REGARDING A CASE OF BLUE COLORATION ON MEAT RABBIT CARCASSES

Boucher S. [1]*, Nicolier A[2], Tatone F.[3], Sauvaget S.[1]

[1] Labovet Conseil (Réseau Cristal), BP 539 85505 Les Herbiers cedex
[2] Vet Diagnostics, 3 avenue de la Victoire, 69260 Charbonnières-les-Bains
[3] Resalab Ouest, 22 rue Olivier des Serres, 85500 Les Herbiers cedex
*Corresponding author: s.boucher@labovet.fr

ABSTRACT

A slaughterhouse told us about the occurrence of a blue coloration of meat from commercial rabbits. It occurs in the summer time, a few days after slaughter, at retailers. In order to highlight the potential etiological agent, histological and bacteriological examinations were carried out at the same time. Four bacteria emerged: *Enterococcus hirae, Escherichia coli, Staphylococcus aureus* and *Pseudomonas libanensis,* which were identified and confirmed using Maldi-tof mass spectrometry technology. The isolated bacteria were then seeded individually at room temperature under a hood on the surface of a healthy rabbit carcass. Coloration occurred after 48 hours only on meat inoculated with *Pseudomonas libanensis*. We conclude that this bacterium is at the origin of the coloration observed in retailers. The literature indicates that a break in the cold chain is necessary for the bacteria to develop at 25 - 30 ° C. A slaughterhouse cleaning procedure and a reminder on the disinfection of the hands of chain operators are in place. Recording thermometers are put in boxes when they leave the slaughterhouse in order to identify where the cold chain has broken.

Key words: Rabbit, *Oryctolagus cuniculus*, carcass, pigment, *Pseudomonas*, *Pseudomonas libanensis*,

INTRODUCTION

The manager of a French slaughterhouse was questioned by some of his customers about the occurrence of blue spots on the rabbit carcasses that he had provided. This is the fourth time this has happened. (Fig. 1). He asked us to find the origin of this problem and to find a practical solution as soon as possible. During slaughter, the meat appeared healthy and not coloured, without any lesions. Three days after death, the blue spots appeared in the refrigerators of retail butchers delivered by the slaughterhouse. The stain, initially small, enlarged as time passed until it covered almost the entire carcass. It was summertime with temperatures approaching 35^0 C. Given the unknown severity of this problem, the slaughterhouse informed the Veterinary State Services. According to its survey, spots seemed to have developed only on rabbits coming from a large farm which provides the bulk of its supply. What follows will show that cases have also occurred in rabbits from other farms. Contacted by the veterinarians in charge of the dossier at the Departmental Directorate of Population Protection (DDcsPP), we decided to set up an experiment aimed at identifying the causes of this abnormality. A histological examination can tell us about the nature of the lesion and tell us whether it is pre- or post-mortem (usually if a lesion was created during the animal's lifetime, there should be traces of inflammation). This examination will also guide us towards the nature of the potential contaminating agents. This examination will be followed by a bacteriological examination to possibly demonstrate the presence of bacteria revived after thawing. Finally, Rossel indicated that such colourings may be due to algae to (Rosell et al., 2000).

MATERIALS AND METHODS

The slaughterer gave us only one sample, so the study focused on this frozen blue rabbit. It was given to us in parchment paper which itself tinted slightly blue on the parts that covered the lesions on the carcass. For the rest of the protocols, we used a normal frozen rabbit carcass not related to either the breeder or the slaughterhouse.

Three affected muscles were sampled, fixed in 4% buffered formalin (SAFECAPSULE system 250 ml of the Microm Microtech brand containing 91 ml of PBS buffer and 19 ml of concentrated formalin) and sent to the laboratory. Samples were dehydrated and embedded in paraffin. Sections, 3 µm thick, were mounted on a glass slide and stained with hematoxylin and eosin and PAS. Slides were analyzed using a Nikon Eclipse Ci light microscope.

