomes gangrenous (Adlam *et al.*, 1977), and the infection progresses faster, resulting in the acute form of mastitis. Surprisingly, a *S. aureus* strain only causing chronic, purulent mastitis after natural infection may induce acute, gangrenous lesions in experimentally infected does. Whereas laboratory animals develop

gangrenous mastitis – presumably a toxigenic infection – naturally infected farm animals may possess natural or acquired resistance which results in lower severity of the disease (Adlam *et al.*, 1976).

The mammary gland has been shown to be highly susceptible to infection, but only when lactating (Adlam *et al.*, 1976). The reason for this hypersusceptibility remains unknown, but may be associated with the inability of milk PMN to kill staphylococci (Russell and Reiter, 1975). Milk fat globules and casein may inhibit the phagocytosis and destruction of *S. aureus* by PMN (Paape and Wergin, 1977).

### Abscesses

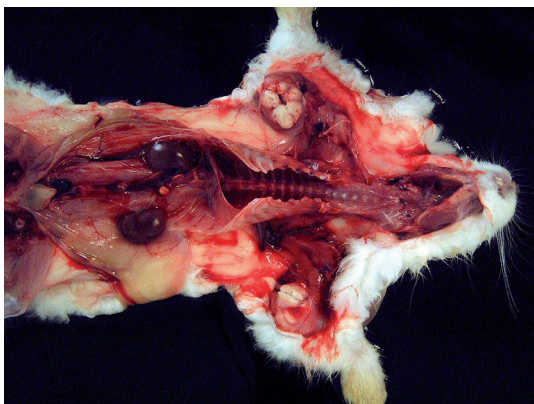
Sometimes the presence of abscesses is the main lesion associated with staphylococcal infection (Devriese *et al.*, 1996). In animals with abscesses, the death rate during the fattening period is not usually higher than normal (less than 10 percent) (Okerman *et al.*, 1984), but at times this situation can change. Subcutaneous abscesses occurred in two-to three-week old rabbits during an outbreak of staphylococcosis in two small commercial rabbitries, and many animals of this age died. There was also a high incidence of mastitis among does. Postmortem examination of litters revealed abscesses in the lungs as well as subcutaneous and interdigital abscesses (Carolan, 1986). This condition has recently been highlighted as one of the most frequent causes of culling bucks in Spain (Rosell and de la Fuente, 2009).

Abscesses originate from traumatic infections (insect bites, scratches by other rabbits, wounds caused by abrasive cage floors) (Marcato and Rosmini, 1986), and are found in rabbits of all ages (Segura *et al.*, 2007). The earliest clinical symptoms are observed in two-day-old kits and they are characterised by purulent lesions on the toes of the forelegs. Usually lesions are detected on the forelegs within the first week of life. They are also frequently found at the base of the nails, on the skin of the head, and commonly around the nares and chin. Upon weaning at around 30 d of age, small abscesses are visible on the back and on the sides (Devriese *et al.*, 1996). In a study carried out on four farms in adult rabbits, teats, mammary glands, skin and footpads were frequently the most affected areas. Abscesses in the distal parts of the forelegs were more common in does than in bucks, while abscesses in the mandibular region were more common in bucks (Devriese *et al.*, 1996). In a survey where *S. aureus* was isolated from 78.5% of the does with abscesses, the neck was the most frequently affected location (Table 1).

In the field, abscesses may appear as spherical, swollen nodules of variable size. They are mobile if they are located subcutaneously and they discharge abundant white-yellowish pus (Figure 6). The subcutaneous tissue around the abscesses is cyanotic due to local circulatory disorders, which may be followed by necrosis and skin ulceration. Depending on their location, subcutaneous abscesses cause

**Table 1:** Anatomical distribution of abscesses in does where *S. aureus* was isolated (n=49).

Anatomical locations	Number
Dorsal neck	17
Ventral neck	8
Lumbar zone	7
Shoulder zone	6
Legs	5
Jaw	3
Abdomen	2
Muzzle	1
TOTAL	49



**Figure 6:** Several 1-2 cm subcutaneous abscesses discharging abundant white-yellowish pus.



**Figure 7:** Hyperkeratotic and ulcerative pododermatitis associated with *S. aureus* infection. The picture shows well circumscribed ulcerated lesions covered by a dry reddish-blackish crust.

several metabolic disturbances, which may lead to weight loss, cachexia and death (Marcato and Rosmini, 1986).