A bacteriological examination was carried out. Using a sterile loop, the surface of the carcass was scraped at the level of the lesion. Then, the sample was inoculated on two types of dishes, a non-selective and rich medium, with agar supplemented with sheep blood, and a selective medium for non-demanding Gram-negative bacilli, with Drigalski agar, a lactose isolation medium. They were incubated at 37°C or 30°C in an oven for 24 to 48 hours. At the same time, a nutritive broth was inoculated and then incubated for 24 hours. It was then inoculated on the same types of agar at the same temperatures. The day after, the colonies were sub-cultured to isolate and purify the bacteria of interest. The colonies were then analyzed using a mass spectrometer technology (Maldi-tof) which directly analyzes the macromolecules of different bacteria, in particular proteins of ribosomal origin. The bacterial strains were deposited on a stainless steel plate and mixed with a matrix which has two functions: to protect the colonies from the laser and to positively charge the proteins once the laser has carried out the physicochemical desorption of each bacterium. The proteins fly in an evacuated tube and a sensor positioned at the end of the flight tube is able to transform the signal (molecular weight and the speed at which the molecule has reached the sensor) into proteomic curves / fingerprints. The device measures the abundant proteins found in microorganisms and their characteristics, then matches them to a database (NCBI - National Center for Biotechnology information) to accurately identify the microorganism.

As for the bacteriological examination, using a sterile loop, we spread the scraping product from the carcass taken from the bluish lesions on a so-called Sabouraud agar which allows the cultivation of fungi, yeasts but also algae of the genus *Prototheca*. It was incubated at 30 ° C for 5 days. In case of positive culture, direct examination under an optical microscope was considered. It should be noted that the prototheca could also have grown on a blood agar or on a specific “PIM” medium (Prototheca isolation medium), which we did not have (Swinne et al., 2002).

Once the various bacterial agents had been isolated, the colonies were taken directly using a sterile swab and then placed on the rabbit carcass that showed no lesion. The carcass was left at room temperature for 72 hours. It was placed under a hood on a cleanable and disinfectable tray and then covered with a transparent glass container allowing its evolution to be observed. At the end of the experiment the carcasses were cremated and the equipment was cleaned and disinfected.

RESULTS AND DISCUSSION

The muscle was of normal appearance, without any inflammation or necrosis. A thick layer of polymorphic bacteria, mainly cocci, was observed on its surface, admixed with rare yeast-like elements (Fig.2) and fungal pseudohyphae visible in PAS staining.

Figure 1. Rabbit carcass with blue spots

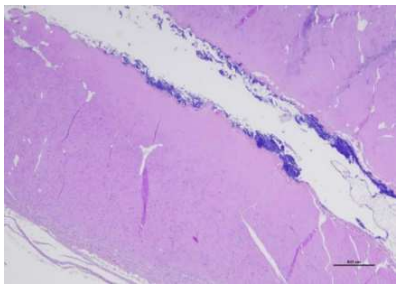

Figure 2 Histological features of the muscles: A thick layer of basophilic bacteria covers the normal appearing muscle. Hematoxylin and eosin (X2)

Four bacteria were isolated and then confirmed. Among them, *Enterococcus hirae, Escherichia coli* and *Staphylococcus aureus* which are potentially pathogenic agents frequently encountered in rabbit farming and *Pseudomonas libanensis*, not known to be pathogenic for this species (Boucher and Nouaille, 2013). Nothing grew on the boxes placed at 37 ° C for 48 hours. *Pseudomonas* grew (without enrichment) on Drigalski agar placed at 30 ° C for 24 hours. It fluoresced in black light (Fig. 3). But it is following the enrichment by the nutrient medium and the subculturing on a Drigalski agar that the staphylococcus and the *E. coli* grew at 37 ° C, while the same *E. coli* and the *enterococcus* grew on this agar at 30 ° C in 24 hours.

Figure 3 Bacteria passed under a Wood's lamp to reveal the fluorescence

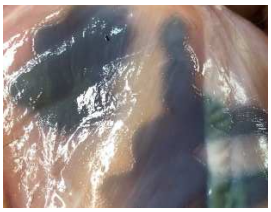

Figure 4 Appearance of the meat 48 hours after inoculation: blue spots appear where *P. libanensis* has been deposited.

No algae or fungus could be detected after 5 days by this culture under the conditions mentioned, neither on Sabouraud agar, nor on blood agar at 30 ° C. The literature mentions, in case of positive protothecal algae, a growth in 24 hours (Swinne et al. 2002).

The meat doesn't change in appearance nor in colour as a result of the deposition of *Enterococcus hirae, Escherichia coli* or *Staphylococcus aureus*. In contrast, it takes on a slightly yellowish tint on the first day and then turns blue after 48 hours on surfaces where *Pseudomonas libanensis* has been deposited (Fig. 4). It is concluded that this bacterium, which developed at room temperature, is the cause of the blue colouring of the carcasses.

Pseudomonas contains an impressive number of species, considering that there are more than 2000. Most are part of the commensal flora of mammals, but the rabbit is known to be also infected with *Pseudomonas areruginosa*. As in humans, the bacteria can contaminate the skin if it is constantly moistened, creating dermatitis. The bacteria then proliferate on the skin creating a greenish halo on the hairs surrounding the lesion. Among humans with weakened immunity, it can create pulmonary, urinary and sepsis infections (Garibaldi, 1990, O'Donoghue 1971, Schoenbaum 1981). Daboussi et al. (1999) proposed to rename, on phenotypic and phylogenic criteria, a strain of *Pseudomonas fluorescens* to *Pseudomonas libanensis* thus naming a new species. It has been deposited at the Institut Pasteur (CIP 105460T) (Dabboussi, 1999). The difference between *P. aeruginosa* and *P. libanensis* is based on the production of pyocyanin. *P. libanensis* can be differentiated from other *P. fluorescens* by the assimilation of alpha amino-butyrate (Anzaï, 2000; Meyer, 2000). However, its clinical significance is unknown to date and there is no publication linking the bacterium to disease. *Pseudomonas fluorescens* forms colonies

that are fluorescent (hence its name). *P. Libanensis* retained this property due to the production of pyoverdin. Its optimum growth temperature is between 25 and 30 ° C. This bacterium is known to be able to contaminate and then colour different foodstuffs such as cheese or meat (Andreani, 2015). The very chemical nature of the pigment found on the carcasses, however, was not investigated in this experiment. It should also be noted that *Pseudomonas* in general, in addition to the production of pigments, can also produce enzymes that can alter meat or change its taste (Andreani, 2015).
The few rare yeast-like elements were observed. They are probably contaminants. No algae were observed.
It is considered that the meat of a slaughtered rabbit must be brought to 2 ° C in one hour in a cooling tunnel. It must then be stored at 4 ° C maximum (Collectif, 2009). *Pseudomonas*, on the other hand, grow at temperature ranges of between 25 and 30 ° C. There must therefore have been a necessary break in the cold chain to promote the development of the bacterium on the carcasses. Any increase in temperature causes and accelerates microbial growth and shortens the shelf life of the product (DGCCRF 2018).
The lesions observed are compatible with post-mortem modifications previously described in rabbits, linked to a break in the cold chain. The literature cites cases of meat staining blue with overgrowth of *Pseudomonas fluorescens* and sometimes other Pseudomonas (Andreani, 2015; Anonymous, 2020; Rosell et al., 2000, Sarale et al., 2011). These *Pseudomonas* produce a yellow pigment which upon lipid peroxidation of meat and fat can turn into a bluish pigment (Frazier et al., 1984).
The slaughterhouse was therefore advised to equip its packages with recording chips (thermobutton) in order to detect where the rupture of the cold chain could occur. This system was difficult to set up due to the multiplicity of resellers and the difficulty in targeting one of them. In fact, the slaughterhouse sells to a wholesaler who himself resells to a multiplicity of retailers. Disposable recording equipment have been purchased and packages will be able to be traced. At the same time, DDcsPP veterinarians revealed a poor practice of hand washing among operators, which favored the inoculation of *P libanensis* on carcasses. The disinfection procedures with hydroalcoholic gel have thus been strengthened. Finally, it was also advised to search for *Pseudomonas libanensis* in the work environment of chain operators. The slaughterhouse preferred to set up a cleaning procedure using enzymatic foam and disinfection of the entire chain, including the slaughterhouse, cold rooms, evaporators. The water is regularly analyzed using agar slides to highlight any *Pseudomonas.*