Microscopically, abscesses consist of three components: (1) a central mass of granulocytes undergoing purulent liquefaction; (2) an intermediate layer of reactive tissue infiltrated with granulocytes and macrophages; (3) and an external layer (capsule) of connective tissue (Marcato and Rosmini, 1986).

### **Pododermatitis or “Sore hocks”**

This condition is one of the most common causes of culling in both does and bucks (Rosell and de la Fuente, 2009). Okerman *et al.* (1984) isolated *S. aureus* from all examined cases of pododermatitis. Similarly, in a recent study carried out by Segura *et al.* (2007), *S. aureus* was isolated from 95% of does with pododermatitis. This lesion is caused by a fault in the animal's protection against its environment (the cage) (Drescher and Schlender-Böbbis, 1996). There are several predisposing factors including the wire floors of the cages (especially if sharp pieces are present), genetic factors (heavyweight breeds), various bacterial agents (responsible for the severity of lesions) and stagnation of urine in the bedding, which favour cutaneous maceration (Marcato and Rosmini, 1986). It has been suggested that *S. aureus* invades the primary traumatic lesions in young animals (sometimes under three months of age, of a fairly resistant breed, and housed on good quality wiring) (Okerman *et al.*, 1984). Adult breeding rabbits sometimes have a high incidence of pododermatitis (Okerman *et al.*, 1984) which is accompanied by multiple interdigital abscesses (Holliman and Girvan, 1986).

Lesion development begins with hyperplasia of the epidermis (parakeratosis). Then ischaemic necrosis develops from pressure to the skin, which may then ulcerate (Figure 7). The lesions associated with pododermatitis have been classified into different stages. This classification was based on macroscopical and histological findings and evidences the progressing loss of histological structures which end in total necrosis (Drescher and Schlender-Böbbis, 1996). Lesions may become infected with *S. aureus*, which leads to the development of abscesses under the crusty debris covering the ulceration (Marcato and Rosmini, 1986). Necrotic-gangrenous lesions and ulcerative lesions are less frequent in forelimbs than in hind legs (Marcato and Rosmini, 1986). Pressure ischaemia of the skin occurs more frequently in accordance with the protruding processes of the epiphyses of the metatarsal bones. Necrosis rarely spreads to the entire leg following tendon sheaths after secondary infection (Marcato and Rosmini, 1986).



### Other lesions associated with *S. aureus*

#### *Torticollis*

This chronic ear infection caused by *S. aureus* and/or *Pasteurella multocida* is often preceded by an ear-mite infestation. It predominantly occurs in unhygienic conditions and is spread by carrier animals. The first stages of the disease are characterized by periodically scratching one or both ears with frequent head-shaking. Signs of severity of the disease progressively increase. Finally, the head is held in a turned position so that feeding becomes progressively more difficult and affected rabbits die of starvation (Zumpt, 1976).

#### *Infections of the female genital tract*

These conditions may also be caused by *S. aureus* and *P. multocida*. They are common in some rabbitries and are usually associated with a high incidence of mastitis (Zumpt, 1976). *Staphylococcus aureus* has been isolated from 15.9% of the animals with pyometra (Segura *et al.*, 2007). The presence of staphylococcal suppurative metritis has been associated with high neonatal mortality (Holliman and Girvan, 1986) since complete or incomplete abortions are common, and such animals invariably die (Zumpt, 1976). Does often have a purulent vaginal discharge and a lethargic appearance (Zumpt, 1976).

In the appropriate conditions, *S. aureus* can also provoke lesions in other locations (Segura *et al.*, 2007). In a study carried out in rabbit does, 30.8% of cases of pneumonia were associated with *S. aureus* infections (Segura *et al.*, 2007). This bacterium has also been isolated from cases of arthritis (Viana *et al.*, 2007) and conjunctivitis (Holliman and Girvan, 1986; Viana *et al.*, 2007).