CONCLUSION

Cases of blue coloring in rabbit meat are rare. Indeed, for these cases to emerge, both the presence of *Pseudomonas* and a break in the cold chain are required. The case presented here has the particularity of being due to a *Pseudomonas* whose species (*libanensis*) was recently redefined. Better application of slaughterhouse hygiene rules and special attention to the cold chain have made it possible not to reproduce the phenomenon

ACKNOWLEDGEMENTS

Thanks to the team of bacteriologists from the Resalab Ouest laboratory who made it possible to identify the concerned agent.

REFERENCES

Andreani N.A. 2015. Into the blue: Spoilage phenotypes of *Pseudomonas fluorescens* in food matrices. *PhD thesis Universita degli studi di Padovia, Scuola di dottorato in scienze veterinarie. 240p*

Anonymous 2020. The Curious Case of the Blue Rabbit *http://www.cresa.cat/blogs/sesc/el-curios-cas-del-conill-blau/?lang=en consulté le 14 10 2020*

Anzai Y. et al. 2000. Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. *Int J Syst Evol Microbiol.50(4): 1563–89.*

Boucher S., Nouaille L. 201.,Maladie des lapins, *3ème édition, France agricole, Paris,306p*

Dabboussi F., Hamze M., Elomari M., Verhille S., Baida N., Izard D., Leclerc H.. 1999. *Pseudomonas libanensis* sp. nov., a new species isolated from Lebanese spring waters. *Int J Syst Bacteriol,49:1091-1101*.

DGCCRF 2018.Température de conservation des aliments. *https://www.economie.gouv.fr/dgccrf/Publications/Vie-pratique/Fiches-pratiques/Temperature-de-conservation lue le 14 10 2020*

Frazier WC, Westhoff DC., Vanitha NM.1984. Food microbiology, *McGraw-Hill Education Ed., New York, 886p*

Garibaldi BA, Fox JG, Musto DR. 1990. Atypical moist dermatitis in rabbits. *Lab Anim Sci.40(6):652-653*.

Meyer JM, 2000.Pyoverdines : pigments, siderophores and potential taxonomic markers of fluorescent Pseudomonas species.*Archives of Microbiology,174(3),135-142*

O'Donoghue PN, Whatley BF., 1971. *Pseudomonas aeruginosa* in rabbit fur. *Lab Anim.5(2):251-255.*

Rosell JM., Facchin E., Tantina M. 2000. Capitulo X, Salud Publica p 502 – 503, *In Rosell et col. Enfermedades del conejo Tomo 1, Madrid, 605p*

Sarale, A., Grassi, M.A., Civera, T., 2011. *Pseudomonas gessardii* causative agent for blue spot formation on rabbit carcasses. *Italian Journal of Food Safety. 1. 10. 261-262.*

Schoenbaum M. 1981. *Pseudomonas aeruginosa* in rabbit fur. *Lab Anim.15(1): 5- 6.*

Swinne D., Moubamba D., Lagneau PE., Geerts S. 2002. Nouveaux cas d'infections par Prototheca zopfii chez la vache laitière en Belgique. *Ann. Med. Vet.146: 311-315.*