## DIAGNOSIS AND CONTROL

Diagnosis of staphylococcosis is usually accomplished by isolating the bacterium from the lesions. After growth on media containing bovine or ovine blood, the *S. aureus* colonies show a typical white to yellow colour. Practically all the *S. aureus* strains have haemolytic properties. Alpha, beta, gamma and delta haemolysins may be produced. Alpha and delta haemolysins provide a clear zone surrounding the bacterial colony. The additional production of beta haemolysin is responsible for an outer zone of incomplete haemolysis or rather discolouring with sharply demarcated edges. The DNase test is used to confirm the species identification (Devriese and Hajek, 1980). *S. aureus* is the only DNase-positive species of staphylococci found in rabbits (Devriese *et al.*, 1981). It is necessary to distinguish between HV and LV strains to complete the diagnosis. Until recently, this was usually done by biotyping followed by the laborious and time-consuming phage-typing technique. A much quicker and less laborious multiplex PCR assay allowing the identification of high-virulence strains after biotyping has been described by Vancraeynest *et al.* (2007). The test is based on the detection of the *bbp* and the *selm* genes, which have been shown to occur specifically in HV isolates (Vancraeynest *et al.*, 2004; 2006b). A third target is a sequence designated “flank”, which was derived from a previously generated HV-specific RAPD pattern (Hermans *et al.*, 2001). Furthermore, the *femA* gene, which is specific for *S. aureus* (Vannuffel *et al.*, 1995), is incorporated in order to avoid false negative results due to insufficient DNA preparation. PFGE may also be used for the identification of highly virulent strains, but this technique is expensive and less readily available. Rabbits infected with LV *S. aureus* strains may be treated by draining and cleaning the subcutaneous abscesses in combination with administration of antimicrobial agents. This treatment is often restricted to pet rabbits and not performed in commercial rabbitries. Effective treatment of rabbit flocks infected with HV *S. aureus* strains is not possible. Thorough cleaning and disinfecting cages and materials, together with antimicrobial treatments such as 800 mg tetracycline HCl per kg feed over a 7-day period, may reduce disease and mortality. These measures do not eliminate the bacterium from the

flock. After cessation of the therapy, the problems will arise again (Carolan, 1986; Holliman and Girvan, 1986; Devriese *et al.*, 1987; Rossi *et al.*, 1995). Culling of diseased or suspected animals is even less successful (Devriese *et al.*, 1987; Devriese *et al.*, 1996). The only solution after intake of HV *S. aureus* strains in a rabbitry is to slaughter the entire flock, clean and disinfect the stable thoroughly, and start all over again with new rabbits from an unaffected rabbitry.

Prevention of HV staphylococcosis in rabbits is therefore of utmost importance, but a difficult task. A restricted introduction of new animals and limited contact between rabbitries may lower the risk of infection (Devriese *et al.*, 1987; Devriese *et al.*, 1996). Currently, this is the most feasible method to cope with rabbit staphylococcosis.

New animals should be tested for the presence of HV strains before being introduced into the flock. Rabbit carrier sites of *S. aureus* may be the nose, the ear, the skin between the toes and the skin of the foreleg, the axillar and inguinal skin regions, the skin around the nipples, the perineum, the vagina and the preputium. Rabbits may be discretely to highly positive for *S. aureus* at one to nine of these body sites (Hermans *et al.*, 1999). Therefore, a high number of samples should be taken to avoid false-negative results. The perineum seems to be an important body site for sampling since it is often intensely colonized (Hermans *et al.*, 1999). The samples should be inoculated on a non-selective blood agar as well as on a suitable selective medium such as modified Baird-Parker medium (Devriese, 1981) since abundant microflora is present on most body sites which may overgrow the media, thus making the identification of *S. aureus* difficult (Hermans *et al.*, 1999).

Unfortunately, screening for HV strains is also complicated by the fact that almost all rabbits are carriers of LV *S. aureus* strains (Hermans *et al.*, 1999). To differentiate HV from LV strains, the multiplex PCR described by Vancraeynest *et al.* (2007) may prove useful. To date, however, this assay has only been shown to be reliable if pure cultures of *S. aureus* are tested, and it remains to be determined whether it can also be applied on mixed cultures obtained after inoculation of media with samples from the aforementioned body sites.

Several authors have described studies in rabbits with bacterins against staphylococcosis (Hinton, 1977; Cameron *et al.*, 1979; Rossi *et al.*, 1995; Matthes, 1995; Meulemans *et al.*, 2008). These vaccines may induce partial protection against an experimental *S. aureus* infection. In the field, autogenous vaccines are sometimes used and some rabbit producers report less severe problems with high-virulence strains after autovaccination. If we bear in mind that the pathogenesis of *S. aureus* infections is highly complex and that the preparation and application of autogenous vaccines is empirical, it is not surprising that the results obtained with these vaccines are inconsistent.

At present development of an effective vaccine against staphylococcosis is a difficult matter, as it is not known which antigens are important for protection. Further research in this field is therefore required.

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